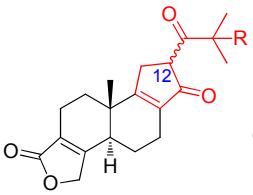
RSC Advances

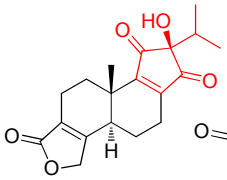
ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

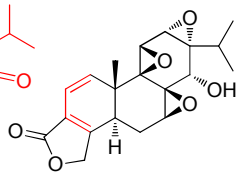
Tripterlides A–F were new abietane derivatives, obtained from *Tripterygium wilfordii*. **1**–**4** were novel 14(13→12),18(4→3)-*diabeo*-abietanoids possessing a 6/6/5 tricyclic ring system.



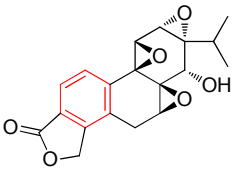
Tripterlide A (**1**) 12*R* R=H
Tripterlide B (**2**) 12*S* R=H
Tripterlide C (**3**) 12*R* R=OH



Tripterlide D (**4**)



Tripterlide E (**5**)



Tripterlide F (**6**)

Journal Name

RSCPublishing

ARTICLE

Bioactive 18(4→3)-*abeo*-abietanoids Derivatives from the Leaves of *Tripterygium Wilfordii*

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Chao Wang,[ab] Chuang-Jun Li,[a] Jie Ma,[a] Jing-Zhi Yang,[a] Xiao-Guang Chen,[a] Li Li,[a] and Dong-Ming Zhang*[a]

Tripterlides A–F (**1**–**6**), six new abietane diterpenoids derivatives, together with six known abietane diterpenoids (**7**–**12**) were obtained from the leaves of *Tripterygium wilfordii*. Especially that tripterlides A–C (**1**–**3**) were novel 14(13→12),18(4→3)-*diabeo*-abietanoids possessing a 6/6/5 tricyclic ring system. These unusual structures, including their absolute configurations, were determined using UV, IR, HRESIMS, 1D-, 2D-NMR data and through comparisons of the experimental and calculated electronic circular dichroism (ECD) spectra. The plausible biosynthetic pathway of **1**–**3** was proposed. Furthermore, in an *in vitro* bioassay, **5**, **6**, **8**, **10**–**12** showed significant cytotoxic effects against five human cell lines, as well as that **6**, **8**, **10**–**12** exhibited moderate inhibitory activities on hypoxia-inducible factor 1 (HIF-1), which were relevant to the tumor development.

Introduction

Tripterygium wilfordii Hook. f. is widely distributed in the southern parts of China, which is known as Lei Gong Teng (Thunder God Vine) in traditional Chinese medicine. The roots of *T. wilfordii* have been traditionally used to treat rheumatoid arthritis, cancer and kill garden insects.[1] Recently, an extract, derived from a water-chloroform extract of the roots (the so-called "total multi-glycoside") has been used in the clinical treatments of rheumatoid arthritis and other inflammatory and autoimmune diseases.[2,3] Previous chemical investigations of this plant have revealed the presences of sesquiterpene polyol esters, sesquiterpene alkoids, diterpenes, triterpenes, and lignans.[4-7] Triptolide (**10**), the typical compound of the 18(4→3)-*abeo*-abietane diterpenoids was isolated from the roots of *T. wilfordii* by Kupchan.[8] Since then, several triptolide analogues have been obtained from *T. wilfordii*.[9-12] Anti-inflammatory, immunosuppressive and anti-cancer activities of those diterpene lactone epoxide compounds were extensively reported.[13] These findings prompted us to systematically investigate the leaves of *T. wilfordii*. As a result, six new abietane derivatives tripterlides A–F (**1**–**6**), together with six known 18(4→3)-*abeo*-abietanoids were isolated from the ethnolic extract of the leaves of *T. wilfordii* by several chromatographic technologies (Figure 1). Their structures were determined by spectroscopic analyses. Tripterlides A–C (**1**–**3**) possessed an unusual 6/6/5 tricyclic ring system and α,β-unsaturated-γ-lactone, which might be biosynthetically from 18(4→3)-*abeo*-abietane diterpenoid lactone by migration of the C-14/C-13 bond to the C-14/C-12 as the key step. It is the first time to obtain these abietane derivatives from natural products. The inhibitory effects on hypoxia-inducible factor 1 (HIF-1) in U251-HRE and cytotoxicities against five human cancer cell lines of **1**–**12** were also evaluated. We present herein the isolation and structural characterization of tripterlides A–F, as well as their bioactivities.

Results and discussion

Tripterlide A (**1**) was obtained as a pale yellow powder. Its molecular formula $C_{20}H_{24}O_4$ was determined by the HRESIMS at *m/z* 351.1567 $[M + Na]^+$ (calcd for $C_{20}H_{24}NaO_4$, 351.1573), implying 9 degrees of unsaturation. The UV spectrum of **1** exhibited an absorption maximum at 246 nm, which was characteristic of an α,β-unsaturated ketone. The carbonyl (1749, 1701 cm^{-1}) and methyl (1462, 1378 cm^{-1}) groups were also observed in the IR spectrum of **1**.

The ^1H NMR spectrum revealed the presences of 24 protons (Table 1) and indicated the presence of an isopropyl moiety [δ_H 1.20 (3H, d, J = 7.2 Hz), 1.14 (3H, d, J = 6.6 Hz), 3.04 (1H, m)],

[a] State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, People's Republic of China

[b] College of Pharmacy, Dalian Medical University, Dalian 116044, China

E-mail: zhangdm@imm.ac.cn; Tel/fax: +86-10-63165227.

†Electronic Supplementary Information (ESI) available: The spectra including 1D-, 2D-NMR, HRESIMS of compounds **1-6** as well as related original ECD calculation data for tripterlides A (**1**), B (**2**), and C (**3**). See DOI: 10.1039/b000000x/

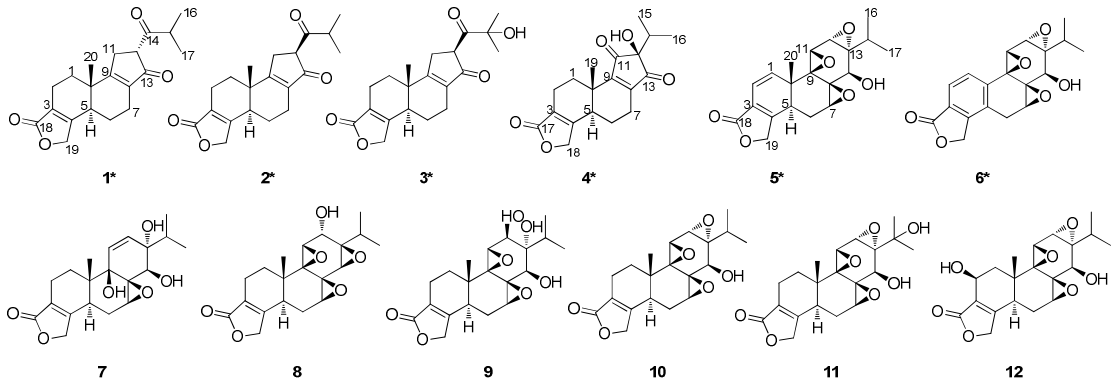


Figure 1. Abietane diterpenoids derivatives (**1**–**12**) obtained from the leaves of *Tripterygium wilfordii*.

a tertiary methyl [δ_H 0.93 (3H, s)]. Additionally, the oxymethylene [δ_H 4.84 (2H, m)] of an α,β-unsaturated-γ-lactone was also observed by the analysis of the ^{1}H NMR spectroscopic data. The ^{13}C NMR spectrum of **1** exhibited 20 carbon signals, which could be attributed to three methyls, six methylenes (including one oxygenated), three methines, and eight quaternary carbons (including four sp^2 carbons and three carbonyls) by the DEPT spectrum. Analyses of these carbon signals indicated the presences of an α,β-unsaturated-γ-lactone [δ_C 173.9, 124.7, 163.1, 71.0], an α,β-unsaturated ketone [δ_C 206.0, 140.2, 176.0], and an isolated ketone [δ_C 214.2]. These spectroscopic data suggested that compound **1** was a diterpenoid with a tricyclic ring system, similar to the known 18(4→3)-*abeo*-abietane, such as triptolide analogues.[8-12]

The HMBC correlations of H-1/C-3 (δ_C 124.7); H-2/C-3 (δ_C 124.7), and C-4 (δ_C 163.1); H-19/C-3 (δ_C 124.7), and C-4 (δ_C 163.1); and H-20/C-1 (δ_C 32.2), C-5 (δ_C 43.4), and C-10 (δ_C 38.1) established ring A, and confirmed the locations of the α,β-unsaturated-γ-lactone and the tertiary methyl [H-20 (δ_H 0.93)], respectively (Figure 2). The ring B also could be established by the long-range correlations between H-6 and C-8 (δ_C 140.2); H-7 and C-8 (δ_C 140.2), C-9 (δ_C 176.0); H-20 and C-9 (δ_C 176.0) observed in the HMBC spectrum. The ^{1}H-^{1}H COSY correlation of H-11/H-12, and the HMBC correlations of H-11/C-9 (δ_C 176.0), and C-13 (δ_C 206.0); and H-12/C-8 (δ_C 140.2), C-9 (δ_C 176.0), and C-13 (δ_C 206.0) established the unusual five membered ring C (Figure 2). The ^{1}H-^{1}H COSY correlations of H-15/H$_3$-16, and H$_3$-17 confirmed the isopropyl moiety, and the HMBC correlations of H-15/C-14 (δ_C 214.2), and H-16/C-14 (δ_C 214.2) established the isobutyl ketone group. The HMBC long-range correlations of H-11/C-14, and H-12/C-14 suggested that the isobutyl ketone was connected to C-12 of ring C.

It was reported that plenty of abietane type diterpenoids including ent-abietanes and abietanes were isolated from various plants.[14-19] In consideration of the chemical investigations of *T. wilfordii*, several abietanes and 18(4→3)-*abeo*-abietanes were obtained.[8-12] The 5α-H, 20β-CH$_3$ and α,β-unsaturated-γ-lactone were the structural characteristics of these 18(4→3)-*abeo*-abietanes. The spectroscopic data analyses indicated that compound **1** was derivative of 18(4→3)-*abeo*-abietanes similar to these isolated diterpenoids from *T. wilfordii*. The NOESY correlations of H-5/H-1a and H-20/H-1b revealed the *trans*-fused relationship of A/B, same as triptolide analogues.[8-12] Therefore, the planar structure of **1** was established as a 14(13→12),18(4→3)-*diabeo*-abietane diterpenoid possessing a 6/6/5 tricyclic ring skeleton that likely derived from 18(4→3)-*abeo*-abietane on the basis of above spectroscopic data.

Compound **2** had the same molecular formula as **1** deduced by the HRESIMS at *m/z* 329.1753. The planar structure of **2** was established same as that of **1** on the basis of the spectroscopic data (Table 1). Therefore, compound **2** was defined to be a stereo-isomer of **1**.

Table 1. NMR Spectroscopic Data of Compounds **1**, **2**, and **3**.

	1		**2**		**3**	
position	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]	δ_H[c]	δ_C[d]
1	2.12 m	32.1	1.82 m	32.1	1.70 m	31.6
	1.82 m		1.54 m		1.59 m	
2	2.36 m	18.4	2.30 m	18.5	2.41m	17.8
	2.26 m		2.21 m		2.28 m	
3	-	124.7	-	124.7	-	124.9
4	-	163.1	-	163.2	-	161.5
5	2.64 brd (12.6)	43.3	2.79 brd (13.6)	42.4	2.86 brd (13.0)	41.4
6	1.99 m	19.1	2.00 m	19.5	1.93 m	19.1
	1.82 m		1.76 m		1.74 m	
7	2.38 m	21.0	2.20-2.33 m	20.8	2.44 m	20.1
	2.22 m					
8	-	140.2	-	139.7	-	140.1
9	-	176.0	-	176.7	-	175.7
10	-	38.1	-	38.4	-	37.8
11	2.60 dd (18.0, 7.2)	41.2	2.66 dd (18.0, 7.6)	41.0	2.73 dd (18.0, 7.5)	40.8
	2.12 dd (18.0, 1.8)		2.08 m		2.21 dd (18.0, 2.0)	
12	4.41 m	49.7	4.44 m	49.3	4.58 m	43.8
13	-	206.0	-	206.0	-	205.7
14	-	214.2	-	214.2	-	213.8
15	3.04 m	42.5	3.00 m	42.3	-	77.5
16	1.20 d (7.2)	18.6	1.17 d (7.2)	18.7	1.51 s	28.1
17	1.14 d (6.6)	18.2	1.13 d (6.8)	18.3	1.47 s	27.4
18	-	173.9	-	173.9	-	173.6
19	4.84 m	71.0	4.84 m	70.9	4.70 m	70.3
20	0.93 s	19.2	1.07 s	19.5	1.02 s	19.5

[a] In actone-d_6 (600 MHz for **1**, 400 MHz for **2**). [b] In actone-d_6 (150 MHz). [c] In chloroform-d_1 (500 MHz). [d] In chloroform-d_1 (125 MHz).

Compound **3** showed the molecular formula $C_{20}H_{24}O_5$ by HRESIMS at *m/z* 345.1701. The ^{1}H NMR spectrum indicated the presences of three methyl groups [δ_H 1.51, 1.47, 1.02 (each 3H, s)], and the oxymethylene [δ_H 4.70 (2H, m)] of an α,β-unsaturated-γ-lactone. The ^{13}C NMR spectrum of **3** exhibited 20 carbon signals, which were similar to those of **2**, as well as

the down shift of C-15 from δ_C 42.3 (**2**) to δ_C 77.5 (**3**). Analyses of the spectroscopic data (Table 1) of **3** suggested that the planar structure of **3** was also similar to that of **2**, except for the hydroxylation of C-15 (δ_C 77.5).

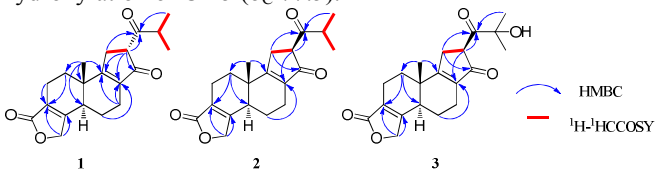


Figure 2. 2D NMR correlations of **1–3**.

It was known that triptolide analogues as the natural products containing the 18(4→3)-*abeo*-abietane skeleton were just obtained from *T. Wilfordii*. Their fine stereo-structures were clarified by X-ray crystallographic analyses. As a result, the stereochemistry of 18(4→3)-*abeo*-abietane skeleton was identified as 5*R*, 10*S*.[8,20] The absolute configurations of **1–3** were determined as 5*R*, 10*S*, 12*R* (**1**), 5*R*, 10*S*, 12*S* (**2**), 5*R*, 10*S*, 12*R* (**3**) by comparisons of the experimental ECD spectra and calculated ECD data using the time-dependent density functional theory (TD-DFT) method at the B3LYP/6-31G(d) level.

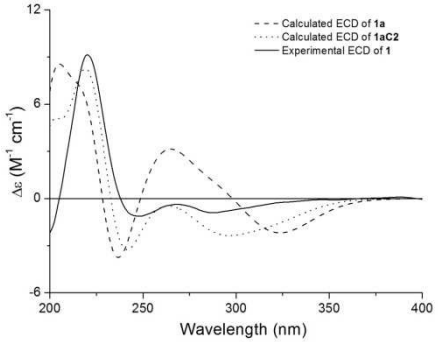


Figure 3. Calculated ECD spectra of 5*R*, 20*S*, 12*R* **(1a)**, and one of the optimized conformations (**1ac2**), and the experimental ECD spectrum of **1**.

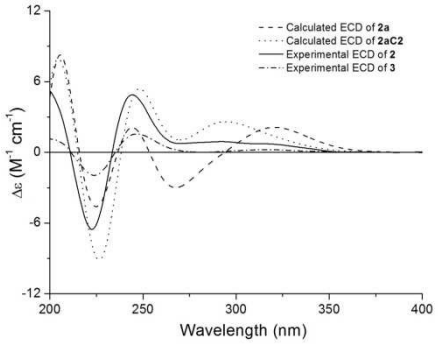


Figure 4. Calculated ECD spectra of 5*R*, 20*S*, 12*S* **(2a)**, and one of the optimized conformations (**2ac2**), and the experimental ECD spectra of **2**, and **3**.

The calculated ECD curves of 5*R*, 10*S*, 12*R* **(1a)**, 5*R*, 10*S*, 12*S* **(2a)** matched well with experimental data of **1** and **2**, respectively (Figures 3 and 4). Additionally, the conformations analyses of **1a** and **2a** (Figure s29, s30) suggested that the optimized conformations **1ac1** (92.09%) and **2ac1** (88.67%) were major conformations, which have important roles in the calculated ECD of **1a** and **2a**. Meantime, it was found that the calculated ECD of conformations **1ac2** (5.71%) and **2ac2** (9.98%) also matched very well with experimental data, which were illustrated in Figures 3, 4, and 5.

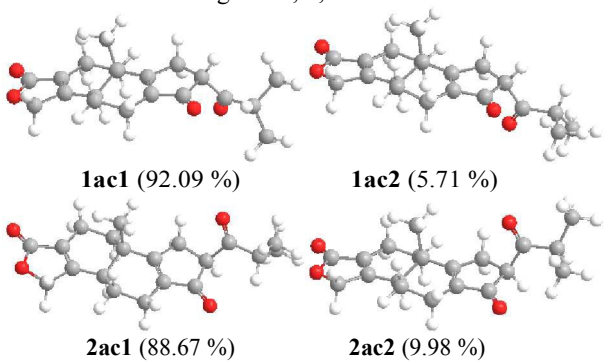
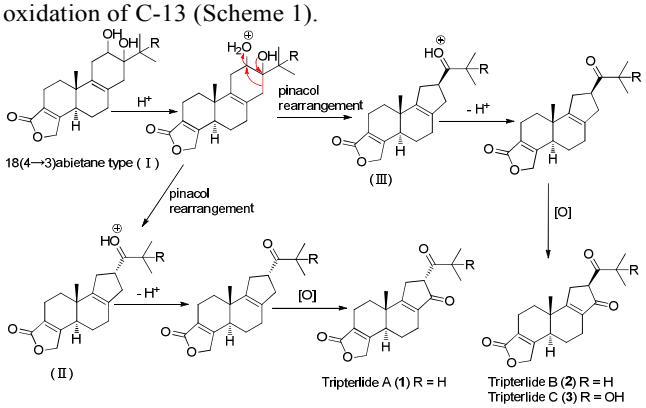


Figure 5. The optimized conformations of **1a**, and **2a**.

Plausible biogenetic pathways to compounds **1–3** are proposed as shown in Scheme 1. The biosynthetic precursor of **1–3** could be traced back to a proposed 18(4→3)-*abeo*-abietane type (**I**). After protonation, **I** would undergo Pinacol rearrangement to give the key intermediates **II** and **III**, which would readily produce **1–3** respectively by loss of a proton and oxidation of C-13 (Scheme 1).



Scheme 1. Plausible Biogenetic Pathway of **1–3**.

Compound **4**, was obtained as a pale yellow solid. Its molecular formula was established as $C_{19}H_{22}O_5$ by the HRESIMS with the ion peak at *m/z* 331.1543 $[M + H]^+$ (calcd for 331.1540), implying 9 unsaturation degrees. The UV spectrum exhibited the absorption of unsaturated ketone (λ_{max} 243 nm). ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum (Table 2) indicated the presences of one isopropyl moiety [δ_H 0.953 (3H, d, J = 6.6 Hz), 0.946 (3H, d, J = 7.2 Hz), 2.08 (1H, m)], one tertiary-methyl [δ_H 1.14 (3H, s)], one oxygenated methene [δ_H 4.82, 4.74 (each 1H, s)], one methine [δ_H 2.71 (1H, brd, J = 13.8 Hz)], and four methylene groups. ^{13}C NMR spectroscopic data (Table 2) exhibited 19 carbon signals, including one α,β-unsaturated-γ-lactone (δ_C 173.5, 125.8, 162.0, 70.3), and one α,β-unsaturated diketone (δ_C 156.3, 162.5, 200.8, 200.7). On the basis of above spectroscopic data, compound **4** was established as a nor-diterpenoid with 19 carbons, which was similar to the abietane diterpenoids isolated from *T. wilfordii*.[8-12]

^{1}H-^{1}H COSY spectrum displayed three separated spin-spin systems (H-1/H-2, H-5/H-6/H-7, H-14/H-15/H-16). In the HMBC spectrum, the long-range correlations of H-1 (δ_H 2.92)/C-2 (δ_C 18.0), C-3 (δ_C 125.8), C-5 (δ_C 42.3), C-10 (δ_C 35.7), and C-19 (δ_C 17.98) established the ring A and

confirmed the location of the α,β-unsaturated-γ-lactone. Ring B could be established by the HMBC correlations of H-6/C-5, C-10, H-7/C-8, and C-9. The five membered ring C was deduced by the long-range correlations of H-19/C-9, H-7/C-13, H-14/C-13, and H-16/C-12. The isopropyl moiety was established by the ^{1}H-^{1}H COSY spectroscopic data (H-14/H-15/H-16) and HMBC correlations (H-15/C-14, H-16/C-14). Analyses of the 1D, 2D NMR data revealed that compound **4** was a nor-diterpene with 18(4→3)-*abeo*-abietane skeleton, possessing a 6/6/5 tricyclic ring system.

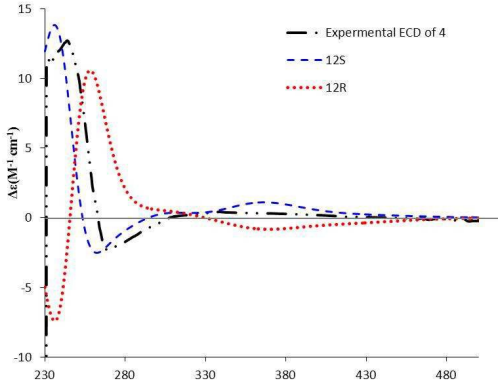


Figure 6. The experimental ECD of **4**, and calculated ECD spectra of **4** (12*S*) and its 12-epimer (12*R*).

The absolute configuration of **C-12** was determined to be 12*S* by the comparison of the experimental ECD and calculated CD curves of **4** and its 12-epimer (Figure 6). Therefore, compound **4** was elucidated as shown, named tripterlide D.

Compound **5**, obtained as a white, amorphous solid, had the molecular formula $C_{20}H_{22}O_6$, deduced from the $[M + H]^+$ ion peak at *m/z* 359.1489. The IR spectrum displayed the absorptions of hydroxyl (3550 cm^{-1}), carbonyl (1764 cm^{-1}), methyl groups (1444, 1371 cm^{-1}). ^{1}H NMR spectrum (Table 2) exhibited the presences of cyclic olefinic bond [δ_H 5.56 (1H, d, J = 9.6 Hz), 6.17 (1H, d, J = 9.6 Hz)], one isopropyl [δ_H 1.02 (3H, d, J = 7.2 Hz), 0.89 d (3H, d, J = 7.2 Hz), 2.23 (1H, m)], one tertiary-methyl [δ_H 1.20 (3H, s)], one oxygenated methene [δ_H 4.82 (2H, m)], and four oxygenated methines [δ_H 3.36, 3.44, 3.54, 3.76]. ^{13}C NMR spectroscopic data (Table 2) exhibited 20 carbon signals, which could confirm above moieties and revealed the presence of one α,β-unsaturated lactone [δ_C 170.8, 157.9, 123.8, 69.5]. Analyses of the spectroscopic data indicated that compound **5** was an 18(4→3)-*abeo*-abietane diterpenoid, which was similar to triptolide.[8]

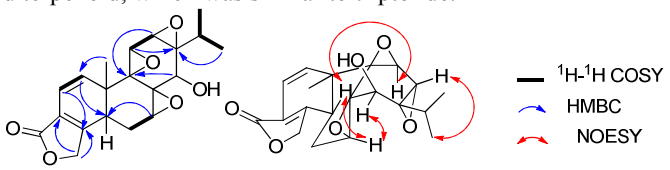


Figure 7. Key ^{1}H-^{1}H COSY, HMBC and NOESY Correlations of Compound **5**.

The long-range correlations of δ_H 1.02/δ_C 65.6 (C-13), δ_H 1.20/δ_C 42.0 (C-10), δ_H 4.82/δ_C 123.8 (C-3), 157.9 (C-4) observed in the HMBC experiment (Figure 7) confirmed the 18(4→3)-*abeo*-abietane skeleton. Compared with triptolide, it was obvious that there was one more cyclic olefinic bond in **5**. Additionally, the HMBC correlations of δ_H 5.56/δ_C 123.8 (C-3), δ_C 39.9 (C-5), δ_H 6.17/δ_C 123.8 (C-3), δ_C 157.9 (C-4), δ_C 42.0 (C-10) suggested that the cyclic olefinic bond located at C-1/C-2. The relative configuration could be established by the NOESY experiment. The NOE correlations of H-5(α)/H-7, H-11, H-7/H-14, H-12/H-16 (Figure 7), and the coupling constant of H-11/H-12 (J = 3.0 Hz) indicated that the relative configuration of **5** was same as triptolide and its analogues.[8] Thus, **5** was elucidated as shown, named tripterlide E.

Table 2. NMR Spectroscopic Data of Compounds **4**, **5**, and **6**.

	4		**5**		**6**	
No.	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]
1	2.92 dd (13.2, 6.6) 1.60 m	29.4	5.56 d (9.6)	134.9	7.32 d (7.2)	123.9
2	2.47 m	18.0	6.17 d (9.6)	115.3	7.80 d (7.2)	124.7
3	-	125.8	-	123.8	-	126.1
4	-	160.0	-	157.9	-	145.3
5	2.71 brd (13.8)	42.3	3.12 m	39.9	-	127.5
6	2.00 m 1.78 m	18.4	2.27 m	22.1	3.33 d (18.0) 3.20 d (18.0)	26.3
7	2.83 dd (20.4, 6.0) 2.43 m	21.1	3.37 d (5.4)	59.4	3.62 m	57.6
8	-	156.3	-	60.7	-	59.6
9	-	162.5	-	64.9	-	60.9
10	-	35.7	-	42.0	-	137.4
11	-	200.8	3.76 d (3.0)	59.8	3.66 m	64.0
12	-	78.2	3.54 d (3.0)	54.6	3.62 m	54.9
13	-	200.7	-	65.6	-	65.9
14	2.08 m	34.6	3.44 d (10.8)	73.3	3.78 d (10.4)	72.7
15	0.953 d (6.6)	17.3	2.23 m	28.2	2.31 m	28.5
16	0.946 d (7.2)	17.3	1.02 d (7.2)	16.9	0.95 d (6.0)	17.0
17	-	173.5	0.89 d (7.2)	17.8	1.08 d (6.0)	17.9
18	4.82 m 4.74 m	70.3	-	170.8	-	170.5
19	1.14 s	17.98	4.82 m	69.5	5.28 d (14.4) 5.21 d (14.4)	68.5
20	-	-	1.19 s	15.3	-	-

[a] In chloroform-d_1 (600 MHz for **4**, **5**, 400 MHz for **6**).[b] In chloroform-d_1 (150 MHz for **4**, **5**, 100 MHz for **6**).

Compoud **6** showed the molecular formula $C_{19}H_{18}O_6$ by the HRESIMS. Analyses of the spectroscopic data indicated that **6** possessed the same 18(4→3)-*abeo*-abietane skeleton as that of **5**, except for ring A. Compared with **5**, one extra olifinic bond (δ_C 137.4, 127.5) was observed, as well as the absence of CH_3-20. Ring A could be established as an aromatic ring by the long-range correlations of δ_H 7.80/δ_C 145.3, 137.4, δ_H 7.32/δ_C 126.1, 127.5 in its HMBC spectrum. On the basis of its 1D, and 2D NMR spectroscopic data, it was suggested that **6** had the same ring B, and C as **5**. Therefore, **6** was established as tripterlide F.

The six known diterpenes were identified as [5a*S*-(5aα,5bα,8β,9α,9aR*,10aβ)]-4,5a,5b,8,9,10a,11,11a-octahydro-5b,8,9-trihydroxy-5a-methyl-8-(1-methylethyl)-1H-oxireno[8a,9]phenanthro[1,2-c]furan-3(5)-one (**7**),[21] isotriptolide (**8**),[22]

triptriolide (**9**),[23] triptolide (**10**),[8] triptolidenol (**11**),[24] tripdiolide (**12**),[8] from their spectroscopic data upon comparisons with values reported in the literatures.

Hypoxia-inducible factor-1 (HIF-1) inhibitory effects of 1–12.

Hypoxia-inducible factor-1 (HIF-1), a critical transcription factor to reduce O_2 availability, has been demonstrated to be extensively involved in tumor survival, and considered as a potential anticancer target.[25, 26] In an *in vitro* bioassay, Compounds **6**, **8**, and **10**–**12** could inhibit the hypoxia-inducible factor 1 (HIF-1) in U251-HRE with the IC_{50} 9.82, 5.87, 0.02, 0.06, 0.17 μM, respectively (Table 3).

Cytotoxic activities of compounds 1–12.

In bioassay experiments using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method,[27] **5**, **6**, **8**, and **10**–**12** exhibited significant cytotoxicities against five human cell lines HCT-116, HepG2, BGC-823, H460, and SK-OV-3 as shown in Table 3.

Table 3. Cytotoxicities and Inhibitory Effects of HIF-1 on the U251-HRE Cells of compounds **1**–**12**.

	Cytotoxicity IC_{50} (μM)					HIF-1 inhibitory IC_{50} (μM)
No.	HCT-116	HepG2	BGC-823	H460	SK-OV-3	U251-HRE
1	>10	>10	>10	>10	>10	>10
2	>10	>10	>10	>10	>10	>10
3	>10	>10	>10	>10	>10	>10
4	>10	>10	>10	>10	>10	>10
5	0.97	1.63	3.16	0.93	8.47	>10
6	0.90	0.63	0.85	0.17	2.30	9.82
7	>10	>10	>10	>10	>10	>10
8	<0.10	0.15	0.34	>10	>10	5.87
9	>10	>10	>10	>10	>10	>10
10	<0.10	<0.10	<0.10	<0.10	<0.10	0.02
11	0.39	0.77	0.20	2.16	0.41	0.06
12	0.73	1.65	0.91	34.58	1.36	0.17

Experimental

General experimental produces.

^{1}H-, Optical rotations were measured on a JASCO P-2000 polarimeter. UV spectra were measured on a JASCO V650 spectrophotometer. IR spectra were recorded on a Nicolet 5700 FT-IR microscope instrument (FT-IR microscope transmission). CD spectra were obtained from a JOUAN Mark II spectropolarimeter. NMR spectra were acquired with VNS-600, Bruker-500, and Mercury-400 spectrometers. HRESIMS spectra were collected on an Agilent 1100 series LC/MSD ion trap mass spectrometer. Preparative HPLC was performed on a Shimadzu LC-6AD instrument with a SPD-20A detector, using a YMC-Pack ODS-A column (2×25 cm, 5 μm). Column chromatography was performed with silica gel (200−300 mesh, Qingdao Marine Chemical Inc., Qingdao, People's Republic of China) and ODS (50 μm, YMC, Japan). TLC was carried out on glass precoated silica gel GF254 plates. Spots were visualized under UV light or by spraying with 10% sulfuric acid in EtOH followed by heating.

Plant material.

The leaves of *Tripterygium wilfordii* were collected in Taining, Fujian, China, in September 2009. A voucher specimen (No. 20090034) was identified by Professor Lin Ma from the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College and was deposited at the herbarium of the Institute of Material Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, China.

Extraction and isolation.

Air-dried leaves of *Tripterygium wilfordii* (50 kg) were extracted with 80% ethanol (400 L × 2h × 3). After evaporation of EtOH in vacuo, the aqueous residue was diluted with water and then partitioned with EtOAc (30 L × 3). The EtOAc extract (4000 g) was subjected to passage over polyamide by elution with water and 30%, 60%, and 95% EtOH−water in sequence to give fractions A_1 (478 g), A_2 (743 g), A_3 (828 g), and A_4 (1000 g). Fraction A_1 (478 g) was subjected to column chromatography on silica gel with $CHCl_3$−MeOH (1:0−10:1) to afford 10 fractions (B_1−B_{10}). Fraction B_7 (52 g) was separated by a silica gel column (200−300 mesh) eluted with $CHCl_3$−MeOH (80:1−10:1) to afford 43 fractions (F_1−F_{43}). Subfraction F_{12} (2.3 g) was passed over an RP-18 column with MeOH−water (20−80%) and finally purified by preparative HPLC (detected at 210 nm, 8 mL/min) to give **1** (6 mg), **2** (7 mg), **3** (4 mg), **4** (3 mg), **5** (2 mg), **6** (4 mg), **7** (2 mg), **8** (6 mg), **9** (3 mg), **10** (34 mg), **11** (21 mg), and **12** (7 mg).

Structure characterization.

Tripterlide A (1): pale yellow powder; $[\alpha]_D^{25}$ + 39.2 (*c* 0.1 $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 246 (3.75) nm; IR (microscope) ν_{max} 2958, 2927, 1749, 1701, 1462, 1378, 1020, 753 cm^{-1}; CD (CH_3CN) λ_{max} ($\Delta\varepsilon$) 287 (- 1.10), 248 (- 1.37), 220 (+ 11.20), 199 (- 2.80) nm; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 351.1567 (calcd for $C_{20}H_{24}NaO_4$, 351.1573).

Tripterlide B (2): pale yellow powder; $[\alpha]_D^{25}$ + 56.8 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 245 (3.90) nm; IR (microscope) ν_{max} 2968, 2932, 1754, 1702, 1464, 1385, 1071, 1019, 754 cm^{-1}; CD (CH_3CN) λ_{max} ($\Delta\varepsilon$) 295 (+ 1.82), 244 (+ 9.07), 222 (- 11.42), 206 (+ 3.32) nm; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 329.1753 (calcd for $C_{20}H_{25}O_4$, 329.1747).

Tripterlide C (3): pale yellow powder; $[\alpha]_D^{25}$ + 30.6 (*c* 0.1 $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 245 (3.71) nm; IR (microscope) ν_{max} 3431, 2928, 2852, 1747, 1702, 1460, 1385, 1073, 1021, 756 cm^{-1}; CD (CH_3CN) λ_{max} ($\Delta\varepsilon$) 315 (+ 0.49), 247 (+ 3.47), 224 (- 4.01) nm; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 345.1701 (calcd for $C_{20}H_{25}O_5$, 345.1697).

Tripterlide D (4): pale yellow solid, $[\alpha]_D^{25}$ + 58.4 (*c* 0.08 $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 243 (4.25) nm; CD ($CHCl_3$) λ_{max} ($\Delta\varepsilon$) 239 (+ 12.05), 267 (- 2.25), 333 (+ 0.486) nm; ^{1}H NMR and ^{13}C NMR data see table 2; HRESIMS *m/z* 331.1543 (calcd for $C_{19}H_{23}O_5$, 331.1540).

Tripterlide E (5): white amorphous solid, $[\alpha]_D^{25}$ − 17.5 (*c* 0.17 $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 233 (0.93), 275 (2.12) nm; IR (microscope) ν_{max} 3550, 2966, 2933, 1764, 1444, 1371, 1034, 1020, 745 cm^{-1}; ^{1}H NMR and ^{13}C NMR data see table 2; HRESIMS *m/z* 359.1489 (calcd for $C_{20}H_{23}O_6$, 359.1496).

Tripterlide F (6): white amorphous solid, $[\alpha]_D^{25}$ − 50.27 (*c* 0.09 $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 245 (4.67), 277 (0.24), 286 (0.24) nm; IR (microscope) ν_{max} 3456, 2958, 2919, 1777, 1763, 1467, 1399, 1078, 1038 cm^{-1}; ^{1}H NMR and ^{13}C NMR data see table 2; HRESIMS *m/z* 343.1176 (calcd for $C_{19}H_{19}O_6$, 343.1179).

Inhibitory Effects on hypoxia-inducible factor 1 (HIF-1) in U251-HRE.

Compounds **1**–**12** were tested for their ability to inhibit hypoxia-inducible factor 1 (HIF-1) in U251-HRE cells. This assay was carried out as previously described.[28]

Cytotoxicties Assay.

Compounds **1**–**12** were tested for cytotoxicities against HCT-116 (human lung carcinoma), HepG2 (human liver carcinoma), BGC-823 (human stomach carcinoma), H460 (human colon carcinoma), and SK-OV-3 (human ovarian carcinoma) cell lines by means of the MTT method as described in the literature.[25]

Conclusions

In conclusion**,** twelve abietane derivatives were obtained from the traditional Chinese medicine *Tripterygium wilfordii* Hook. f.. Especially, three novel 14(13→12),18(4→3)-*diabeo*-abietane diterpenoids, tripterlides A–C (**1**–**3**), were isolated, which possessed 6/6/5 tricyclic ring skeleton that likely derived from 18(4→3)-*abeo*-abietane. It is the first time to report these abietane derivatives isolated from natural products. Additionally, these diterpenoids displayed potential HIF-1 inhibitory effects, which were suggested to be the main bioactive substance of *T. wilfordii*. On the basis of the structures and bioactivities, preliminary structure & activity relationship was deduced. It was suggested that the epoxy groups of the abietanoids may play important role in the cytotoxicties against cancer cells and HIF-1 inhibitory effects. These findings prompt us to pay more attentions to trace bioactive diterpenoids in chemical studies of medicinal plants.

Acknowledgements

This work was supported by the National Key Technology R & D program of the Ministry of Science and Technology of China (No. 2011BAI01B05), the National Natural Science Foundation of China (No. 21132009), and the Beijing Natural Science Foundation (No. 7121010).

Notes and references

1 Jiangsu New Medical College. *Dictionary Traditional Drugs (Zhong Yao Da Ci Dian)*; Shanghai Scientific and Technical Press: Shanghai, 1977; Vol. 2, pp 2469−2470.
2 M. Xiang, C. L. Zhang, *Chin. Tradit. Herbal Drugs*, 2005, **36**, 458−461.
3 Y. G. Wan, W. Sun, H. L. Zhang, Q. J. Yan, P. Chen, C. H. Dou, H. M. Yang, M. Ge, *Nephron Exp. Nephrol.*, 2010, **114**, e7−e14.
4 H. Q. Duan, Y. Takaishi, H. Momota, Y. Ohmoto, T. Taki, M. Tori, S. Takaoka, Y. F. Jia, D. Li, *Tetrahedron* 2001, **57**, 8413−8424.
5 H. Q. Duan, Y. Takaishi, Y. F. Jia, D. Li, *Phytochemistry* 2001, **56**, 341−346.
6 Y. G. Luo, M. Zhou, Q. Ye, Q. Pu, G. L. Zhang, *J. Nat. Prod.* 2012, **75**, 98−102.
7 X. Cao, C. J. Li, J. Z. Yang, B. X. Wei, S. P. Yuan, Y. M. Luo, Q. Hou, D. M. Zhang, *Fitoterapia* 2012, **83**, 343−347.
8 S. M. Kupchan, W. A. Court, R. G. Dailey, C. J. Gilmore, R. F. Bryan, *J. Am. Chem. Soc.* 1972, **94**, 7194−7195.
9 C. P. Zhang, X. Y. Lv, P. C. Ma, Y. Chen, Y. G. Zhang, Z. Yan, G. F. Chen, Q. T. Zheng, C. H. He, D. Q. Yu, *Acta Pharm. Sin.* 1993, **28**, 110−115.
10 H. Q. Duan, Y. Takaishi, H. Momota, Y. Ohmoto, T. Taki, Y. F. Jia, D. Li. *J. Nat. Prod.* 1999, **62**, 1522−1525.
11 F. J. Guo, M. L. Xi, Y. C. Li. *Tetrahedron Lett* 1999, **40**, 947−950.
12 S. Lin, X. Y. Yu, H. Q. Que, Z. Chen, D. L. Xie, Y. C. Li. *Acta Pharm. Sin.* 2005, **40**, 632−635.
13 J. R. Zheng, K. X. Gu, L. F. Xu, J. W. Gao, Y. H. Yu, M. Y. Tang, *Acta Acad. Med. Sin.* 1991, **13**, 391−397.
14 H. Haba, C. Lavaud, H. Harkat, A. A. Magid, L. Marcourt, M. Benkhaled. *Phytochemistry* 2007, **68**, 1255−1260.
15 J. M. Amaro-Luis, J. R. Herrera, J. G. Luis. *Phytochemistry* 1998, **47**, 895−897.
16 C. T. Che, T. X. Zhoua, Q. G. Ma, G. W. Qin, I. D. Williams, H. M. Wu, Z. S. Shi. *Phytochemistry* 1999, **52**, 117−121.
17 R. Tanaka, H. Ohtsu, S. Matsunaga. *Phytochernistry* 1997, **46**, 1051−1057.
18 J. Z. Xu, J. Lu, F. Sun, H. Z. Zhu, L. J. Wang, X. Y. Zhang, Z. J. Ma. *Phytochemistry* 2011, **72**, 1482–1487.
19 N. Crespi-Perellino, L. Garofano, E. Arlandini, V. Pinciroli. *J. Nat. Prod.* 1996, **59**, 773–776.
20 Y. Lv, Q. T. Zheng, Z. Y. Tian, P. C. Ma, C. P. Zhang, Y. Chen, J. R. Zheng, *Nat. Prod. Res. Dev.* 1998, **10**, 1−5.
21 J. M. Jung, M. Wickramaratne, M. Hepperle [P]. US 5972998, 1999.
22 D. Q. Yu, D. M. Zhang, H. B. Wang, X. T. Liang, *Acta Pharm.Sin.* 1992, **27**, 830−836.
23 P. C. Ma, C. L. Yang, *Acta Botanica Sin.* 1993, **35**, 637-643.
24 F. X. Deng, S. Q. Huang, J. H. Cao, Z. L. Xia, S. Lin, D. Y. Zhu, S. H. Jiang, Y. L. Zhu, *Acta Botanica Sin.* 1985, **27**, 516−519.
25 Z. L. Zhou, Z. G. Luo, B. Yu, Y. Jiang, Y. Chen, J. M. Feng, M. Dai, L. J. Tong, Z. Li, Y. C. Li, J. Ding, Z. H. Miao, *Mol. Cancer* 2010, **9**, 268.
26 M. Zhang, Z. H. Xu, *J. Int. Oncol.* 2007, **34**, 484−487.
27 L. Yu, J. Z. Yang, X. G. Chen, J. G. Shi, D. M. Zhang, *J. Nat. Prod.* 2009, **72**, 866−870.
28 L. W. Lang, K. Tang, Y. Li, X. Y. Liu, C. Wang, X. G. Chen, *Chin. J. Chin. Mater. Med.* 2012, **37**, 2151−2155.

