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The isolation, absolute configuration and activities of 18(4 → 3)-*abeo*-abietane lactones from *Tripterygium wilfordii*

Lin Ni[a,b], Li Li[a], Yingda Zang[a], Chuang-jun Li[a], Jie Ma[a], Tiantai Zhang[a], Dong-ming Zhang[a,*]

[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 Plant Protection, Fujian Agriculture and Forestry University, Fuzhou 350002, Fujian Province, People's Republic of China

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ABSTRACT

Phytochemical studies on the leaves of *Tripterygium wilfordii* led to the isolation of seven new 18(4 → 3)-*abeo*-abietane lactones, triptergulides E – K (**1** – **7**). The structure of the new compounds was elucidated on the basis of their spectroscopic analysis, and the absolute configurations of compounds were confirmed by ECD, calculated ECD, and X-ray crystallographic analysis using anomalous scattering of Cu Kα radiation. Some compounds showed moderate inhibitory activities against NO, IL-6, and TNF-α production in LPS RAW 264.7 macrophage *in vitro*.

1. Introduction

Tripterygium wilfordii was a plant from the family of Celastracrae and used as the traditional Chinese medicine, known as "leigongteng" over the past years [1]. Chemical components studies revealed a series of ingredients, including dihydroagarofuran sesquiterpenes [2–5], triterpenes [6], diterpenes [7], megastigmanes [8,9], and lignans [10], providing many ingredients for anti-tumor, anti-fertility, anti-inflammatory, anti-HIV, and immunosuppressive [11]. Among the ingredients, 18(4 → 3)-*abeo*-abietane lactones (ABALs) were the most compelling because of their remarkably activities, which seemed to show all the traditional effects of leigongteng [12]. However, no more than 30 ABALs have been isolated from plant and they seemed to exist only in the genus *tripterygium* from the review literature [13–17].

Our group devoted ourselves to how to get more of ABALs from the *T. wilfordii* and study on these derivatives characteristics and activities. Previous work had led the isolation of four ABALs, triptergulides A-D [13]. As we all known, a ring of α, β-unsaturated-γ-lactone was an important functional group in these derivatives and could be positive reaction through kedde reagents, a mixture with 1 N KOH and 2.0% 3,5-dinitrobenzoic acid methanol solution (1:1), on the TLC [18]. Thus, it was used as "detector" for the isolation of ABALs from the leaves of *T. wilfordii*, and now, seven new ones (Fig. 1), triptergulides E – K (**1** – **7**) were isolated. Meanwhile, their absolute configurations were determined by X-ray, ECD and calculated ECD analysis, and their inhibitory activities against NO, IL-6 and TNF-α production in LPS-induced RAW 264.7 macrophage *in vitro* were evaluated.

2. Results and discussion

Triptergulide E (**1**), was isolated as colorless needles, and assigned to a molecular formula of $C_{20}H_{28}O_8$ on the basis of HRESIMS at *m/z* 419.1677 $[M+Na]^+$ (calcd $C_{20}H_{28}NaO_8$, 419.1676), corresponding with seven degrees of unsaturation. The IR spectrum exhibited absorption for hydroxy (3499 cm^{-1}), unsaturated ketone (1736 cm^{-1}) and olefinic bond (1670 cm^{-1}). In the spectrum of ^1H NMR (Table 1), it showed three methyl groups [δ_H 0.82 (3H, d, J = 6.7 Hz), 0.87 (3H, d, J = 6.8 Hz) and 1.10 (3H, s)] and four oxygenated methine [3.45 (1H, d, J = 7.4 Hz), 3.70 (1H, m), 3.70 (1H, m), and 3.76 (1H, br s)]. The ^{13}C NMR and HSQC spectra exhibited three methyl groups, three methenes, six methines, nine quaternary carbons including two olefinic carbons (Table 2). Obvious signals, a ring of α, β-unsaturated-γ-lactone [δ_H 4.82 (2H, m, H-19); δ_C 173.5 (C-18), 70.5 (C-19), 123.3 (C-3), and 164.6 (C-4)] and an isopropyl group [δ_H 0.82 (3H, d, J = 6.7 Hz, H_3-16), 0.87 (3H, d, J = 6.8 Hz, H_3-17), and 2.28 (1H, m, H-15); δ_C 28.9 (C-15), 15.7 (C-16), and 16.3 (C-17)] were observed. These spectral characteristics suggested **1** was an ABAL derivative and similar to triptergulides A-D [13–15], which could contain five hydroxy groups and an oxygenate ring to satisfy the number of hydrogen deficiency except the ABAL carbon skeleton. The ABAL carbon skeleton with four rings was confirmed by ^1H, ^1H COSY correlations of H-1/H-2, H-5/H-6/H-7, H-11/H-12, H_3-16/H-15/H_3-17 and HMBC correlations of H_2-19 [δ_H 4.82 (2H,

* Corresponding author.
E-mail address: zhangdm@imm.ac.cn (D.-m. Zhang).

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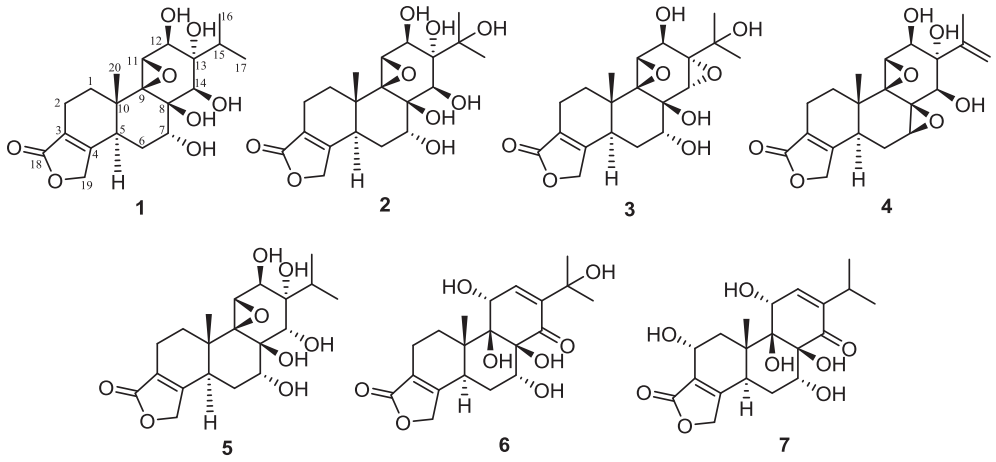

Fig. 1. Structure of compounds **1 – 7.**

Table 1
^{1}H NMR data of compounds **1 – 7**.

No.	1[a]	2[a]	3[a]	4[a]	5[a]	6[a]	7[a]
1α	1.30, m	1.26, m	1.25, m	1.27, m	1.32, m	1.80, m	2.00, m
1β	1.41, m	1.41, m	1.36, m	1.38, m	1.40, m	2.09, m	2.11, m
2α	2.03, m	2.03, m	2.00, m	1.98, m	2.06, m	2.09, m	
2β	2.15 (overlap)	2.13, m	2.15, m	2.13, m	2.19, m	2.21, m	4.37, m
5	2.93, br d (12.7)	2.89, d (13.0)	3.03, br d (12.9)	2.67, br d (13.1)	3.14, br d (13.2)	3.81, br d (13.8)	3.90 (overlap)
6α	1.58, d (12.7)	1.59, d (13.0)	1.59, m	1.85, m	1.62, m	1.48, m	1.49, m
6β	2.15 (overlap)	2.17, m	2.06, m	2.13, m	2.06, m	2.01, m	2.00, m
7	3.76, br s	3.81, br s	3.79, br s	3.37, d (6.0)	3.76, d (2.6)	3.86, m	3.90 (overlap)
11	3.70, m	3.69, d (5.5)	3.25, d (1.8)	3.71, d (5.4)	3.52, d (3.4)	4.24, dd (10.5, 6.1)	4.19, br s
12	3.70, m	3.97, br s	4.29, br d (6.3)	3.98, m	4.00, dd (6.7, 3.4)	7.03, d (6.1)	6.66, d (6.0)
14	3.45, d (7.4)	3.78, br s	2.93, s	3.22, dd (7.3, 2.0)	4.27, d (5.2)		
15	2.28, m				1.86, m		2.77, m
16	0.82, d (6.7)	1.22, s	1.22, s	5.17, d (2.0); 4.94, d (2.0)	1.00, s	1.29, s	0.98 (overlap)
17	0.87, d (6.8)	1.30, s	1.18, s	1.78, s	1.02, s	1.31, s	0.98 (overlap)
19	4.82, m	4.82, m	4.81, m	4.83, m	4.83, m	4.79, m	4.78, m
20	1.10, s	1.10, s	1.05, s	0.96, s	1.07, s	1.00, s	1.02, s

[a] In DMSO-d_6 (500 MHz).

Table 2
^{13}C NMR data assignment of compounds **1 – 7.**

No.	1[a]	2[a]	3[a]	4[a]	5[a]	6[a]	7[a]
1	30.4	30.3	28.8	29.9	29.4	26.0	38.0
2	17.1	16.9	17.2	16.8	17.1	17.2	60.8
3	123.3	123.1	123.1	123.2	122.9	122.4	125.0
4	164.6	164.2	164.8	162.7	165.1	166.0	167.3
5	35.8	35.6	36.5	39.2	35.7	34.5	34.9
6	28.0	27.8	26.5	22.6	26.1	26.7	26.2
7	73.1	72.9	71.3	60.5	70.2	72.3	72.0
8	72.8	72.8	71.6	61.5	74.8	79.0	78.1
9	67.9	67.8	63.2	65.6	65.4	74.6	74.3
10	36.6	36.4	36.5	35.0	36.8	41.1	45.0
11	61.8	61.8	60.1	58.0	61.3	67.9	68.0
12	68.4	69.7	62.5	68.3	67.1	142.1	140.9
13	72.1	70.7	65.5	73.6	74.4	142.5	141.6
14	73.7	73.2	62.8	77.1	66.2	201.8	201.8
15	28.9	75.9	69.8	145.3	34.3	69.5	25.6
16	15.7	25.1	26.4	113.1	16.6	28.6	21.5
17	16.3	25.9	26.7	18.7	18.5	29.3	21.5
18	173.5	173.3	173.5	173.3	173.3	173.6	172.0
19	70.5	70.3	70.3	70.4	70.3	70.2	69.1
20	15.3	15.1	14.4	14.1	15.2	13.7	14.5

[a] In DMSO-d_6 (125 MHz).

m, H-19]/C-3 (δ_C 123.3), C-18 (δ_C 173.5); H_3-20 [δ_H 1.10 (3H, s)]/C-1 (δ_C 30.4), C-5 (δ_C 35.8), C-9 (δ_C 67.9), C-10 (δ_C 36.6); H-14 [δ_H 3.45 (1H, d, J = 7.4 Hz)]/C-8 (δ_C 72.8), C-9, C-15 (δ_C 28.9); H-7 [δ_H 3.76 (1H, br s)]/C-5, C-9, C-14 (δ_C 73.7); H_3-16 [δ_H 0.82 (3H, d, J = 6.7 Hz)]/C-13 (δ_C 72.1), C-15, C-17 (δ_C 16.3) (Fig. 2). Five hydroxy groups were situated in C-7, C-8, C-12, C-13, and C-14, which could be verified by ^{1}H, ^{1}H COSY correlations of H-7/7-OH [δ_H 7.08 (1H, s)], H-12/12-OH [δ_H 4.12 (1H, d, J = 8.5 Hz)], H-14/14-OH [δ_H 5.16 (1H, d, J = 8.0 Hz)] and HMBC correlations of 8-OH [δ_H 4.55 (1H, s)]/C-7 (δ_C 73.1), C-9 and of 13-OH [δ_H 6.60 (1H, s)]/C-12 (δ_C 68.4), C-13 (Fig. 2). Thus, the oxygenate rings was only set up by an oxide bridge between C-9 and C-11.

The relative configuration of **1** was determined by NOESY experiment (Fig. 2). The observed NOESY correlations of H-5/H-1α/H-11 and H-12, H-14/13-OH, accordingly, the structure of **1** was proposed as shown. Fortunately, compound **1** was recrystallized in the CH_3OH to yield colorless needles. On the basis of single crystal X-ray diffraction data, the absolute configuration of **1** was determined to be 5*S*, 7*R*, 8*R*, 9*S*, 10*S*, 11*S*, 12*R*, 13*S*, and 14*R* (Fig. 3). Meanwhile, the obvious Cotton effects were at λ_{max} ($\Delta\varepsilon$) 220 (+5.67) and 247.5 (−1.89) nm in the ECD spectrum.

Triptergulide F (**2**), was isolated as colorless crystalline powder, and a molecular formula of $C_{20}H_{28}O_9$ as determined by HRESIMS ion at *m/z* 435.1635 $[M+Na]^+$ ($C_{20}H_{28}O_9Na$, calcd 435.1626), which was 16 mass units (a oxygen atom) more than that of **1**. The NMR data of **2** (Tables 1 and 2) were similar to those of **1** except that two methyl doublets of H_3-16 and H_3-17 for **1** were replaced by two singlets [δ_H 1.22 (3H, s, H-16) and 1.30 (3H, s)] of **2** and that the methane carbon of C-15 assigned to isopropyl unit of **1** was replaced by an oxygenated quaternary carbon (δ_c 75.9). These data suggested that **2** was an analogue of **1** with one more hydroxy at C-15, which was further confirmed

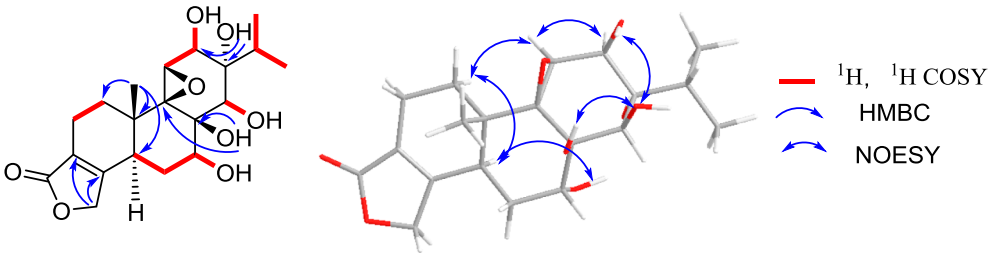

Fig. 2. Key ^{1}H, ^{1}H COSY (red thick lines), HMBC (blue arrows, from ^{1}H to ^{13}C) and NOESY (blue double-headed arrows) correlations of compounds **1**. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

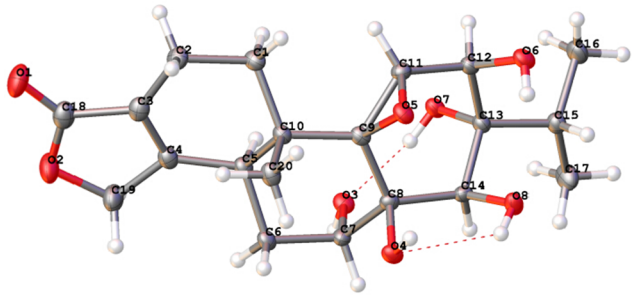

Fig. 3. Single-crystal structure of **1**.

by HMBC correlation from H_3-16 to C-13 (δ_c 73.2), C-15 (δ_c 75.9), and C-17(δ_c 25.9). Because of the similar ECD spectrum [λ_{max} ($\Delta\varepsilon$) 221 (+6.18), 246.5 (−2.06)], the absolute configuration was assumed to be identical with those of **1**.

Triptergulide G (**3**), was isolated as colorless oil, and a molecular formula of $C_{20}H_{26}O_8$ as determined by HRESIMS ion at *m/z* 417.1528 $[M+Na]^+$ ($C_{20}H_{26}O_8Na$, calcd 417.1520), corresponding with eight degrees of unsaturation. It shared many spectral feature (IR, ^{1}H and ^{13}C NMR) in common with **1**, which suggested **3** was also an ALBL derivative and contained four hydroxy groups and two oxygenate rings (Tables 1 and 2). The detailed 2D NMR analyses established the planar structure of **3** (Fig. 4). The four hydroxy groups were assigned to C-7, C-8, C-12, and C-15 because of HMBC correlations from 8-OH [δ_H 5.33 (1H, s)] to C-7 (δ_c 71.3) and C-9 (δ_c 63.2); from 15-OH [δ_H 4.11 (1H, s)] to C-16 (δ_c 26.4) and C-17 (δ_c 26.7); ^{1}H-^{1}H COSY correlations from 7-OH [δ_H 5.48 (1H, d, J = 2.8 Hz)] to H-7 [δ_H 3.79 (1H, br s)]; from 12-OH [δ_H 4.64 (1H, d, J = 8.5 Hz)] to H-12 [δ_H 4.29 (1H, br d, J = 8.5 Hz)]. Therefore, a trisubstituted epoxide emerged between C-13 and C-14, and the other one emerged between C-11 and C-12, which led the four carbons to upfield. In the NOESY spectrum, the correlations of H-5/H-1/H-11/H-12 indicated *α*-orientations. The correlation of H-7/H-14/8-OH/12-OH indicated *β*-orientation. Thus, in view of NMR data and ECD spectrum which exhibited Cotton effects at λ_{max} ($\Delta\varepsilon$) 219 (+5.35), and 246.5 (−1.64), the absolute configuration of **3** was deduced as 5*S*, 7*R*, 8*R*, 9*S*, 10*S*, 11*S*, 12*R*, 13*S*, and 14*R*.

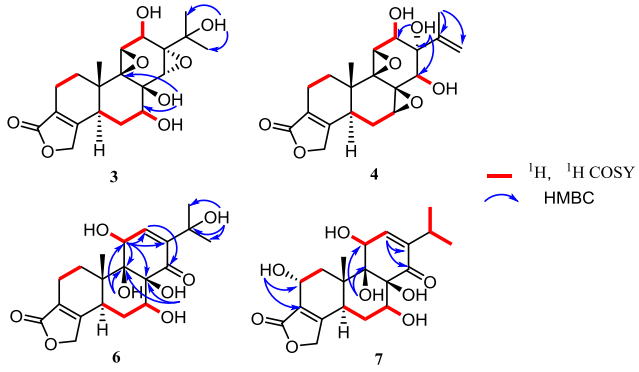

Fig. 4. Key ^{1}H, ^{1}H COSY (red thick lines) and HMBC (blue arrows, from ^{1}H to ^{13}C) correlations of compounds **3**, **4**, **6**, and **7**. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Triptergulide H (**4**), was isolated as a colorless solid. The molecular formula $C_{20}H_{24}O_7$ was established upon analysis of the HRESIMS peak at *m/z* 399.1417 $[M+Na]^+$; this molecular afforded nine degrees of unsaturation. It was the same carbon skeleton as **1**. The ^{13}C NMR lines observed at δ_C 145.3 (C-15), 113.1 (C-16), and 18.7 (C-17); and ^{1}H NMR peaks at δ_H 5.12 (1H, d, J = 2.0 Hz), and 4.94 (1H, d, J = 2.0 Hz) were confidently assigned to an isopropenyl group. HMBC correlations from H_3-17 [δ_H 1.78 (1H, s)] to C-13 (δ_c 73.6), C-15 (δ_c 145.3), and C-16 (δ_c 113.1) indicated that the isopropenyl group was connected to C-13. HMBC correlations from 13-OH [δ_H 5.04 (1H, s)] to C-13, C-12 (δ_c 68.3), and C-14 (δ_c 77.1); ^{1}H-^{1}H COSY correlations from 12-OH [δ_H 4.54 (1H, d, J = 9.0 Hz)] to H-12 [δ_H 3.98 (1H, m)]; and from 14-OH [δ_H 4.74 (1H, d, J = 7.3 Hz)] to H-14 [δ_H 3.22 (1H, dd, J = 7.3, 2.0 Hz)] suggested that three hydroxy groups were positioned at C-12, C-13, and C-14, respectively. In the NOESY spectrum, the cross-peaks of H-5/H-6*α*/H-7/H-14/13-OH/H-12/H-11 indicated their *α*-orientation. Because of NMR data and ECD spectrum [λ_{max} ($\Delta\varepsilon$) 219.5 (+0.71), 246.0 (−0.37)], the absolute configurations were assumed to 5*S*, 7*S*, 8*S*, 9*S*, 10*S*, 11*S*, 12*R*, 13*S*, and 14*R*.

Triptergulide I (**5**), isolated as a minor constituent and colorless oil. Its HRESIMS, IR, ^{1}H and ^{13}C NMR were the similar to compound **1**. The detailed 1D and 2D NMR analyses could indicated that **5** and **1** were isomers. Compared to **1**, compound **5** chemical shifts of C-13, C-14, and C-15 were downfield 2.3 ppm, upfield 7.5 ppm, and downfield 5.4 ppm, respectively. Meanwhile, the NOESY correlation of H-7/9-OH/14-H could be found. These information could confirmed the absolute configuration of C-14 was different between **1** and **5**. Thus, **5** was elucidated as shown.

Triptergulide J (**6**) was obtained as a colorless oil and gave a quasi-molecular ion peak at *m/z* 417.1529 $[M+Na]^+$, and the molecular formula was determined as $C_{20}H_{26}O_8$ by the HRESIMS. The gross structure of **6** and all of the ^{1}H and ^{13}C chemical shifts associated with the molecule were assigned unambiguously by a series of 2D NMR experiments. HMBC correlation from H-11 [δ_H 4.24 (1H, dd, J = 10.5, 6.1 Hz)] to C-8 (δ_c 79.0), C-9 (δ_c 74.6), C-12 (δ_c 142.1), and C-13 (δ_c 142.5); from H-12 [δ_H 7.03 (1H, d, J = 6.1 Hz)] to C-9, C-11 (δ_c 67.9), C-13, and C-14 (δ_c 201.8), ^{1}H, ^{1}H-COSY correlation between H-11 to H-12 suggested that an *α*,*β*-unsaturated-γ-carbonyl was positioned at C-12, C-13, and C-14. Another five hydroxy groups were located at C-7, C-8, C-9, C-11, and C-15 as confirmed by HMBC correlations from 8-OH [δ_H 5.37 (1H, s)] to C-7 (δ_c 72.3), C-8, and C-9; of 9-OH [δ_H 4.14 (1H, s)] to C-8, C-9, and C-11; of 15-OH [δ_H 4.84 (1H, s)] to C-15 (δ_c 69.5), C-16 (δ_c 28.6), and C-17 (δ_c 29.3) and ^{1}H, ^{1}H-COSY correlations between 7-OH [δ_H 6.36 (1H, d, J = 5.4 Hz)] and H-7 [δ_H 3.86 (1H, m)]; and between 11-OH [δ_H 5.61 (1H, d, J = 10.5 Hz)] and H-11. The relative configuration of **6** was determined by NOE correlations. The NOESY cross-peaks of H_3-20/H-7/8-OH/9-OH indicated that H_3-20, H-7, 8-OH, and 9-OH were present in a *β*-orientation. The correlation of H-5 and 11-OH indicated an *α*-orientation. Finally, the absolute

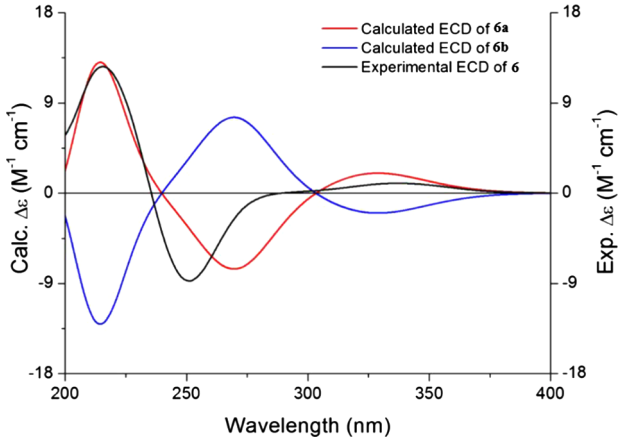

Fig. 5. Experimental and theoretical ECD spectra for triptergulide J (**6**).

configuration of **9** was deduced as 5*S*, 7*R*, 8*S*, 9*S*, 10*S*, 11*R*, and 12*R* by calculated ECD spectra using the TD-DFT method at the B3LYP/6-31G(d) level (Fig. 5) [13,14].

Triptergulide K (**7**) was isolated as colorless oil, and a molecular formula of $C_{20}H_{26}O_8$ as determined by HRESIMS ion at *m/z* 417.1523 $[M+Na]^+$ ($C_{20}H_{26}O_8Na$, calcd 417.1520), corresponding with eight degrees of unsaturation, which was similar to **6**. The detailed 1D and 2D NMR analysis could suggest that **7** and **6** were isomers, and their differences were from the isolated of hydroxy groups. They were assigned to C-2, C-7, C-8, C-9, and C-11 determined by HMBC correlations of 2-OH [δ_H 4.98 (1H, d, J = 6.0 Hz)]/C-1 (δ_c 38.0), C-2 (δ_c 60.8), and C-3 (δ_c 125.0); of 8-OH [δ_H 5.38 (1H, s)]/C-6 (δ_c 26.2), C-7 (δ_c 72.0), and C-8 (δ_c 78.1); of 9-OH [δ_H 4.19 (1H, s)]/C-8, C-9 (δ_c 74.3), and C-11 (δ_c 68.0); and ^{1}H, ^{1}H COSY correlations of H-7 [δ_H 3.90 (1H, m)]/7-OH [δ_H 6.43 (1H, d, J = 4.0 Hz)]; and H-11 [δ_H 4.19 (1H, m)]/11-OH [δ_H 5.60 (1H, d, J = 9.0 Hz)]. The configuration of compound **7** was also similar to **6** except C-2, which was further confirmed by compared C-2 chemical shift to tripdiolide and 2-epitripdiolide. Finally, compound **7** was determined to own 2*α*-OH because the C-2 chemical shift was the same as 2-epitripdiolide [16].

Many chiral carbon atoms make it difficult to determine the absolute configurations of ALBLs. However, there were no exceptions in the orientation of H_3-20*β* and H-5*α*, which could be explained by the ALBLs of biosynthetic approach. In our studies, X-ray analysis, calculated experimental ECD and ECD were employed to confirm their absolute configurations. Notably, we found that an obvious positive Cotton effect near 220 nm appeared at the ECD of ALBLs (Fig. 6). The positive Cotton effect was resulted from the chromophore, a ring of *α*, *β*-unsaturated-γ-lactone by the asymmetric carbon pairs. The "vicinity rule" [19], Snatzke's spheres for *α*, *β*-unsaturated-γ-lactone, could be responsible for explanation of the Cotton effect, and their chiral carbon atoms could

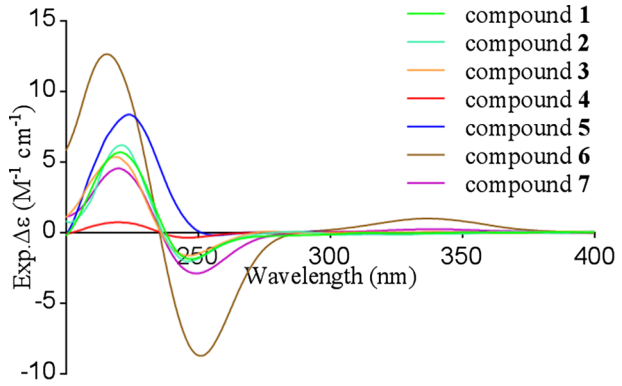

Fig. 6. The ECD spectra of compounds **1** – **7**.

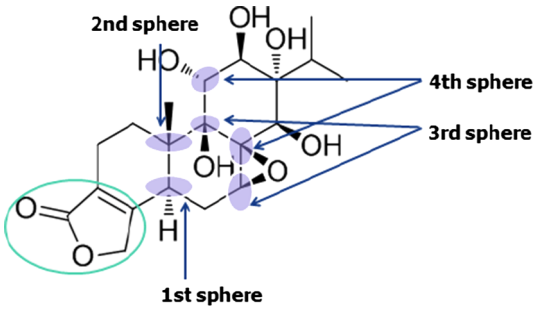

Fig. 7. Snatzke's spheres for *α*,*β*-unsaturated-γ-lactone.

Table 3
Inhibitory activities of compounds **1** – **7** against NO, IL-6, and TNF-*α* production in LPS-induced RAW 264.7 macrophage *in vitro*.

No.	Concentration/μM	NO inhibition (%)	IL-6 inhibition (%)	TNF-*α* inhibition (%)
DEX[a]	10	81.1 ± 6.1	96.6 ± 7.2	98.9 ± 6.4
1	10	< 30.0	< 30.0	65.0 ± 7.8
2	10	< 30.0	31.6 ± 4.3	< 30.0
3	10	53.0 ± 5.4	50.39 ± 4.4	57.0 ± 6.8
4	10	64.0 ± 4.7	37.7 ± 5.2	84.3 ± 7.3
5	10	< 30.0	< 30.0	< 30.0
6	10	85.2 ± 7.5	< 30.0	< 30.0
7	10	45.6 ± 6.3	48.6 ± 5.7	77.4 ± 8.2

[a] Dexamethasone, a positive control.

be arranged in order by impact, 1 > 2 > 3 > 4, as shown Fig. 7. Thus, the positive Cotton effect near 220 nm could be applied in the absolute configurations of ALBL derivatives.

Meanwhile, all the ALBLs were evaluated for their inhibitory activities against NO, IL-6, and TNF-*α* production in LPS-induced RAW 264.7 macrophages *in vitro*. Detailed data was exhibited in the Table 3. Compounds **3**, **4**, and **7** showed moderate inhibitory activities on the inflammatory factors. Compound **1** showed inbibition against TNF-*α*, but no inhibition against NO and IL-6. Compound **6** showed inhibition against NO, but no inhibition against IL-6 and TNF-*α*. These results implied compounds **1** and **6** displayed selective inhibition against the inflammatory factors.

3. Experimental section

3.1. General

Bruker APEX DUO diffractometer with Cu Kα radiation was used to collected the X-ray data of compound **1**. JASCO P-2000 polarimeter, JASCO V650 spectrophotometer, and XT-5B micro melting point apparatus were used to collected Optical rotations, UV spectra, and melting point, respectively. Bruker-500 spectrometers was used to collected NMR spectra. Agilent 1100 series LC/MSD ion trap mass spectrometer was used to collect HRESIMS spectra. 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), polyamide (60–100 mesh, Changfeng Chemical Inc., Jiangsu, People's Republic of China), and ODS (50 μm, YMC, Japan).

3.2. Plant material

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

deposited at the herbarium of the Institute of Material Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, China.

3.3. Extraction and isolation

The air dried and powdered leaves of *Tripterygium wilfordii* (100 kg) were extracted two times with 80% EtOH (800 L) at 80 °C for 2 h. After filtration and evaporation ethanol under reduced pressure at 50 °C, the aqueous residue was diluted with H_2O and then partitioned three times with ethyl acetate (300 L). The remaining water extract was subjected to passage over polyamide by elution with water and 30%, 60%, and 95% EtOH-H_2O. Then the water elution was subjected to passage over D101 macroporous resin by elution successively with H_2O, 30%, 60%, and 95% EtOH-H_2O (Fractions B_1-B_4). Four fractions were analyzed TLC and employed kedde reagents as color developing agent, and Fraction B_3 was positive reaction. Fraction B_3 (338.5 g) was subjected to passage over a bergmeal column eluting successively with ethyl acetate, ethanol, and methanol (Fractions C_1-C_3), and only Fraction C_2 was positive reaction through the kedde reagents. Fraction C_2 (151.3 g) was chromatographed on a silica gel column eluting successively with a solvent gradient system ($CHCl_3$–MeOH, 15:1-0:1) to afford 7 fractions (D_1–D_7), and fractions D_4 and D_5 were positive reaction. Thus, fraction D_4 (3.36 g) was passed over an PRP-512A macroporous resin column with MeOH-H_2O (10%, 30%, 60% and 100%) to give four fractions (E_1-E_4), and the fraction E_3 (0.45 g) was purified by preparative HPLC (MeOH-H_2O, 35:65, v/v, detected at 210 nm, 8 mL/min) to give **1** (25.8 mg), **2** (14.3 mg), and **3** (4.0 mg). fraction D_5 (1.36 g) was subjected by preparative HPLC (MeOH-H_2O, 35:65, v/v, detected at 210 nm, 8 mL/min) and purified by preparative HPLC (CH_3CN-H_2O, 15:85, v/v, detected at 210 nm, 8 mL/min) to give **4** (6.2 mg), **5** (1.3 mg), **6** (18.6 mg), **7** (1.5 mg).

3.4. Spectral data

Triptergulide E (**1**): colorless needles (MeOH); mp: 217–218 °C; [α] −64.8 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 223 (3.19) nm; IR (microscope) ν_{max} 3499, 3282, 2971, 1736, 1670, 1452, 1042, 971, and 941 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 220 (+5.67), 247.5 (−1.89) nm; ^1H NMR (DMSO-d_6, 500 MHz) see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 2; HRESIMS *m/z* 419.1677 $[M+Na]^+$ (calcd for $C_{20}H_{28}NaO_8$, 419.1676).

Single crystals data of triptergulide E (**1**): $C_{21}H_{34}O_{10}S_{0.5}$, M = 462.51, orthorhombic, a = 10.7577(7) Å, b = 11.0431(11) Å, c = 36.8863(17) Å, U = 4382.0(6) $Å^3$, T = 98.5, space group $P2_12_12_1$ (no. 19), Z = 8, μ(Cu Kα) = 1.357, 15,966 reflections measured, 8398 unique (R_{int} = 0.0339) which were used in all calculations. The final w$R(F_2)$ was 0.1231 (all data). Flack parameter was 0.01(2).

Crystallographic data for the structure of triptergulide E (**1**) have been deposited in the Cambridge Crystallographic Data Centre (deposition numbers: CCDC 1846481). Copies of the data can be obtained free of charge via www.ccdc.cam.ac.uk.

Triptergulide F (**2**): colorless crystalline powder (MeOH); [α] −57.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 220 (3.08) nm; IR (microscope) ν_{max}3317, 2926, 1768, 1680, 1408, 1172, 1055, and 927 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 221 (+6.18), 246.5 (−2.06); ^1H NMR (DMSO-d_6, 500 MHz) see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 2; HRESIMS *m/z* 435.1639 $[M+Na]^+$ (calcd for $C_{20}H_{28}NaO_9$, 435.1626).

Triptergulide G (**3**): colorless oil (MeOH); [α] −78.9 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 218 (3.10) nm; IR (microscope) ν_{max} 3379, 2928, 1721, 1669, 1443, 1287, 1049, and 921 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 219 (+5.35), 246.5 (−1.64); ^1H NMR (DMSO-d_6, 500 MHz) see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 2; HRESIMS *m/z* 417.1528 $[M+Na]^+$ (calcd for $C_{20}H_{26}NaO_8$, 417.1520).

Triptergulide H (**4**): colorless solid (MeOH); [α] −73.7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 219 (3.82) nm; IR (microscope) ν_{max} 3355, 2935, 1767, 1676, 1441, 1171, 1030, 994, and 901 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 219.5 (+0.71), 246.0 (−0.37); ^1H NMR (DMSO-d_6, 500 MHz) see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 2; HRESIMS *m/z* 399.1417 $[M+Na]^+$ (calcd for $C_{20}H_{24}NaO_7$, 399.1414).

Triptergulide I (**5**): colorless oil (MeOH); [α] −54.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 219 (3.12) nm; IR (microscope) ν_{max} 3379, 2919, 1722, 1669, 1442, 1068, 1014, 920, and 598 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 224 (+8.35), 256.5 (−0.20); ^1H NMR (DMSO-d_6, 500 MHz) see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 2; HRESIMS *m/z* 419.1692 $[M+Na]^+$ (calcd for $C_{20}H_{28}NaO_8$, 419.1690).

Triptergulide J (**6**): colorless oil (MeOH); [α] −35.4 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 223 (3.52) nm; IR (microscope) ν_{max} 3443, 2986, 1717, 1660, 1442, 1317, 1052, and 959 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 2; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 215.5 (+12.63), 251 (−8.75), 337 (+0.98); HRESIMS *m/z* 417.1529 $[M+Na]^+$ (calcd for $C_{20}H_{26}NaO_8$, 417.1520).

Triptergulide K (**7**): colorless oil (MeOH); [α] −41.7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 220 (3.72) nm; IR (microscope) ν_{max}3286, 2925, 1743, 1654, 1535, 1394, 1239, 1083, and 950 cm^{-1}; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 220 (+4.54), 249.5 (−2.91), 339 (+0.23); ^1H NMR (DMSO-d_6, 500 MHz) see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 2; HRESIMS *m/z* 417.1523 $[M+Na]^+$ (calcd for $C_{20}H_{26}NaO_8$, 417.1520).

3.5. Biological activities

The inhibitory effects of three compounds on NO, IL-6, and TNF-α production in LPS-induced RAW264.7 macrophage were evaluated. Dexamethasone was used a positive control. Concrete operation method was according to described in the literature [20,21].

4. Conclusions

Phytochemical studies on the leaves of *T. wilfordii* led to the isolation of seven new 18(4 → 3)-*abeo*-abietane lactones, triptergulides E – K (**1** – **7**) by using kedde reagents as a detector. The ECD, calculated ECD and X-ray crystallographic analysis were employed to determined the absolute configurations of the new compounds, and a valuable positive Cotton effect near 220 nm could be applied in the following studies. Some compounds showed moderate inhibition activities against NO, IL-6, and TNF-α production in LPS-induced RAW 264.7 macrophage *in vitro*.

Conflict of interest

The authors declare no compete in financial interests.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.09.031.

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