Two New Abietane Diterpenoids from the Roots of *Tripterygium wilfordii* HOOK. f.

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Two new abietane-type diterpenoids, named triptobenzene R (**1**) and triptobenzene S (**2**), together with three known abietane-type diterpenoids, triptophenolide (**3**), triptonodiol (**4**), and triptonoterpene methyl ether (**5**), were isolated from the roots of *Tripterygium wilfordii* HOOK. f. Their structures and relative configurations were established by detailed spectral studies, including 1D- and 2D-NMR (HSQC, HMBC, and NOESY), and HR-ESI-TOF-MS, and by comparison with published data. Their absolute configurations were assigned by the CD technique, applied for the first time to abietane diterpenes from *Tripterygium wilfordii*. Compound **2** is the first abietane-type norditerpenoid isolated from the genus *Tripterygium*.

Introduction. – *Tripterygium wilfordii* HOOK. f. (Celastraceae) is a perennial woody vine plant native to Eastern and Southern China, Korea, and Japan [1]. *T. wilfordii*, known as lei gong teng ('Thunder God Vine') in China, has a long history of use in traditional Chinese Medicine (TCM) as an anticancer drug and insecticide [2]. Recently, significant pharmacological activities, including antifertility [3], antirheumatoid arthritis [4], immunosuppressive [5], repair of burn wound [6], and anti-AIDS (anti-HIV) [7] activities were found for *T. wilfordii*. So far, more than 116 diterpenoids isolated from *Tripterygium* have been reported [8]. These diverse biological activities of diterpenoids were an incentive to reinvestigate the roots of *T. wilfordii* collected from the Guangxi Province of China.

In this article, we describe the isolation and structure elucidation of two new compounds of the abietane-type from the roots of *T. wilfordii*, named triptobenzene R (**1**) and triptobenzene S (**2**), along with three known abietane-type diterpenoids, triptophenolide (**3**) [9], triptonodiol (**4**) [10], and triptonoterpene methyl ether (**5**) [11] (*Fig. 1*). The known compounds have already been isolated from this species. Compound **2**, an abietane-type norditerpenoid, is the first norditerpeniod of this type isolated from the genus *Tripterygium.*

Results and Discussion. – Repeated column chromatography (CC) and semi-prep. HPLC of the AcOEt-soluble fraction from the 95% (*v*/*v*) EtOH extracts of the dried

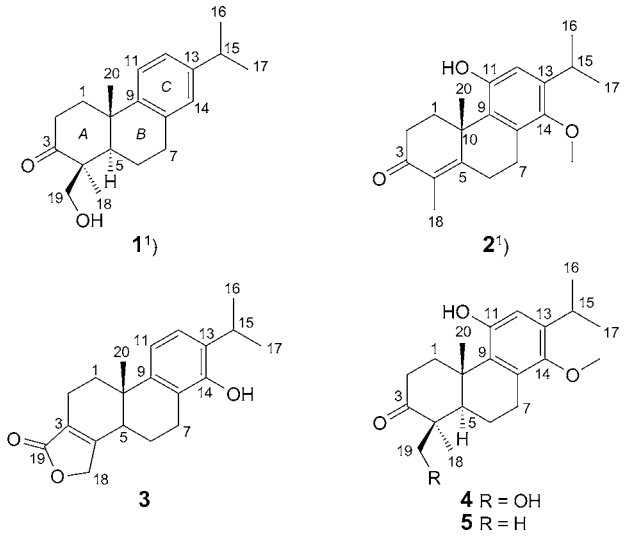


Fig. 1. *Compounds* **1**–**5**, *isolated from* Tripterygium wilfordii HOOK. *f.*

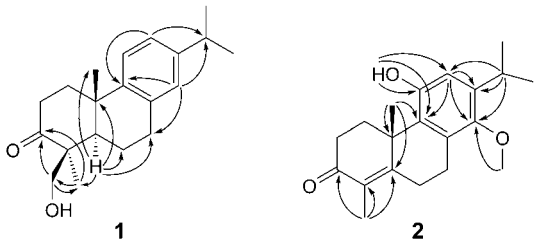
roots of *T. wilfordii* yielded two new compounds, triptobenzene R (**1**) and triptobenzene S (**2**), and the three known compounds **3**–**5**.

Compound **1** was obtained as a white amorphous powder, and its molecular formula was deduced to be $C_{20}H_{28}O_2$ from the HR-ESI-TOF-MS *m/z* 301.2159 ($[M+H]^+$), requiring seven degrees of unsaturation. The ^{1}H-NMR spectrum (*Table*) of **1** revealed the presence of an isopropyl group at δ(H) 1.23 (*d*, $J = 6.9$ Hz, 6 H) and 2.83 (*sept.*, $J =$ 6.6 Hz, 1 H), two Me groups at δ(H) 1.26 and 1.35 (2*s*), a pair of CH_2 H-atoms at δ(H) 3.52 and 4.08 (*d*, $J = 11.4$ Hz, each 1 H) bearing an OH group, and a typical *ABX* coupling system of three aromatic H-atoms at δ(H) 7.03 and 7.16 (*d*, $J = 8.4$ Hz, each 1 H) and 6.90 (br. *s*, 1 H). The ^{13}C-NMR spectrum of **1** (*Table*) showed a C=O signal at δ(C) 220.6, in addition to trisubstituted-benzene C-atom signals, a CH_2 group attached to an O-atom at δ(C) 65.9, four Me signals, four CH_2 groups, two CH groups, and two quaternary C-atoms. These groups accounted for five of the seven degrees of unsaturation deduced from the molecular formula, suggesting that **1** had three rings, among them a benzene ring. From this information, compound **1** was assumed to be an abietane-type diterpene, such as triptobenzenes A – Q and triptobenzene Y isolated from *T. wilfordii* var. *regelii* [12], *T. wilfordii* HOOK. f. [2][10b][13], *T. hypoglaucum* (LEVL.) HUTCH [14] and *T. doianum* [15]. The ^{13}C-NMR data of **1** were similar to those of triptobenzene A [12], triptobenzene M [10b], and triptobenzene O [15] concerning the rings *A* and *B*, as well as to those of triptobenzene D [12] and triptobenzene L [10b] concering the ring *C* and the isopropyl group especially. Analysis of the ^{1}H- and ^{13}C-NMR, and HSQC data of **1** helped to allot the H- to their bonded C-atoms, and further, the planar structure of **1** was deduced from the HMBC experiment (*Fig. 2*). In the HMBC spectrum of **1**, the H-atom at δ(H) 2.14 (*m*, H–C(5)) showed long-range

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

Table. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *of* **1** *and* **2**[1]). Recorded at 300 and 75 MHz, resp.; δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1)	2.47–2.49 (m, H_β), 2.03–2.07 (m, H_α)	37.3	3.12–3.18 (m, H_β), 1.80–1.91 (m, H_α)	32.5
CH_2(2)	2.59–2.63 (m, H_β), 2.70–2.72 (m, H_α)	35.0	2.43–2.45 (m, H_β), 2.68–2.75 (m, H_α)	34.4
C(3)	–	220.6	–	199.7
C(4)	–	51.1	–	126.9
H–C(5)	2.14 (br. d, J = 12.6)	51.6	–	165.0
CH_2(6)	1.67–1.71 (m, H_β), 1.86–1.90 (m, H_α)	19.9	2.21–2.31 (m, H_β), 2.93–2.98 (m, H_α)	26.7
CH_2(7)	2.90–2.92 (m, H_β), 2.83–2.87 (m, H_α)	31.3	3.30–3.32 (m, H_β), 2.48–2.53 (m, H_α)	25.6
C(8)	–	134.3	–	131.4
C(9)	–	144.2	–	129.5
C(10)	–	37.0	–	40.8
H–C(11)	7.16 (d, J = 8.4)	125.9	–	150.9
H–C(12)	7.03 (d, J = 8.4)	124.7	6.47 (s)	112.8
C(13)	–	146.5	–	139.8
H–C(14)	6.90 (br. s)	126.9	–	148.8
H–C(15)	2.83 (*sept.*, J = 6.9)	33.6	3.24 (*sept.*, J = 6.6)	26.4
Me(16)	1.23 (d, J = 6.9)	24.1	1.18 (d, J = 6.9)	23.7
Me(17)	1.23 (d, J = 6.9)	24.1	1.20 (d, J = 6.9)	24.0
Me(18)	1.35 (s)	22.4	1.88 (s)	10.9
CH_2(19)	4.08 (d, J = 11.4, H_β), 3.52 (d, J = 11.4, H_α)	65.9	–	–
Me(20)	1.26 (s)	25.9	1.70 (s)	22.6
MeO	–		3.65 (s)	61.1



1 **2**

Fig. 2. *Key HMBC interactions* (H → C) *of compounds* **1** *and* **2**

correlations with the C-atoms at δ(C) 51.1 (C(4)), 19.9 (C(6)), 31.3 (C(7)), 37.0 (C(10)), 22.4 (C(18)), 65.9 (C(19)), and 25.9 (C(20)), the H-atoms at δ(H) 1.35 (s, Me(18)) with the C-atoms at δ(C) 220.6 (C(3)) and 65.9 (C(19)), and the H-atom at δ(H) 4.08 (d, H_β–C(19)) with the C-atoms at δ(C) 220.6 (C(3)) and 22.4 (C(18)). These facts confirmed the presence of a ketone and CH_2OH moiety at C(3) and C(4), respectively. Further, the H-atom at δ(H) 6.90 (H–C(14)) correlated with the C-atoms at δ(C) 144.2 (C(9)), 31.3 (C(7)), and 33.6 (C(15)), the H-atom at δ(H) 7.03 (H–C(12)) with the signals at δ(C) 144.2 (C(9)) and 33.6 (C(15)), the H-atom signal at δ(H) 7.16 (H–C(11)) with the C-atoms at δ(C) 134.3 (C(8)), 37.0 (C(10)), and 146.5 (C(13)), and the H-atom at δ(H) 2.83 (H–C(15)) with the C-atoms at δ(C) 24.1 (C(16)

and C(17)), 124.7 (C(12)), 146.5 (C(13)), and 126.9 (C(14)). From these observations, the location of the isopropyl group at C(13) and the substituent pattern of the benzene ring were clearly inferred. In the NOESY plot, the H-atoms at δ(H) 3.52 and 4.08 (CH_2(19)) showed correlation with the H-atoms at δ(H) 1.26 (*s*, Me(20)); moreover, the H-atoms at δ(H) 1.35 (*s*, Me(18)) correlated with the H-atom at δ(H) 2.14 (H–C(5)) (*Fig. 3*). This confirmed the β-orientation of CH_2OH at C(4) and Me(20) at C(10), further demonstrating the α-orientation of H–C(5) and Me(18). The CD spectrum (*Fig. 4*) of **1** showed a positive *Cotton* effect at 292 nm, which is consistent with the octant rule of a saturated cyclohexanone[16]. Therefore, the absolute configuration of **1** was elucidated as (4*S*,5*R*,10*S*), and **1** was named triptobenzene R[1]). Assignments of the detailed ^{1}H- and ^{13}C-NMR data (*Table*) were made on the basis of the 2D-NMR spectra, including HSQC, HMBC, and NOESY.

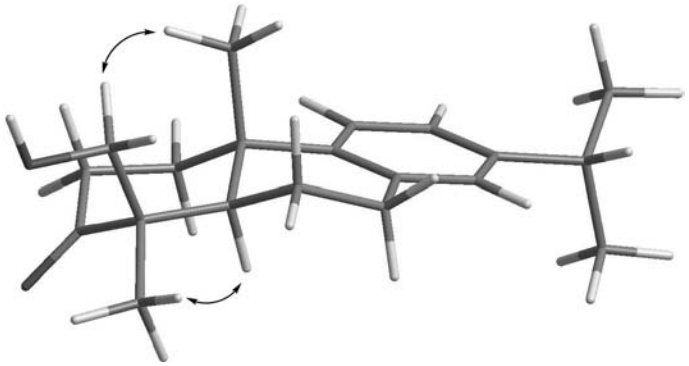


Fig. 3. *Key NOE correlations* (H ↔ H) *of compound* **1**[2])

Compound **2** was isolated as colorless oil. The HR-ESI-TOF-MS of **2** showed the quasimolecular-ion peak at *m*/*z* 313.1805([$M-H$]$^-$), corresponding to the molecular formula $C_{20}H_{26}O_3$ with eight degrees of unsaturation. In the ^{1}H-NMR spectrum (*Table*), an isopropyl group at δ(H) 1.18 (*d*, J = 6.9 Hz, 3 H), 1.20 (*d*, J = 6.9 Hz, 3 H), and 3.24 (*sept.*, J = 6.6 Hz, 1 H), two quaternary Me groups at δ(H) 1.70 (*s*, 3 H) and 1.88 (*s*, 3 H) were observed. Moreover, an MeO group at δ(H) 3.65 (*s*), an OH group at δ(H) 5.36 (br. *s*, 1 H), and a lone aromatic H-atom at δ(H) 6.47 (*s*) were also observed. In addition to the MeO group at δ(C) 61.1, the ^{13}C-NMR spectrum showed 19 C-atom signals, including that of a conjugated ketone at δ(C) 199.7 attached to a C=C bond appearing at δ(C) 126.9 and 165.0, together with those of a pentasubstituted benzene ring, four Me groups, four CH_2 groups, one CH group, and one quaternary C-atom. The rough analysis of the ^{1}H- and ^{13}C-NMR spectrum of **2** accounted for six of the eight degrees of unsaturation established by the molecular formula. From these facts, the structure of **2** was deduced to have an abietane-type norditerpenoid skeleton having 20 C-atoms, one of which was arising from a MeO group. Also the ^{13}C-NMR data of **2**, **4**, and **5**, neotriptophenolide [9], triptobenzene H [2], and (3β)-14-methoxyabieta-

[2]) Structure optimized with MM2 to minimize steric energy, by means of ChemBio3D Ultra (version 12.0).

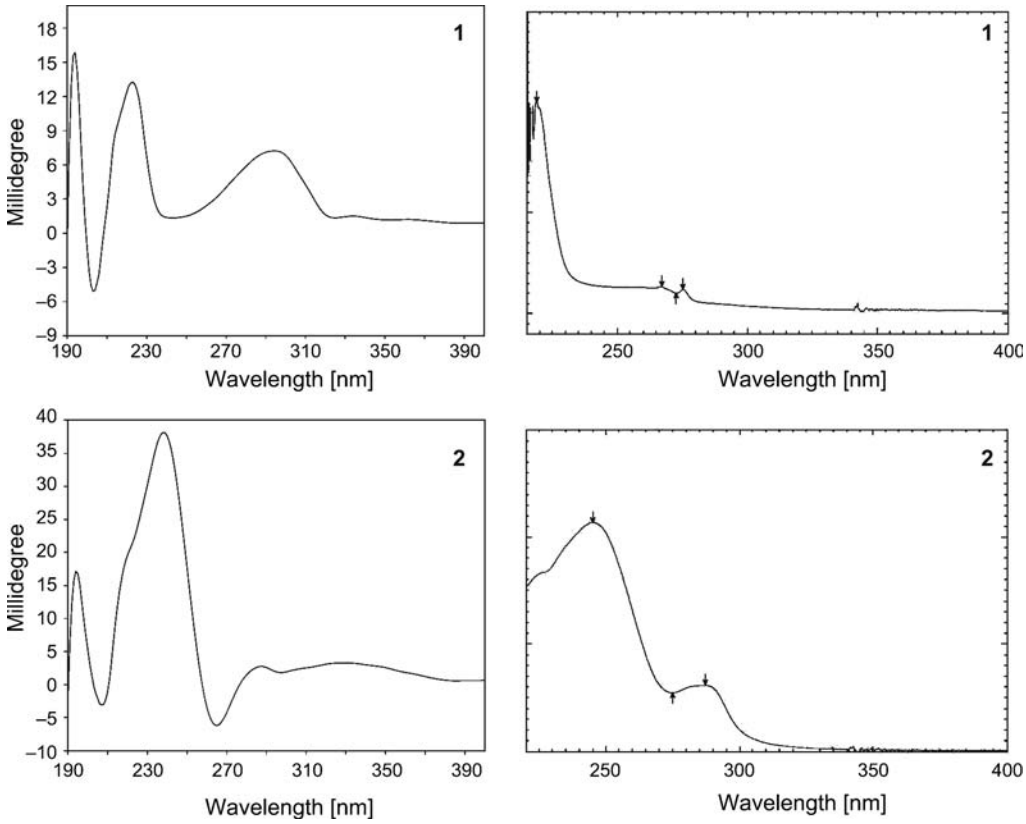


Fig. 4. *CD* (left) *and UV Curves* (right) *of compounds* **1** *and* **2**

8,11,13-triene-3,11,19-triol [17] showed similar chemical shifts of ring *C* and the isopropyl group. In the HMBC spectrum of **2** (*Fig. 2*), the following correlations were observed: δ(H) 3.65 (*s*, MeO–C(14))/δ(C) 148.4 (C(14)), δ(H) 3.24 (H–C(15))/δ(C) 139.8 (C(13)), 112.8 (C(12)), and 148.4 (C(14)), δ(H) 6.47 (*s*, H–C(12))/δ(C) 148.4 (C(14)), 139.8 (C(13)), 150.9 (C(11)), and 129.5 (C(9)), and δ(H) 5.36 (br. *s*, OH)/δ(C) 112.8 (C(12)), 129.5 (C(9)), and 150.9 (C(11)). These facts clearly established the substituent pattern of the benzene ring with the OH group at C(11), the lone aromatic H-atom at C(12), the isopropyl group at C(13), and the MeO group at C(14). Further, the correlations δ(H) 1.70 (*s*, Me(20))/δ(C) 32.5 (C(1)), 40.8 (C(10)), 129.5 (C(9)), and 165.0 (C(5)), and δ(H) 1.88 (*s*, Me(18))/δ(C) 126.9 (C(4)), 165.0 (C(5)), and 199.7 (C(3)) were consistent the positions of the ketone and double bond groups at C(3) and C(4). Assignments of the detailed ^{1}H- and ^{13}C-NMR data (*Table*) were made on the basis of the 2D-NMR spectra, including HSQC and HMBC. There is only one stereogenic C-atom center in the structure of **2**. Its CD spectrum (*Fig. 4*) showed a positive *Cotton* effect at 238 nm, which is consistent with the helicity rule of an α,β-unsaturated 6-membered-ring ketone [16b][18] (*Fig. 5*). Thus, the absolute config-

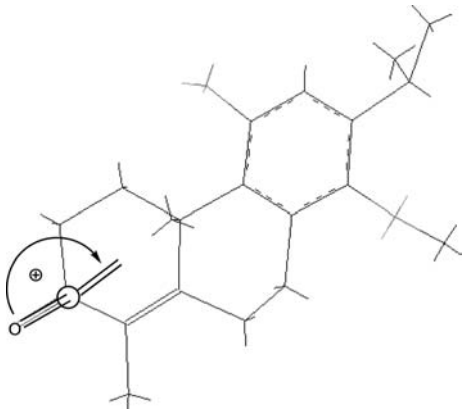


Fig. 5. Cotton *effect of the α,β-unsaturated cyclic-ketone moiety of compound* **2**

uration of **2** was elucidated as (10*S*), and **2** was named triptobenzene S[1]) which is the first abietane-type norditerpenoid isolated from the genus *Tripterygium*.

Further studies to evaluate the biological activities of the new compounds are currently underway in our laboratory.

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Experimental Part

General. TLC: silica gel precoated glass plates (SiO_2 *GF*$_{254}$; *Qingdao Marine Chemical Inc.*, Qingdao, P. R. China); visualization under UV light or by spraying with 10% H_2SO_4 in EtOH with vanillin, followed by heating. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Marine Chemical Inc.*, Qingdao, P. R. China) or SiO_2 *H* (10–40 μm; *Qingdao Marine Chemical Inc.*); *MCI* gel (*CHP20P*, 75–150 μm; *Mitsubishi Chemical Corporation*, Japan); reversed-phase C_{18} silica gel (60–80 μm; *Merck*, Germany). Semi-prep. HPLC: *Hitachi-655–15* liquid chromatograph pump, *Hitachi-L-2490 RI* detector (*Japan Analytical Industry Co., Ltd.*); column *YMC ODS-A* (5 μm, 10 × 250 mm; *YMC*, Japan); t_R in min. CD Spectra: *Bio-Logic-CD-MDS450* spectrophotometer; in CH_2Cl_2; λ_{max} ($\Delta\varepsilon$) in nm. UV Spectra: *Shimadzu-UV-1700* spectrophotometer; λ_{max} (log ε) in nm. ^{1}H-, ^{13}C-, and 2D-NMR Spectra: *Bruker-AV-600* and *-ARX-300* spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-TOF-MS: *MicroTOF* spectrometer (*Bruker Daltonics*); in *m*/*z*.

Plant Material. The roots (11 kg) of *Tripterygium wilfordii* Hook. f. were collected in September 2009 from the Guangxi Province, P. R. China, and authenticated by Prof. *Qishi Sun*, Department of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University. A voucher specimen (No. 20090905) was deposited with the Nature Products Laboratory of Shenyang Pharmaceutical University, Shenyang, P. R. China.

Extraction and Isolation. Air-dried roots of *Tripterygium wilfordii* Hook. f. (11 kg) were extracted with 95% (*v*/*v*) EtOH (3 × 66 l, 4 h each time) under reflux to give a crude extract (473.0 g). The extract was suspended in H_2O (12 l) and extracted successively with AcOEt (4 × 15 l) and BuOH (4 × 15 l) to yield a AcOEt-soluble fraction (262.0 g) and a BuOH-soluble fraction (136.0 g). The AcOEt-soluble fraction was subjected to CC (SiO_2, CH_2Cl_2/MeOH 100:0 → 0:100): *Fractions A–F* (by TLC monitoring). *Fr. B* (57.3 g) was further separated by CC (*MCI* gel *CHP20P*, MeOH/H_2O 6:4 → 100:0): *Frs. B1–B5*. *Fr. B2* (23.6 g) was purified by CC (SiO, CH_2Cl_2/MeOH 100:0 → 0:100) and CC

(reversed-phase C_{18} SiO_2; MeOH/H_2O 3:7 → 100:0), and finally separated by recersed-phase HPLC (MeOH/H_2O 7:3 → 9:1): **1** (with 80% MeOH; t_R 33.4; 5 mg), **2** (with 71% MeOH; t_R 49.4; 9 mg), **3** (with 71% MeOH; t_R 38.0; 35 mg), **4** (with 64% MeOH; t_R 60.5; 40 mg), and **5** (with 71% MeOH; t_R 77.1; 25 mg).

Triptobenzene R (=*(1S,4aS,10aR)-3,4,4a,9,10,10a-Hexahydro-1-(hydroxymethyl)-1,4a-dimethyl-7-(1-methyl)-phenanthren-2(1H)-one*; **1**): White amorphous powder. CD (CH_2Cl_2): 292 (+6.37 mdeg, c = 1 mg/ml). UV (CH_2Cl_2): 223 (7.31). ^{1}H- and ^{13}C-NMR: *Table.* HR-ESI-TOF-MS: 301.2159 ([M + H]$^+$, $C_{20}H_{29}O_2^+$; calc. 301.2162).

Triptobenzene S (=*(4aS)-4,4a,9,10-Tetrahydro-5-hydroxy-8-methoxy-1,4a-dimethyl-7-(1-methylethyl)-phenanthren-2(3H)-one*; **2**): Colorless oil. CD (CH_2Cl_2): 238 (+37.17 mdeg, c = 1 mg/ml). UV (CH_2Cl_2): 243 (7.11), 287 (3.93). ^{1}H- and ^{13}C-NMR: *Table.* HR-ESI-TOF-MS: 313.1805 ([M – H]$^-$, $C_{20}H_{25}O_3^-$; calc. 313.1809).

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