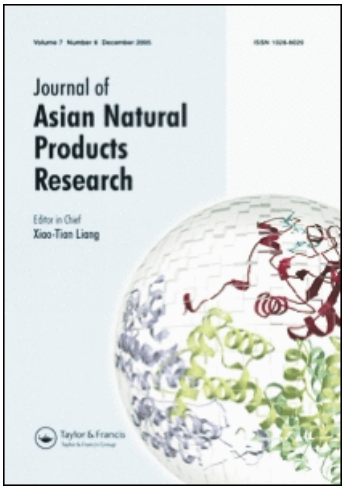


Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Terpene alkaloids from *Tripterygium wilfordii*

Xiao-Dong Wang[a]; Wei Jia[b]; Wen-Yuan Gao[a]; Rong Zhang[a]; Yan-Wen Zhang[c]; Jun Zhang[c]; Yoshihisa Takaishi[d]; Hong-Quan Duan[c]
[a] College of Pharmaceuticals and Biotechnology, Tianjin University, Tianjin, China [b] School of Pharmacy, Shanghai Jiao Tong University, Shanghai, China [c] School of Pharmacy, Tianjin Medical University, Tianjin, China [d] Faculty of Pharmaceutical Sciences, University of Tokushima, Tokushima, Japan

To cite this Article Wang, Xiao-Dong , Jia, Wei , Gao, Wen-Yuan , Zhang, Rong , Zhang, Yan-Wen , Zhang, Jun , Takaishi, Yoshihisa and Duan, Hong-Quan(2005) 'Terpene alkaloids from *Tripterygium wilfordii*', Journal of Asian Natural Products Research, 7: 5, 755 — 759
To link to this Article: DOI: 10.1080/1028602042000325618
URL: http://dx.doi.org/10.1080/1028602042000325618

Journal of Asian Natural Products Research, Vol. 7, No. 5, October 2005, 755–759

Terpene alkaloids from *Tripterygium wilfordii*

XIAO-DONG WANG†, WEI JIA‡, WEN-YUAN GAO†, RONG ZHANG†, YAN-WEN ZHANG§, JUN ZHANG§, YOSHIHISA TAKAISHI¶ and HONG-QUAN DUAN§*

†College of Pharmaceuticals and Biotechnology, Tianjin University, Tianjin 300072, China
‡School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200030, China
§School of Pharmacy, Tianjin Medical University, Tianjin 300070, China
¶Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi 1-78, Tokushima 770-8505, Japan

(Received 30 March 2004; revised 1 June 2004; in final form 4 July 2004)

Two new sesquiterpene alkaloids, 1β-hydroxy-2β,5α,11-triacetoxy-7β-nicotinoyl-8β-benzoyl-dihydroagarofuran (**1**), and 1β,5α,11-triacetoxy-7β-nicotinoyl-8β-benzoyl-dihydroagarofuran (**2**) were isolated from the xylem of *Tripterygium wilfordii*, together with six known compounds. Their structures were elucidated on the basis of spectroscopic studies.

Keywords: *Tripterygium wilfordii*; Xylem; Sesquiterpene alkaloid

1. Introduction

Plants of the genus *Tripterygium* (Celastraceae) have been used in traditional Chinese medicine for treatment of cancer and as an insecticide for hundreds of years. Over the past several decades, the plants of the genus *Tripterygium*, particularly, their xylem extracts, have widely been used in clinical treatment of rheumatoid arthritis, skin disorders, male-fertility control, and other inflammatory and autoimmune diseases [1–3]. We have previously reported some anti-HIV agents, triptonines A and B, along with several related compounds from *Tripterygium wilfordii* in our studies on the bioactive metabolites of this genus [4–6]. This paper deals with the isolation and structure determination of two new sesquiterpene alkaloids named wilforsinines A (**1**) and B (**2**), as well as six known compounds (**3**–**8**) from the xylem of *Tripterygium wilfordii*. Compounds **3**–**8** were isolated from the xylem of this plant for the first time.

2. Results and discussion

Wilforsinine A (**1**) was obtained as maize crystal, having a molecular formula of $C_{34}H_{39}O_{12}N$ from HREIMS. There was an ester carbonyl band at 1735 cm^{-1} in the IR spectrum, and the UV

*Corresponding author. Email: duanhq@tijmu.edu.cn

Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2005 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/1028602042000325618

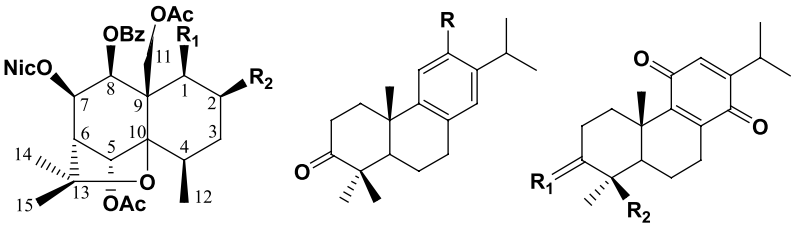
spectrum showed the presence of an aromatic moiety (225 and 265 nm). The ^{1}H NMR spectral data of **1** revealed the presence of three acetyl methyls (δ_H 2.12, 2.02, and 1.94), a nicotinoyl group [δ_H 9.25 (1H, d, $J = 1.6$ Hz), 8.81 (1H, br t, $J = 3.3$ Hz), 8.40 (1H, br d, $J = 8.0$ Hz), 7.47 (1H, m)], a benzoyl group [δ_H 7.85 (2H, d, $J = 7.8$ Hz), 7.51 (1H, *t*, $J = 7.4$ Hz), 7.34 (2H, dd, $J = 7.8, 7.4$ Hz)], an oxygenated methylene [δ_H 5.26, 4.94 (each 1H, d, $J = 12.4$ Hz)], as well as five methine protons (δ_H 6.23, 5.96, 5.86, 5.23 and 4.39). The ^{13}C NMR spectral data of **1** revealed the presence of six methyls, one oxygenated methylene, and five oxygenated methine carbons, in addition to two methines, five ester carbonyl carbons, three quaternary carbons, one nicotinoyl group [δ_C 164.5 (s), 153.6 (d), 151.0 (d), 137.3 (d), 125.9 (s), 123.5 (d)], and one benzoyl group [δ_C 165.2 (s), 133.2 (d), 129.8 (s), 129.5 (d), 128.4 (d)]. From the above information, compound **1** should be a sesquiterpene polyol ester having a dihydroagarofuran skeleton as found in the genus *Tripterygium* [7–9].

The ^{1}H–^{1}H COSY spectrum of **1** revealed two separated spin–spin system (H-1/H-2/H-3/H-4, H-6/H-7/H-8) in the dihydroagarofuran skeleton. The remaining dihydroagarofuran proton signal at δ_H 6.23 (H-5) was correlated with the carbon signals at δ_C 53.2 (C-6), 73.7 (C-7), 50.8 (C-9), 89.8 (C-10) and 81.4 (C-13) in the HMBC spectrum.

From the HMBC spectrum, the proton signal of benzoyl (δ_H 7.85) and the methine proton signal (δ_H 5.96, H-8) were correlated with the carbonyl carbon signal at δ_C 165.2, and the proton signal at δ_H 5.86 (H-7) with the resonance at δ_C 164.5 (nicotinoyl), while the signals at δ_H 5.23 (H-2), 6.23 (H-5) and 4.94 (H-11a) were correlated with the acetyl carbonyl carbons at δ_C 170.6, 169.7, and 169.8, respectively. From above observations, the nicotinoyl and benzoyl groups were assigned at positions C-7 and C-8, and three acetyl groups were assigned at positions C-2, C-5 and C-11, respectively. Acetylation of **1** afforded **1a**, the proton signal at δ_H 4.39 (H-1, in **1**) shifted to the downfield region at δ_H 5.60 in **1a**. Thus, the hydroxyl group was located at position C-1.

In the NOESY spectrum of **1**, the proton signal at δ_H 4.39 (H-1) correlated with the signals at δ_H 5.96 (H-8) and 5.23 (H-2), the proton signal at δ_H 5.96 (H-8) with the signal at δ_H 5.86 (H-7) and 1.64 (H_3-14), and the proton signal at δ_H 5.26 (H-11b) correlated with the signals at δ_H 6.23 (H-5) and 1.23 (H_3-12). Thus, the relative stereochemistry of the ester and hydroxyl groups were elucidated as having the 1β, 2β, 5α, 7β and 8β configurations. The ^{1}H and ^{13}C NMR assignments were obtained by 2D NMR spectra including NOESY. Therefore, the structure of wilforsinine A (**1**) was determined as shown in figure 1.

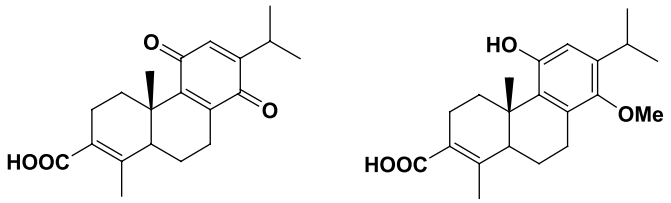
Wilforsinine B (**2**), $C_{34}H_{39}O_{11}N$, revealed signals for three acetyl groups (δ_H 2.17, 1.97, and 1.49), a benzoyl group [δ_H 7.93 (2H, d, $J = 7.1$ Hz), 7.58 (1H, t, $J = 7.4$ Hz), 7.43 (2H, dd, $J = 7.4, 7.1$ Hz)], and a nicotinoyl group [δ_H 9.14 (1H, br s), 8.75 (1H, m), 8.31 (1H, br d, $J = 8.0$ Hz), 7.55 (1H, m)], as well as four methine protons [δ_H 6.82, 5.84, 5.81, 5.47] in its ^{1}H NMR spectrum. The ^{13}C NMR spectral data were similar to those of **1**, except for the C-1, -2 and -3 carbon signals (table 1). Compound **2** was also a dihydroagarofuran polyol ester and was presumed to be 1-acetyl-2-deacetoxy of **1**. In the HMBC spectrum of **2**, the proton signals at δ_H 8.31 (nicotinoyl) and 5.84 (H-7) correlated with the carbonyl carbon signal at δ_C 165.9, and the signals at δ_H 7.93 (benzoyl group) and 5.81 (H-8) with the carbon signal at δ_C 166.4, while the signals at δ_H 5.47 (H-1), 6.82 (H-5) and 4.83 (H-11a) correlated with the acetyl carbon signals at δ_C 171.8, 171.7 and 172.2, respectively. Thus, the nicotinoyl and benzoyl groups were assigned at positions C-7 and C-8, and three acetyl groups were located at positions C-1, C-5 and C-11. In the NOESY spectrum, the proton signal at δ_H 4.83 (H-11a) correlated with the proton signals at 6.82 (H-5) and 1.07 (H_3-12), and the proton



	R_1	R_2
1	OH	OAc
1a	OAc	OAc
2	OAc	H

	R
3	H
5	OH

	R_1	R_2
4	2H	COOH
8	O	CH_2OH



6 **7**

Figure 1. Structures of compounds **1**–**8**.

signal at δ_H 5.81 (H-8) with the signals at δ_H 5.47 (H-1) and 1.63 (H_3-14), while the signal at δ_H 5.84 (H-7) correlated with the signal at δ_H 1.63 (H_3-14). Therefore, the relative configurations of ester groups of **2** were determined as 1β, 5α, 7β and 8β (figure 1).

The known compounds were identified by spectral comparison with 8,11,13-abietatriene-3-one (**3**) [10], triptoquinone F (**4**) [11], hinokione (**5**) [12], triptoquinone A (**6**) [11], triptobenzene H (**7**) [13] and triptoquinone B (**8**) [11], respectively.

Table 1. ^{1}H NMR and ^{13}C NMR spectral data of **1** and **2**.

	1 *($CDCl_3$)*		***2*** *(CD_3OD)*	
No.	^{13}C	^1H	^{13}C	^1H
1	76.6	4.39 (m)	80.7	5.47 (m)
2	73.6	5.23 (m)	24.3	1.83, 1.68 (m)
3	33.2	2.30, 1.90 (m)	27.5	2.24, 1.55 (m)
4	31.2	2.33 (m)	34.9	2.32 (m)
5	74.7	6.23 (s)	76.1	6.82 (s)
6	53.2	2.70 (d, 4.1)	54.6	2.72 (d, 3.8)
7	73.7	5.86 (dd, 5.6, 4.1)	73.2	5.84 (dd, 5.9, 3.8)
8	72.2	5.96 (d, 5.6)	74.4	5.81 (d, 5.9)
9	50.8	–	52.4	–
10	89.8	–	92.2	–
11	64.0	5.26, 4.94 (d, 12.4)	62.0	4.83, 4.72 (d, 13.3)
12	18.1	1.21 (d, 7.7)	15.6	1.07 (d, 7.5)
13	81.4	–	82.5	–
14	24.5	1.64 (s)	24.8	1.63 (s)
15	30.4	1.46 (s)	30.8	1.52 (s)

3. Experimental

3.1 General experimental procedures

NMR experiments were run on a Bruker AVANCE 300 instrument. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz) both had teramethylsilane as an internal standard. MS data were obtained on a JEOL JMS-SX102A instrument. Column chromatography was performed on silica-gel (Qingdao Haiyang Chemical Co. Ltd) and Sephadex LH-20 (Amersham Pharmacia Biotech). HPLC was a JASCO Gulliver Series with PU-1580 (pump), RI-1530 and UV-1575 (detector). Preparative HPLC column was used as follows: ODS (YMC-Pack ODS-A, SH-343-5), GPC (Shodex, Asahipak GS-310, 20G, MeOH), Si-HPLC$_1$ (Hibar RT 250-25, Lichrosorb, Si60 7 μm), and Si-HPLC$_2$ (YMC-pack SIL-06, SH-043-5-06). IR spectra were recorded on a 1710 Infrared Fourier Transform spectrometer (Perkin-Elmer). UV spectra were obtained on a UVIKON$_{XS}$ recording spectrometer (Bio-Tek). Optical rotation was measured with a MC 241 digital polarimeter (Perkin-Elmer).

3.2 Plant material

The xylem rhizomes of *Tripterygium wilfordii* were purchased from Yueyang, Hunan province, and were identified by Professor Wen-Yuan Gao, Department of Pharmacognosy and Natural Medicines, Tianjin University. A voucher specimen (D20021018) is deposited at the College of Pharmaceuticals and Biotechnology, Tianjin University, China.

3.3 Extraction and isolation

The xylem rhizomes (10 kg) of *T. wilfordii* were refluxed three times with 95% EtOH (15 l each) for 2 h. The extract was concentrated under reduced pressure to give a residue (390 g) which was partitioned between chloroform and H_2O. The $CHCl_3$ layer was concentrated to a residue of 112 g. Chromatographic separation was performed with a silica gel column and solvents of increasing polarity as mobile phase [petroleum ether/EtOAc (8:1, 5:1, 3:1, 1:1, 1:2, 1:4), EtOAc, EtOAc/MeOH (19:1, 9:1, 4:1), MeOH] to give 16 frs. Fraction 10 (2 g) was chromatographed on Sephadex LH-20 (MeOH) to give 3 frs. (fr. 10.1–10.3). Fr. 10.1 (840 mg) was separated by HPLC (ODS, MeOH/H_2O 8:2) to give 12 frs. (fr. 10.1.1.1–10.1.1.12). Fr. 10.1.1.5 (80 mg) and Fr. 10.1.1.9 (26 mg) were separated respectively by HPLC (ODS, MeOH/H_2O 7:3) to give **1** (6.5 mg) and **2** (8.5 mg). Fraction 7 (2.4 g) was chromatographed on a silica column [$CHCl_3$/MeOH (97:3, 9:1)] to give 10 frs. (fr.7.1–7.10). Fr. 7.7 (85 mg) was separated by HPLC (GPC, MeOH) to give **3** (6.5 mg). Fraction 7.3 (210 mg) was separated by Sephadex LH-20 (MeOH) to give **4** (21 mg). Fraction 6 (2.2 g) was chromatographed on Sephadex LH-20 (MeOH), then separated by Si-HPLC$_1$ ($CHCl_3$/MeOH 97:3) to give **5** (5.0 mg). Fraction 11 (8.5 g) was chromatographed on a silica column to give frs. 11.1–11.8. Fraction 11.5 (800 mg) was separated by GPC (MeOH), then by Si-HPLC$_2$ (hexane/EtOAc 3:1) to give **6** (8.0 mg) and **7** (90 mg). Fraction 13 (5.2 g) was chromatographed with middle pressure silica gel column with $CHCl_3$/MeOH (98:2, 95:5, 9:1) to give 10 frs. (fr. 13.1–13.10). Fraction 13.6 (245 mg) was chromatographed using LH-20 (MeOH), then by Si-HPLC$_2$ (hexane/EtOAc 5:2) to give **8** (50 mg).

Wilforsinine A (**1**) was isolated as a maize crystal. $[\alpha]_D^{25}$ −10.5 (c 0.1, MeOH). UV (MeOH) λ_{max} (log ε): 225 (4.21), 265 (3.57) nm. IR (KBr) ν_{max} cm^{-1}: 3438, 2920, 2851,

1735, 1593, 1371, 1292, 1225, 1106, 1042, 743, 713. ^{1}H-NMR ($CDCl_3$), see table 1; δ 2.02 (2-OAc); 2.12 (5-OAc); 1.94 (11-OAc); 7.85 (2H, d, $J = 7.8$ Hz), 7.51 (1H, t, $J = 7.4$ Hz), 7.34 (2H, dd, $J = 7.8, 7.4$ Hz), (8-OBz); 9.25 (1H, d, $J = 1.6$ Hz), 8.81 (1H, br t, $J = 3.3$ Hz), 8.40 (1H, br d, $J = 8.0$ Hz), 7.47 (1H, m), (7-ONic). ^{13}C-NMR ($CDCl_3$), see table 1; δ 21.3, 170.6 (2-OAc); 21.2, 169.7 (5-OAc); 21.2, 169.8 (11-OAc); 165.2, 129.5, 128.4, 133.2 (8-OBz); 164.5, 125.9, 137.3, 123.5, 153.6, 151.0 (7-ONic). EI-MS: *m/z* 653[M]$^+$(3), 611 (5), 593 (7), 318 (4), 149 (15), 124 (36), 105 (100), 57 (24). HR-EIMS *m/z* 653.2457 (calcd for $C_{34}H_{39}O_{12}N$, 653.2472).

Compound **1** was subjected to acetylation with Ac_2O-pyridine for 4 h at room temperature to give **1a**. ^{1}H-NMR ($CDCl_3$), δ 5.60 (1H, d, $J = 3.2$ Hz, H-1), 5.41 (1H, m, H-2), 2.50 (m, H-3), 1.95 (m, H-3), 2.35 (m, H-4), 6.85 (s, H-5), 2.66 (1H, d, $J = 3.7$ Hz, H-6), 5.79 (1H, m), 5.76 (1H, m), 5.36 (1H, d, $J = 12.5$ Hz, H-11), 4.63 (1H, d, $J = 13.5$ Hz, H-11), 1.16 (1H, d, $J = 7.7$ Hz), 1.53 (3H, s), 1.48 (3H, s), 1.62 (3H, s), 2.08 (3H, s), 2.15 (3H, s), 2.00 (3H, s), 7.90 (2H, d, $J = 7.2$ Hz), 7.70 (1H, m), 7.38 (2H, br t, $J = 7.2$ Hz), (8-OBz); 9.22 (1H, *s*), 8.78 (1H, br t, $J = 7.4$ Hz), 8.27 (1H, br d, $J = 7.4$ Hz), 7.53 (1H, *m*) (7-ONic).

Wilforsinine B (**2**) was isolated as a colourless crystal. $[\alpha]_D^{25}$ -34.1 (c 0.1, MeOH). UV (MeOH) λ_{max} (log ε) δ 225 (4.23), 265 (3.61) nm. IR (KBr) ν_{max} cm^{-1}: 3448, 2927, 1738, 1592, 1452, 1371, 1231, 1097, 1025, 743, 713. ^{1}H-NMR (CD_3OD), see table 1; δ 1.49 (1-OAc); 2.17 (5-OAc); 1.97 (11-OAc); 7.93 (2H, d, $J = 7.1$ Hz), 7.58 (1H, t, $J = 7.4$ Hz), 7.43 (2H, dd, $J = 7.4, 7.1$ Hz), (8-OBz); 9.14 (1H, br s), 8.75 (1H, m), 8.31 (1H, br d, $J = 8.0$ Hz), 7.55 (1H, m) (7-ONic). ^{13}C-NMR (CD_3OD), see table 1; δ 21.2, 171.7 (1-OAc); 21.6, 172.2 (5-OAc); 21.4, 171.8 (11-OAc); 166.4, 131.5, 130.8, 129.9, 134.9 (8-OBz); 165.9, 128.0, 139.2, 125.4, 154.5, 151.5 (7-ONic). EI-MS: *m/z* 637[M]$^+$(27), 595 (78), 124 (43), 106 (44), 105 (100), 77 (15). HR-EIMS *m/z* 637.2556 (calcd for $C_{34}H_{39}O_{11}N$, 637.2523).

References

[1] S.Z. Qian. *Contraception*, **36**, 335 (1987).
[2] S.A. Matlin, A. Belenguer, V.E. Stacey, S.Z. Qian, Y. Xu, J.W. Zhang, J.K.M. Sanders, S.R. Amor, C.M. Pearce. *Contraception*, **47**, 387 (1993).
[3] S.Z. Qian, Y. Xu, J.W. Zhang. *Contraception*, **51**, 121 (1995).
[4] H.Q. Duan, T. Yoshihisa, T. Hiroshi, M. Yasukazu, T. Takao, Y.F. Jia, D. Li. *J. Nat. Prod.*, **64**, 582 (2001).
[5] H.Q. Duan, T. Yoshihisa, I. Yasuhiro, Y.F. Jia, D. Li, K.H. Lee. *J. Nat. Prod.*, **63**, 357 (2000).
[6] H.Q. Duan, T. Yoshihisa, Y.F. Jia, D. Li. *Chem. Pharm. Bull.*, **47**, 1664 (1999).
[7] T. Yoshihisa, K. Tokura, S. Tamai, K. Ujita, K. Nakano, T. Tomimatsu. *Phytochemistry*, **30**, 1567 (1991).
[8] T. Yoshihisa, S. Tamai, K. Nakano, T. Tomimatsu. *Phytochemistry*, **30**, 3027 (1991).
[9] H.Q. Duan, T. Yoshihisa, M. Hiroshi, O. Yasukazu, T. Takao, Y.F. Jia, D. Li. *Phytochemistry*, **53**, 805 (2000).
[10] G. Topca, A. Vlubelen. *Phytochemistry*, **29**, 2346 (1990).
[11] S. Kozo, N. Kimiko, K. Kazuyoshi, T. Yoshihisa. *Phytochemistry*, **35**, 731 (1994).
[12] M. Takashi, U. Shuj, K. Hiroyuk, M. Masanori. *Bull. Chem. Soc. Jpn*, **54**, 581 (1981).
[13] K.H. Li, H.Q. Duan, K. Kazuyoshi, T. Yoshihisa. *Phytochemistry*, **35**, 791 (1997).