Pergamon

0031-9422(94)00916-3

Phytochemistry, Vol. 39, No. 5, pp. 1159–1163, 1995

0031-9422/95 $9.50 + 0.00

D: A-FRIEDO-24-NOROLEANANE TRITERPENOIDS FROM *TRIPTERIGIUM WILFORDII*

TAKASHI MOROTA, CHUN-XIN YANG,* TATSUNORI OGINO, WAN-ZHANG QIN,* TAKAO KATSUHARA, LI-HONG XU,* YASUHIRO KOMATSU, KANG-LI MIAO,* MASAO MARUNO and BING-HUI YANG*

Tsumura Central Laboratories, 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-11, Japan; *Zhong Shan Hospital of Shanghai Medical University, 136 Yi-Xue-Yuan Road, Shanghai 200032, China

(*Received in revised form* 19 *October* 1994)

Key Word Index—*Tripterigium wilfordii*; Celastraceae; wilforols; D:A-friedo-24-noroleananes; triterpenoids.

Abstract—The acetone extract of dried root bark of *Tripterigium wilfordii* afforded D:A-friedo-24-noroleanane triterpenoids including two new compounds named wilforols A and B. Their structures were established on the basis of chemical and spectroscopic studies.

INTRODUCTION

Tripterigium wilfordii Hook f. is commonly known in China as Lei-Gong-Teng (Thunder God vine) and is widely distributed in southern China. Its powdered root has been used as a contact insecticide by gardeners in rural China. Clinically, a water extract of this plant has been used to treat a variety of autoimmune diseases such as rheumatoid arthritis (RA), systematic lupus erythematosus (SLE) and Bechet's disease in China [1]. Several diterpenoids and triterpenoids and other compounds have been isolated from *T. wilfordii* [2].

The triepoxides, diterpenoids, triptolide and triptonide from the roots of this plant have been shown to have significant antileukaemic activity [3]. The immunosuppressive and antifertility activity of some fractions prepared from metabolites of a cell culture line of this plant has also been reported [4]. We have isolated two immunosupressive compounds, demethylzeylasteral (**3**) and celastorol (tripterine, **6**), from this plant (data not shown).

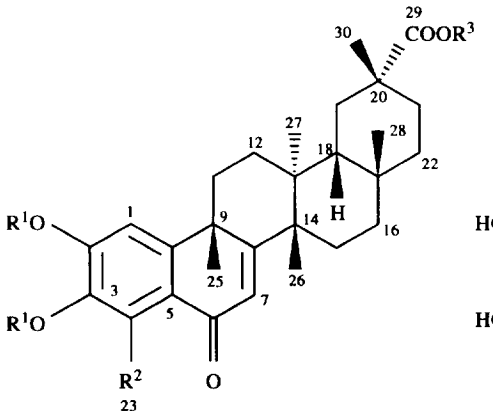
In the course of our study, we isolated two new D:A-friedo-noroleanane triterpenes, 1,2-dihydroxy-6-oxo-D:A-friedo-24-nor-1,3,5 (10), 7-oleanatetraene-29-oic acid (**1**) and 25 (9 → 8)-*abeo*-D:A-friedo-1, 2-dihydroxy-24-nor-1,3,5 (10), 6,8-oleanapetaen-29-oic acid (**2**), which we have named wilfolol A and B, respectively, along with four known triterpenoids: demethylzeylasteral (**3**), 23-nor-6-oxodemethylpristimerol (**4**), demethylzeylasterone (**5**) and celastrol (tripterin, **6**). Compounds **3**–**6** were identified by comparisons of their physico-chemical and spectral data with those in the literature [5–9]. The carbon signals in the ^{13}C NMR spectrum of **3** were completely assigned by 2D-NMR experiments (^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, HMBC, INADEQUATE) as shown in Table 1. In this paper, we present the structural investigations of these novel compounds.

RESULTS AND DISCUSSION

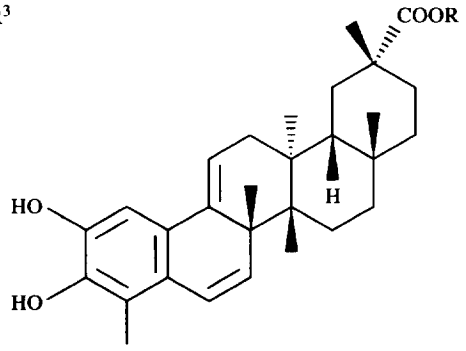
An acetone extract of *T. wilfordii* afforded two new D:A-friedo-24-noroleanane triterpenes [wilforol A (**1**) and wilforol B (**2**)].

Wilforol A (**1**) gave a positive response to both the Liebermann–Burchard test for triterpenoids and the neutral iron (III) chloride test for phenols. In the IR spectrum of **1**, adsorption bands at 3500–3000, 1702 and 1636 cm^{-1} indicated the presence of a hydroxyl group, a carboxyl group and a conjugated ketone, respectively. Acetylation and successive methylation (CH_2N_2) of **1** afforded a monomethyl ester–diacetate (**1a**) whose molecular formula $C_{34}H_{44}O_7$ was determined by HR mass spectroscopy. The ^{13}C NMR spectrum of **1** exhibited 29 carbon signals (Table 1) and the ^{1}H NMR spectrum showed five tertiary methyl signals, one aromatic proton signal (δ6.84, *s*) and one olefinic proton signal (δ6.02, *s*). Since these spectral data were very similar to those of **3**, **1** was presumed to have the same skeleton as **3**. The EIMS of **1** showed fragments at *m/z* 466 $[M]^+$ and 218, derived from cleavage around ring C [5]. These fragments were smaller by 14 amu than those (*m/z* 480, 232) of **3**, indicating that the aldehyde group in **3** was replaced by a methyl group in **1**. By comparisons of the ^{1}H and ^{13}C NMR spectra of **1** with those of **3**, it was confirmed that the aldehyde group (δ_H 11.0; δ_C200.2) in **3** was replaced by an aromatic methyl group (δ_H 2.47; δ_C13.5) in **1**. On the basis of above results, the structure of wilforol A was formulated as 1,2-dihydroxy-6-oxo-D:A-friedo-24-nor-1,3,5 (10), 7-oleanatetraen-29-oic acid (**1**).

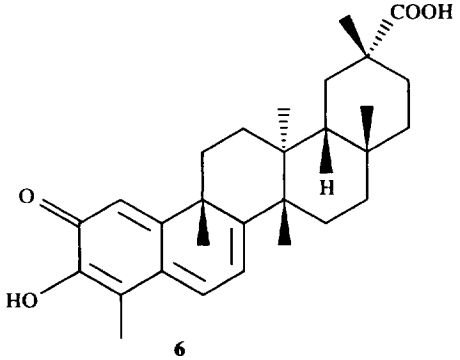
Wilforol B (**2**) was assigned the molecular formula $C_{29}H_{36}O_4$ (HR-FABMS). Analyses of the ^{1}H and ^{13}C NMR spectral data suggested the presence of an aromatized A-ring with hydroxyl groups at the C-2 and C-3 positions and a carboxyl group as in the other



	R^1	R^2	R^3
1	H	Me	H
1a	Ac	Me	Me
3	H	CHO	H
4	H	H	H
5	H	COOH	H



	R
2	H
7	Me



6

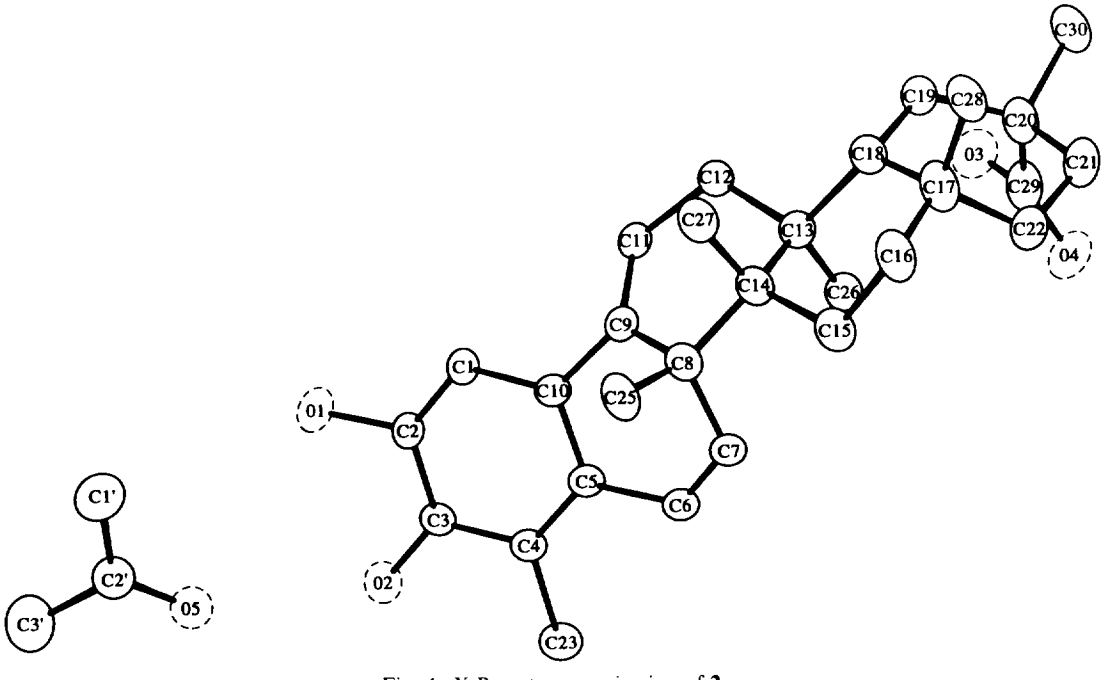


Fig. 1. X-Ray stereoscopic view of **2**.

Table 1. ^{13}C NMR spectral data of triterpenoids **1**–**7** (100 MHz, TMS as int. standard)

C	1*	1a†	2*	3*§	4*	5‡	6†	7†
1	108.4	117.5	108.5	116.6	110.5	112.4	120.7	108.8
2	141.8	139.6	142.9	150.7	144.1	152.6‖	178.3	143.5
3	149.1	145.2	141.9	150.0	148.6	143.5	147.1	142.0
4	124.8	133.9	119.6	117.0	111.3	120.4	120.7	120.3
5	121.0	127.8	122.8	122.3	121.9	121.8	127.5	124.5
6	186.0	186.9	120.9	186.3	183.1	185.8	135.6	122.4
7	125.3	126.0	137.3	125.2	123.4	124.8	118.3	138.6
8	170.2	171.9	43.3	174.4	173.7	177.4¶	172.9	44.2
9	**	40.5	144.1	40.5	39.4	41.7	43.1	143.8
10	149.3	155.0	128.5	150.4	150.9	151.7‖	165.0	130.3
11	33.6	33.9	119.4	33.7	33.1	35.0	33.8	119.8
12	29.3	29.8	32.6	29.6	29.1	30.8	29.5	33.4
13	38.5	39.0	39.4	39.4	38.9	40.7	39.3	40.9
14	44.0	44.9	40.1	45.1	44.5	46.4	45.3	41.1
15	28.0	28.6	23.3	28.7	28.3	30.0	28.7	24.2
16	36.0	36.4	36.7	36.4	36.0	37.6	36.3	37.4
17	30.0	30.5	31.1	30.5	30.0	31.6	30.7	31.9
18	43.6	44.3	46.3	44.3	43.7	45.7	44.2	47.4
19	30.0	30.9	30.7	30.8	30.1	31.9	31.1	31.8
20	39.3	40.4	38.3	40.1	39.1	41.3	39.9	39.3
21	29.3	29.9	29.2	29.8	29.3	30.9	29.3	30.1
22	34.5	34.8	37.2	34.8	34.5	36.0	34.5	38.0
23	13.5	14.5	11.1	200.2		173.1¶	10.5	11.1
25	37.4	37.3	22.4	36.3	36.3	37.4	38.4	22.6
26	20.7	20.3	19.1	20.5	20.3	21.3	21.4	19.6
27	18.0	18.4	18.7	18.7	18.0	19.4	18.7	19.1
28	31.3	31.6	32.7	31.6	31.3	32.1	31.5	33.0
29	179.3	178.7	179.7	181.2	179.4	182.5¶	182.5	180.2
30	32.2	32.8	30.4	32.6	32.2	33.2	32.4	30.8
		Me						OMe
		51.6						51.9
		Ac						
		20.7						
		20.8						
		167.9						
		168.1						

*DMSO-d_6.
†$CDCl_3$.
‡CD_3OD.
§Recorded at 125 MHz.
‖, ¶Assignments may be interchangeable in each column.
**Signal is hidden in a solvent.
††Data were taken from ref. [10].

compounds presented above. The UV data (λ_{max} 251, 255 and 308 nm) of **2** indicated the presence of a styrene-type chromophore as in isopristimerin III (**7**) and isotingenone III [10]. The ^{13}C NMR data of **2** showed good agreement with those of **7** (Table 1), except for the methyl ester signal (δ51.9) in the ^{13}C NMR spectrum of **7**. Thus **2** was presumed to have a C-29 carboxyl group. Finally, the structure of wilforol B was established as 25 (9 → 8)-*abeo*-D:A-friedo-1,2-dihydroxy-24-nor-1,3,5 (10), 6,8-oleanapentaen-29-oic acid (**2**) by X-ray crystallography. Itokawa *et al.* reported that they had isolated the same type triterpenes as **2**, isopristimerin III (**7**) and isotingenone III as natural products [10]. However, there still remains the possibility that wilforol B (**2**) may be an artefact arising from celastrol (**6**) as the result of an acid-catalysed rearrangement [6].

Demethylzeylasteral (**3**), 23-nor-oxopristimerol (**4**) and demethylzeylasterone (**5**) have been isolated from *Kokoona zeylanica* which belongs to the same family (Celastraceae) as *T. wilfordii*.

EXPERIMENTAL

General. Mps: uncorr.; ^1H NMR: 500 or 400 MHz, ^{13}C NMR: 125 or 100 MHz with TMS as int. standard;

2D-NMR; 500 MHz under standard conditions; EIMS: 70 eV; CC: Kieselgel 60 (70–230 mesh).

Plant material. Roots of *Tripterigium wilfordii* were collected in Jingling county and Tailing county (China) and were identified by W. Z. Qin and C. X. Yang.

Isolation procedure. The dried root bark of *T. wilfordii* (30 kg) was extracted (×2) with Me_2CO (150 l) under reflux. The Me_2CO extract was evapd *in vacuo* to give a brown mass (1.5 kg) which was chromatographed over cellulose (2 kg, eluting with *n*-hexane (20 l) then 25% Me_2CO–*n*-hexane (25 l). The 25% Me_2CO–*n*-hexane eluate (527 g) was loaded on an MCI CHP 20P (Mitsubishi Chemical Industries, 75–150 μm, 15 i.d. ×80 cm) column and eluted with 80% MeOH–H_2O (15 l, fr. M1, 185 g), 85% MeOH–H_2O (20 l, fr. M2, 140 g), then 100% MeOH (10 l, fr. M3, 67 g). Fr. M1 was dissolved in 1 l Et_2O and extracted (×3) with 300 ml of 1% HCl. The ethereal layer was washed (×2) with 300 ml satd NaCl soln, dried over $MgSO_4$, filtered and evapd. This fr. (136 g) was chromatographed on silica gel with increasing amounts of MeOH in $CHCl_3$ to give 3 frs (fr. L1: 8.5 g, fr. L2: 12.0 g, fr. L3: 6.6 g). Fr. L3 was subjected to Sephadex LH20 CC (MeOH) and the frs which showed a blue colouration with $FeCl_3$ were combined (2.6 g) and then purified by prep. HPLC (TSK ODS 80 T_M 21 i.d. ×300 mm; mobile phase, MeOH–MeCN–H_2O, 2:1:1) to give 2 compounds. Both were crystallized from Me_2CO to give **4** (1.4 g) and **1** (470 m g), respectively. Fr. M2 was crystallized from Me_2CO and afforded mixed crystals of 2 compounds (60 g). The mixture was repeatedly chromatographed on Sephadex LH-20 ($CHCl_3$–EtOH, 7:3) to give the individual compounds. Each compound was crystallized from Me_2CO to give **2** (22 g) and **3** (15 g). The mother liquor of these compounds was applied to Diaion HP-20 and eluted with H_2O (2 l), MeOH (2 l) then 2% HCl–MeOH (1.5 l). The 2% HCl–MeOH eluate was concd *in vacuo*, the residue dissolved in H_2O (500 ml) and extracted (×3) with EtOAc (250 ml). The EtOAc extract (10 g) was chromatographed on Sephadex LH-20 ($CHCl_3$–EtOH, 7:3) to give a compound which showed a blue colouration with $FeCl_3$. It was crystallized from Me_2CO to give **5** (1.6 g). Fr. M3 was applied to a silica gel column and eluted with 8% then 15% Me_2CO–*n*-hexane. The 15% Me_2CO–*n*-hexane eluate (55 g) was crystallized from EtOH to give **6** (15.5 g).

Wilforol A (**1**). Tan yellow needles (Me_2CO), mp 348° (decomp.). $[\alpha]_D^{27}$ −99° (pyridine; *c* 0.32); UV λ_{max}^{EtOH} nm (log ε): 253 (4.15), 304 (3.93); IR ν_{max}^{KBr} cm^{-1}: 3432, 2944, 1702, 1636; EIMS *m/z* (rel. int.): 466 $[M]^+$ (100), 451 (57), 257 (12), 218 (74); ^1H NMR (DMSO-d_6, 400 MHz): δ0.66 (3H, *s*, H-27), 1.08 (3H, *s*, H-28), 1.12 (3H, *s*, H-30), 1.26 (3H, *s*, H-26), 1.46 (3H, *s*, H-25), 2.47 (3H, *s*, H-23), 6.02 (1H, *s*, H-7), 6.84 (1H, *s*, H-1); ^{13}C NMR: Table 1.

Methyl-diacetyl wilforol A (**1a**). Amorphous powder. $[\alpha]_D^{24}$ −59° ($CHCl_3$; *c* 0.83); IR ν_{max}^{KBr} cm^{-1}: 2944, 1778, 1730, 1654; EIMS *m/z* (rel. int.): 564 $[M]^+$ 564 $[M]^+$ (83, calcd for $C_{34}H_{44}O_7$: 564.30870, found: 564.30839), 522 (79), 480 (199), 465 (46), 260 (60), 218 (74); ^1H NMR (DMSO-d_6 400 MHz): δ0.59 (3H, *s*, H-27), 1.10 (3H, *s*, H-28),1.17 (3H, *s*, H-30), 1.30 (3H, *s*, H-26), 1.58 (3H, *s*, H-25), 2.30 (3H, *s*, Ac), 2.34 (3H, *s* Ac), 2.55 (3H, *s*, H-23), 3.35 (3H, *s*, OMe), 6.29 (1H, *s*, H-7), 7.23 (1H, *s*, H-1); ^{13}C NMR: Table 1.

Wilforol B (**2**). Plates (Me_2CO), mp 194° (decomp.). $[\alpha]_D^{24}$ −46° ($CHCl_3$, *c* 0.68); UV λ_{max}^{EtOH} nm (log ε): 251 (4.53), 255 (4.53), 308 (3.70); IR ν_{max}^{KBr} cm^{-1}: 3452, 3308, 2940, 1710, 1688; EIMS *m/z* (rel. int.): 450 $[M]^+$ (58), 435 (31), 241 (100), 214 (99); ^1H NMR (DMSO-d_6, 400 MHz): δ0.90 (3H, *s*, H-26), 0.94 (6H, *s*, H-27), 0.95 (3H, *s*, H-25), 1.03 (3H, *s*, H-30), 1.13 (3H, *s*, H-28), 2.06 (3H, *s*, H-23), 5.41 (1H, *d*, *J* = 5.2 Hz), 6.22 (1H, *d*, *J* = 10.0 Hz), 6.32 (1H, *d*, *J* = 10.0 Hz), 6.59 (1H, *s*); ^{13}C NMR: Table 1.

X-Ray analysis of **2**. The crystal size of **2** was 0.3 × 0.25 × 0.25 mm. The unit cell dimension was obtained by least-squares refinement using 23 centred reflections for which $18° < 2\theta < 24°$ (graphite monochromatized CuKα, λ = 1.54184 Å). Intensity data were collected at $\omega/2\theta$ scans on an Enraf-Nonius CAD-4 with three check reflection at intervals of 100 reflections. Other crystal data were: $C_{29}H_{38}O_4 \cdot C_3H_6O$, monoclinic, space group $P2_1$, *Z* = 2, *a* = 11.747 (3) Å, *b* = 7.853 (2) Å, *c* = 16.164 (3) Å, β = 100.8481° (3), *V* = 1465 (1) $Å^3$, D_{calc} = 1.154 g cm^{-3} and (CuKα) with 5.0 cm^{-1}. Intensities were measured for 2644 reflections in the range $2° \leqq 2\theta \leqq 130°$ with 2610 considered as observed by the criteria $1 > 3\sigma$ (I). The data were corrected for Lorentz and polarization effects. No absorption correction was applied. The structure was solved by the direct-method program Multan and was refined by full-matrix least-squares, using the Enraf-Nonius SDP programs. All the non-hydrogen atoms were refined anisotropically. Hydrogen atoms were located from difference maps. The last difference Fourier map was essentially featureless with no peaks greater than 0.14[e] $Å^{-3}$. The final discrepancy index was *R* = 0.036.

Acknowledgements—The authors are grateful to Dr K. Sugama, Mrs Y. Tanaka, Miss Y. Imamura and Miss N. Kanda for 1D- and 2D-NMR spectra, Mr K. Kano for MS, and the people in Zhong Shan Hospital for helping to make the plant extract. We are also indebted to Dr T. Tamaki, Hachiouji Medical Center, Tokyo Medical University, and Mr M. Ando, Mr T. Yoshino, Dr H. Kawamura, Dr H. Maruyama and other members in Tsumura & Co. for supporting the present work.

REFERENCES

1. Jia, L. (1985) *Yaoxue Tongbao* **20**, 101.
2. Zhang, L. Zhang, Z. X. and An, D. K. (1990) *J. China Pharmaceut. Univ.* **21**, 251.
3. Kupchan, S. M., Court, W. A., Daiely, R. G., Gilmore, C. J. and Bryan, R. F. (1972) *J. Am. Chem. Soc.* **94**, 7194.
4. Kutney, J. P., Hewitt, G. M., Lee, G., Piotrowska, K., Roberts, M. and Rettig, S. J. (1992) *Can. J. Chem.* **70**, 1455.

5. Gamlath, C. B., Gunaherath, G. M. K. B. and Gunatilaka, A. A. L. (1987) *J. Chem. Soc. Perkin Trans. I* 2849.
6. Gamlath, C. B., Gunaherath, G. M. K. B. and Gunatilaka, A. A. L. (1986) *New Trends Nat. Prod. Chem.* 109.
7. Gamlath, C. B. and Gunatilaka, A. A. L. (1988) *Phytochemistry* **27**, 3221.
8. Gunaherath, G. M. K. B. and Gunatilaka, A. A. L. (1983) *J. Chem. Soc. Perkin Trans. I* 2845.
9. Kutney, J. P., Hewitt, G. M., Kurihara, T., Sarisbury, P. J., Sindelar, R. D. and Stuart, K. L. (1981) *Can. J. Chem.* **59**, 2677.
10. Itokawa, H., Shirota, O., Ikuta, H., Morita, H., Takeya, K. and Iitake, Y. (1991) *Phytochemistry* **30**, 3713.