



SESQUITERPENE ALKALOIDS FROM *TRIPTERIGIUM WILFORDII**

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Key Word Index—*Tripterigium wilfordii*; Celastraceae; root bark; sesquiterpene alkaloids; triptonines A and B.

Abstract—An acetone extract of the dried root bark of *Tripterigium wilfordii* afforded four sesquiterpene alkaloids, including two new compounds, named triptonines A and B. Their structures were established on the basis of chemical and spectroscopic studies.

INTRODUCTION

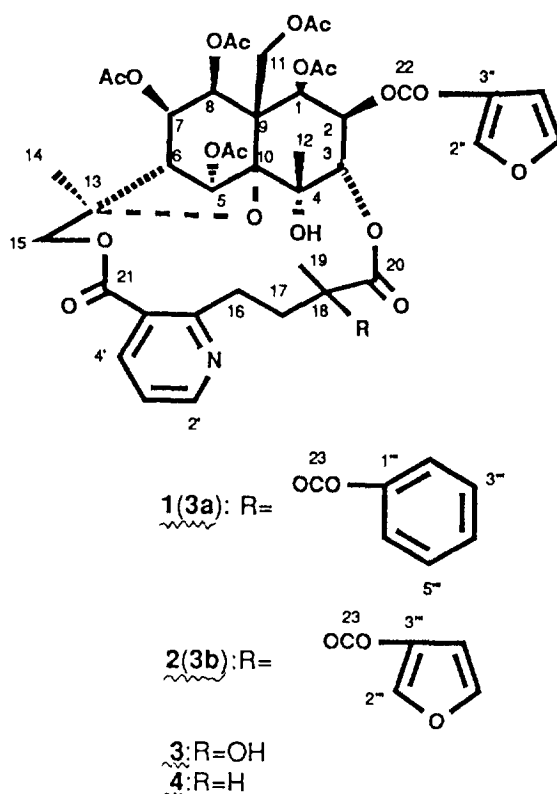
In the previous papers, we reported the isolation of D:A-friedo-24-noroleanane triterpenoids [1], oleanane and D:B-friedooleanane triterpenes [2] from root bark of *Tripterigium wilfordii* HOOK f. In continuing studies on the chemical components of this species, we have isolated two novel sesquiterpene alkaloids, along with two known sesquiterpene alkaloids, wilfortrine (3) and wilforgine (4), from an acetone extract. Compounds 3 and 4 were identified by comparisons of their spectral data with those in the literature [3–6].

A number of sesquiterpene alkaloids have already been isolated from *T. wilfordii* and related species [3–14]. In 1989, Zheng *et al.* reported that wilfortrine and euonine showed immuno-suppressive effects on humoral immunity using the haemolysin reaction [15].

In this paper, we describe the structural elucidation of two novel sesquiterpene alkaloids.

RESULTS AND DISCUSSION

Triptonine A (1) was shown to have the molecular formula $C_{18}H_{51}NO_{21}$ from its HR-FAB-mass spectrum. It showed a positive response to Dragendorff's reagent. In the 1H NMR spectrum of 1, there were five acetoxyl groups (δ 1.69, 1.90, 2.12, 2.16, 2.25, each 3H, s), a disubstituted pyridine ring [δ 7.34 (1H, dd, J = 7.9, 4.8 Hz), 8.11 (1H, dd, J = 7.9, 1.8 Hz), 8.80 (1H, dd, J = 4.8, 1.8 Hz), a 3-furanoyl group [δ 6.90 (1H, dd, J = 2.6, 0.8 Hz), 7.35 (1H, dd, J = 2.6, 1.5 Hz), 8.30 (1H, dd, J = 1.5, 0.8 Hz)] and a highly oxygenated aliphatic ring (Table 1). By comparison of the 1H NMR spectrum



of 1 with that of 3, 1 was assumed to be a compound having one benzoyl group [δ 7.34 (1H, dt), 7.50 (2H, m), 7.73 (2H, m)], in addition to the 3-furanoyl group attached to the C-2 hydroxyl group in 3 (Table 1). Benzoylation of 3 with benzoyl chloride and a catalytic amount of 4,4-dimethylaminopyridine afforded the monobenzoate (3a) of 3, which was identical to 1. Thus, there were two possible positions in 3 which could be

*Part 3 in the series 'Chemical studies on the root bark of *Tripterigium wilfordii*'. For part 2 see ref. [2].

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Table 1. ^1H NMR spectral data of alkaloids from *T. wilfordii* (500 MHz in CDCl_3)*

H	1	2	3
1	5.62 <i>d</i> (4.0)	5.64 <i>d</i> (4.1)	5.70 <i>d</i> (3.5)
2	5.45 <i>dd</i> (4.0, 2.3)	5.43 <i>dd</i> (4.1, 2.2)	5.35 <i>dd</i> (3.5, 2.9)
3	5.05 <i>d</i> (2.3)	5.02 <i>d</i> (2.2)	5.03 <i>d</i> (2.9)
5	6.90 <i>s</i>	6.93 <i>s</i>	6.92 <i>d</i> (0.8)
6	2.27 <i>d</i> (4.1)	2.36 <i>d</i> (4.0)	2.37 <i>dd</i> (3.9, 0.8)
7	5.51 <i>dd</i> (6.0, 4.1)	5.56 <i>dd</i> (6.0, 4.0)	5.54 <i>dd</i> (5.9, 3.9)
8	5.21 <i>d</i> (6.0)	5.26 <i>d</i> (6.0)	5.40 <i>d</i> (5.9)
11	4.28 <i>d</i> (13.5)	4.29 <i>d</i> (13.4)	4.31 <i>d</i> (13.4)
	5.46 <i>d</i> (13.5)	5.47 <i>d</i> (13.4)	5.56 <i>d</i> (13.4)
12	1.59 <i>d</i> (1.2)	1.59 <i>d</i> (1.2)	1.62 <i>d</i> (1.3)
14	1.17 <i>s</i>	1.37 <i>s</i>	1.48 <i>s</i>
15	3.75 <i>d</i> (11.9)	3.83 <i>d</i> (11.9)	3.74 <i>d</i> (12.0)
	5.59 <i>dd</i> (11.9, 1.0)	5.47 <i>d</i> (11.9)	5.83 <i>d</i> (12.0)
16	3.02 <i>m</i>	2.87 <i>ddd</i> (14.5, 8.8, 6.8)	2.86 <i>ddd</i> (14.2, 7.1, 5.0)
	3.76 <i>m</i>	3.76 <i>ddd</i> (14.5, 11.3, 6.8)	4.05 <i>ddd</i> (14.2, 6.9, 4.9)
17	2.36 <i>m</i>	2.27 <i>m</i>	2.22 <i>m</i>
	2.97 <i>m</i>	2.99 <i>ddd</i> (13.5, 8.8, 6.8)	2.48 <i>m</i>
19	1.82 <i>s</i>	1.82 <i>s</i>	1.64 <i>s</i>
2'	8.80 <i>dd</i> (4.8, 1.8)	8.77 <i>dd</i> (4.8, 1.8)	8.69 <i>dd</i> (4.8, 1.8)
3'	7.34 <i>dd</i> (7.9, 4.8)	7.34 <i>dd</i> (7.9, 4.8)	7.20 <i>dd</i> (7.9, 4.8)
4'	8.11 <i>dd</i> (7.9, 1.8)	8.16 <i>dd</i> (7.9, 1.8)	8.23 <i>dd</i> (7.9, 1.8)
2''	8.30 <i>dd</i> (1.5, 0.8)	8.31 <i>d</i> (0.9)	8.26 <i>dd</i> (1.5, 0.8)
4''	6.90 <i>dd</i> (2.6, 0.8)	6.89 <i>d</i> (1.8)	6.84 <i>dd</i> (1.9, 0.8)
5''	7.35 <i>dd</i> (2.6, 1.5)	7.49 <i>dd</i> (1.8, 1.3)	7.49 <i>dd</i> (1.9, 1.5)
2'''	7.73, 2H, <i>m</i>	7.76 <i>d</i> (1.3)	—
6'''	—	—	—
4'''	7.34 <i>dt</i> (8.0, 1.5)	6.56 <i>d</i> (1.8)	—
3'''	7.50, 2H, <i>m</i>	—	—
5'''	—	7.34 <i>dd</i> (1.8, 0.9)	—
1-Ac	1.69 <i>s</i>	1.77 <i>s</i>	1.87 <i>s</i>
5-Ac	2.16 <i>s</i>	2.17 <i>s</i>	2.194 <i>s</i>
7-Ac	2.12 <i>s</i>	2.13 <i>s</i>	2.187 <i>s</i>
8-Ac	1.90 <i>s</i>	1.91 <i>s</i>	1.97 <i>s</i>
11-Ac	2.25 <i>s</i>	2.27 <i>s</i>	2.25 <i>s</i>

*Coupling constants (Hz) given in parentheses.

Assignments are based on ^1H - ^1H , ^{13}C - ^1H COSY (1-3) and HMBC (1) data.

esterified with the hydroxyl groups at the C-4 and C-18 positions. In the ^{13}C NMR spectrum of **1**, two carbon signals showed a significant difference of chemical shift (δ 81.7: + 3.9 and δ 23.6: - 4.5) compared with that of **3** (Table 2). This must be caused by the acylation effect. These signals were assigned to C-18 and C-19, respectively, from HMBC data (Fig. 1). Thus, the location of the benzoyl group was shown to be at the C-18 position. From the available evidence, the structure of triptonine A was formulated as 18-*O*-benzoylwilfortrine (1).

Triptonine B (**2**) analysed for $\text{C}_{46}\text{H}_{49}\text{NO}_{22}$ from its HR-FAB-mass spectrum. It was also presumed to be a sesquiterpene alkaloid having the same skeleton as **1** and **3** based on the resemblance of its ^1H and ^{13}C NMR spectra. In the low-field region of the ^1H NMR spectrum of **2**, resonances due to a 3-furoyl group [δ 6.56 (1H, *d*, J = 1.8 Hz), 7.34 (1H, *dd*, J = 1.8, 0.9 Hz), 7.76 (1H, *d*, J = 0.9 Hz)] were present, in addition to the signals ascribable to the 3-furoyl group attached to the C-2 position (Table 1). The $[\text{M}]^+$ in the FAB-mass spectrum (m/z 967) and the above data suggested that **2** had an extra 3-furoyl group compared with **3**. Esterification of **3** with 3-furoyl chloride, prepared from 3-furoic acid, yielded **3b** which was identical to **2**. On the other hand, the ^{13}C NMR spectrum of **2** showed the same substitution effect that was observed in that of **2**, i.e. the signals assignable to the C-18 position showed a lower shift (+ 3.6 ppm) and that of the C-19 position showed a higher shift (- 4.6 ppm), compared with those of **3** (Table 2). This indicated that **2** had a 3-furoyl group at C-18. From the evidence described, the structure of triptonine B was established as 18-*O*-(3-furoyl) wilfortrine (**2**).

Although many sesquiterpene alkaloids have already been reported, this is the first report of the isolation of 18-*O*-esterified ones as naturally occurring compounds.

EXPERIMENTAL

General. Mps: uncorr. ^1H NMR: 500 MHz, ^{13}C NMR: 125 MHz with TMS as int. standard. EIMS: 70 eV. Silica gel CC was carried out Kieselgel 60 (70-230 mesh). Prep. HPLC: CIG Si-10 (silica gel, 1.5 i.d. \times 30 cm).

Plant material. See ref. [1].

Isolation procedure. The 1% HCl layer of MI. [1] was made alkaline with NH_4OH and extracted (\times 3) with CHCl_3 . Work-up gave an alkaloid fr. (45 g). This fr. (25 g) was divided into three (frs A-1-3) by silica gel CC using 15%, then 20% Me_2CO in benzene as eluants. Fr. A-2 (13 g) contained 2 compounds which were sepd by prep. LC (15% Me_2CO in benzene). The compounds were recrystallized from EtOH to give **3** (2.8 g) and **4** (9.1 g). Fr. A-1 (1.3 g) was repeatedly chromatographed by prep. LC (15% MeCN in CHCl_3 and 15% MeO_2CO in *n*-hexane) and finally purified by reversed phase prep. LC (TOSO-ODS 80T_M, 21.5 i.d. \times 30 cm; mobile phase, 30% H_2O in MeOH) to give 2 compounds. The compounds were recrystallized from EtOH to give **1** (63 mg) and **2** (128 mg).

Triptonine A (1). Needles (EtOH), mp 163-164°. $[\alpha]_{\text{D}}^{27}$ - 36° (Me_2CO : *c* 0.21). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3464, 1744, 1574,

Table 2. ^{13}C NMR spectral data of alkaloids from *T. wilfordii* (125 MHz in CDCl_3)*

C	1	2	3
1	72.1 <i>d</i>	72.0 <i>d</i>	73.1 <i>d</i>
2	69.4 <i>d</i>	69.3 <i>d</i>	68.9 <i>d</i>
3	78.3 <i>d</i>	78.3 <i>d</i>	76.8 <i>d</i>
4	69.6 <i>s</i>	69.6 <i>s</i>	69.8 <i>s</i>
5	73.9 <i>d</i>	73.8 <i>d</i>	73.6 <i>d</i>
6	51.0 <i>d</i>	51.0 <i>d</i>	51.2 <i>d</i>
7	68.5 <i>d</i>	68.4 <i>d</i>	68.7 <i>d</i>
8	72.0 <i>d</i>	71.8 <i>d</i>	70.7 <i>d</i>
9	52.3 <i>s</i>	52.3 <i>s</i>	52.0 <i>s</i>
10	93.5 <i>s</i>	93.5 <i>s</i>	94.1 <i>s</i>
11	60.7 <i>t</i>	60.6 <i>t</i>	60.5 <i>t</i>
12	23.2 <i>q</i>	23.0 <i>q</i>	22.7 <i>q</i>
13	84.3 <i>s</i>	84.2 <i>s</i>	84.8 <i>s</i>
14	17.5 <i>q</i>	17.5 <i>q</i>	17.9 <i>q</i>
15	69.8 <i>t</i>	69.9 <i>t</i>	69.8 <i>t</i>
16	30.8 <i>t</i>	30.5 <i>t</i>	31.4 <i>t</i>
17	37.9 <i>t</i>	37.7 <i>t</i>	38.4 <i>t</i>
18	81.7 <i>s</i>	81.4 <i>s</i>	77.8 <i>s</i>
19	23.6 <i>q</i>	23.5 <i>q</i>	28.1 <i>q</i>
20	171.8 <i>s</i>	171.6 <i>s</i>	172.5 <i>s</i>
21	167.9 <i>s</i>	167.7 <i>s</i>	168.0 <i>s</i>
22	160.6 <i>s</i>	160.6 <i>s</i>	161.0 <i>s</i>
23	161.7 <i>s</i>	161.7 <i>s</i>	—
2'	152.4 <i>d</i>	152.1 <i>d</i>	152.3 <i>d</i>
3'	121.0 <i>d</i>	121.2 <i>d</i>	120.6 <i>d</i>
4'	138.4 <i>d</i>	138.5 <i>d</i>	137.8 <i>d</i>
5'	125.9 <i>s</i>	125.7 <i>s</i>	125.6 <i>s</i>
6'	165.7 <i>s</i>	162.1 <i>s</i>	164.9 <i>s</i>
2''	148.7 <i>d</i>	148.6 <i>d</i>	148.7 <i>d</i>
3''	118.7 <i>s</i>	118.6 <i>s</i>	118.2 <i>s</i>
4''	144.2 <i>d</i>	144.1 <i>d</i>	144.3 <i>d</i>
5''	110.0 <i>d</i>	109.9 <i>d</i>	109.7 <i>d</i>
1'''	129.9 <i>s</i>	—	—
2'''	130.4 <i>d</i>	149.0 <i>d</i>	—
3'''	128.1 <i>d</i>	118.9 <i>s</i>	—
4'''	130.4 <i>d</i>	143.2 <i>d</i>	—
5'''	128.1 <i>d</i>	110.3 <i>d</i>	—
6'''	133.1 <i>d</i>	—	—
1-Ac	20.3 <i>q</i> , 169.8 <i>s</i>	20.3 <i>q</i> , 169.8 <i>s</i>	20.4 <i>q</i> , 169 <i>s</i>
5-Ac	21.6 <i>q</i> , 169.9 <i>s</i>	21.6 <i>q</i> , 169.9 <i>s</i>	21.6 <i>q</i> , 169.8 <i>s</i>
7-Ac	21.1 <i>q</i> , 170.7 <i>s</i>	21.0 <i>q</i> , 170.7 <i>s</i>	21.0 <i>q</i> , 170.6 <i>s</i>
8-Ac	20.4 <i>q</i> , 168.6 <i>s</i>	20.4 <i>q</i> , 168.6 <i>s</i>	20.5 <i>q</i> , 169.0 <i>s</i>
11-Ac	21.3 <i>q</i> , 167.9 <i>s</i>	21.2 <i>q</i> , 168.1 <i>s</i>	21.1 <i>q</i> , 169.6 <i>s</i>

*Assignments based on ^1H – ^1H , ^{13}C – ^1H COSY (1–3) and HMBC (1) data.

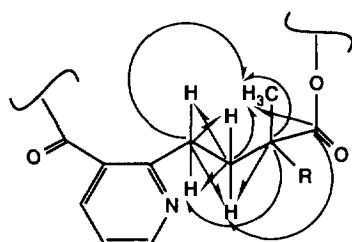


Fig. 1. HMBC data around C-18 of 1.

1510. HR-FABMS m/z : 978 ($[\text{M} + \text{H}]^+$, calcd for $\text{C}_{18}\text{H}_{52}\text{NO}_{21}$: 978.30318. Found: 978.30660). EIMS m/z (rel. int.): 977 ($[\text{M}]^+$ (5), 856 (14), 811 (6), 95 (100). ^1H and ^{13}C NMR: Tables 1 and 2.

Triptonine B (2). Needles (EtOH), mp 160–161°. $[\alpha]_D^{30}$ –13° (Me₂CO: c 0.20). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3492, 1744, 1574, 1510. HR-FABMS m/z : 968 ($[\text{M} + \text{H}]^+$, calcd for $\text{C}_{46}\text{H}_{50}\text{NO}_{22}$: 968.28245. Found: 968.28460). EIMS m/z (rel. int.): 967 ($[\text{M}]^+$ (10), 856 (19), 811 (7), 85 (100). ^1H and ^{13}C NMR: Tables 1 and 2.

Benzoylation of 3. Compound **3** (60 mg) was dissolved in 3 ml of pyridine and a catalytic amount of 4,4-dimethylaminopyridine and 1 ml of benzoyl chloride added to the soln. The reaction mixt. was stirred for 48 hr at room temp. under N₂. Work-up gave 65 mg of residue which was purified by prep. LC (20% Me₂CO in benzene) to afford 58 mg of pure compound. This was recrystallized from EtOH to give needles of **3a** (44 mg). All spectral and physicochemical data were identical to those of **1**.

Preparation of 3-furoyl chloride. A mixt. of 3-furoic acid (3 g) and SOCl₂ (5 ml) in dry benzene (20 ml) was stirred for 2 hr under reflux. Excess SOCl₂ was repeatedly removed by distillation with benzene at 100°. The reaction mixt. was then concd to 5 ml under red. pres.

Esterification of 3 with 3-furoyl chloride. Compound **3** (35 mg) was dissolved in 5 ml of pyridine and a catalytic amount of 4,4-dimethylaminopyridine and 1 ml of the reaction mixt. prep'd above were added to the soln. The reaction mixt. was stirred for 18 hr at room temp. under N₂. Usual work-up gave 43 mg of residue which was purified by prep. LC (25% Me₂CO in benzene) to afford 18 mg of pure compound. This was recrystallized from EtOH to give needles of **3b** (12 mg). All spectral and physicochemical data of **3b** were identical to those of **2**.

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