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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

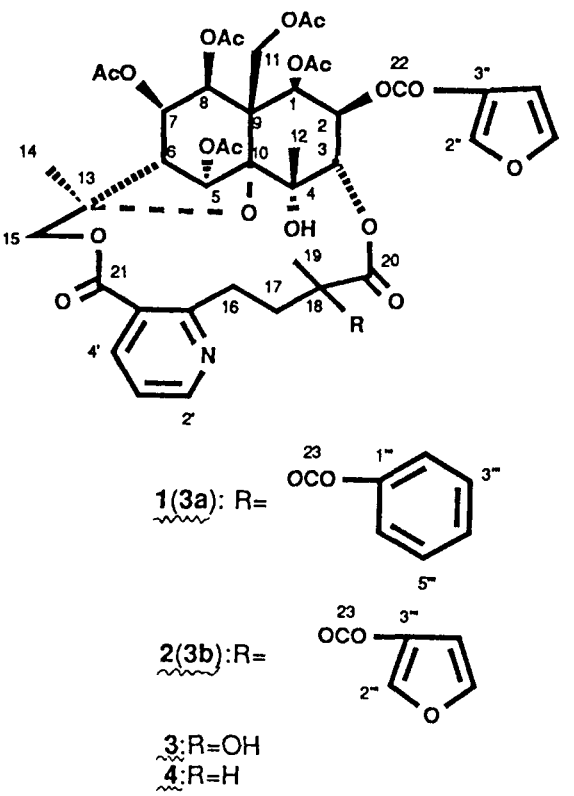
In the previous papers, we reported the isolation of D:A-friedo-24-noroleanane triterpenoids [1], oleanane and D:B-friedooleanane triterpenes [2] from root bark of *Tripterigium wilfordii* HOOK f. In continuing studies on the chemical components of this species, we have isolated two novel sesquiterpene 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 ^{1}H NMR spectrum of **1**, there were five acetoxyl groups (δ1.69, 1.90, 2.12, 2.16, 2.25, each 3H, *s*), a disubstituted pyridine ring [δ7.34 (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 [δ6.90 (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 ^{1}H 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. ^{1}H NMR spectral data of alkaloids from *T. wilfordii* (500 MHz in $CDCl_3$)*

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

*Coupling constants (Hz) given in parentheses.

Assignments are based on ^{1}H–^{1}H, ^{13}C–^{1}H 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 (δ81.7: + 3.9 and δ23.6: − 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 $C_{46}H_{49}NO_{22}$ 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 ^{1}H and ^{13}C NMR spectra. In the low-field region of the ^{1}H NMR spectrum of **2**, resonances due to a 3-furoyl group [δ6.56 (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 $[M]^+$ 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. ^{1}H 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. × 30 cm).

Plant material. See ref. [1].

Isolation procedure. The 1% HCl layer of MI. [1] was made alkaline with NH_4OH and extracted (× 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. × 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°. $[\alpha]_D^{27}$ − 36° (Me_2CO: *c* 0.21). IR ν_{max}^{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 *d*	72.0 *d*	73.1 *d*
2	69.4 *d*	69.3 *d*	68.9 *d*
3	78.3 *d*	78.3 *d*	76.8 *d*
4	69.6 *s*	69.6 *s*	69.8 *s*
5	73.9 *d*	73.8 *d*	73.6 *d*
6	51.0 *d*	51.0 *d*	51.2 *d*
7	68.5 *d*	68.4 *d*	68.7 *d*
8	72.0 *d*	71.8 *d*	70.7 *d*
9	52.3 *s*	52.3 *s*	52.0 *s*
10	93.5 *s*	93.5 *s*	94.1 *s*
11	60.7 *t*	60.6 *t*	60.5 *t*
12	23.2 *q*	23.0 *q*	22.7 *q*
13	84.3 *s*	84.2 *s*	84.8 *s*
14	17.5 *q*	17.5 *q*	17.9 *q*
15	69.8 *t*	69.9 *t*	69.8 *t*
16	30.8 *t*	30.5 *t*	31.4 *t*
17	37.9 *t*	37.7 *t*	38.4 *t*
18	81.7 *s*	81.4 *s*	77.8 *s*
19	23.6 *q*	23.5 *q*	28.1 *q*
20	171.8 *s*	171.6 *s*	172.5 *s*
21	167.9 *s*	167.7 *s*	168.0 *s*
22	160.6 *s*	160.6 *s*	161.0 *s*
23	161.7 *s*	161.7 *s*	—
2′	152.4 *d*	152.1 *d*	152.3 *d*
3′	121.0 *d*	121.2 *d*	120.6 *d*
4′	138.4 *d*	138.5 *d*	137.8 *d*
5′	125.9 *s*	125.7 *s*	125.6 *s*
6′	165.7 *s*	162.1 *s*	164.9 *s*
2″	148.7 *d*	148.6 *d*	148.7 *d*
3″	118.7 *s*	118.6 *s*	118.2 *s*
4″	144.2 *d*	144.1 *d*	144.3 *d*
5″	110.0 *d*	109.9 *d*	109.7 *d*
1‴	129.9 *s*	—	—
2‴	130.4 *d*	149.0 *d*	—
3‴	128.1 *d*	118.9 *s*	—
4‴	130.4 *d*	143.2 *d*	—
5‴	128.1 *d*	110.3 *d*	—
6‴	133.1 *d*	—	—
1-Ac	20.3 *q*, 169.8 *s*	20.3 *q*, 169.8 *s*	20.4 *q*, 169 *s*
5-Ac	21.6 *q*, 169.9 *s*	21.6 *q*, 169.9 *s*	21.6 *q*, 169.8 *s*
7-Ac	21.1 *q*, 170.7 *s*	21.0 *q*, 170.7 *s*	21.0 *q*, 170.6 *s*
8-Ac	20.4 *q*, 168.6 *s*	20.4 *q*, 168.6 *s*	20.5 *q*, 169.0 *s*
11-Ac	21.3 *q*, 167.9 *s*	21.2 *q*, 168.1 *s*	21.1 *q*, 169.6 *s*

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

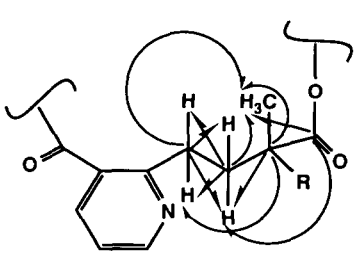

Fig. 1. HMBC data around C-18 of **1**.

1510. HR-FABMS *m/z*: 978 ($[M + H]^+$, calcd for $C_{18}H_{52}NO_{21}$: 978.30318. Found: 978.30660). EIMS *m/z* (rel. int.): 977 $[M]^+$ (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° (Me_2CO: *c* 0.20). IR ν_{max}^{KBr} cm^{-1}: 3492, 1744, 1574, 1510. HR-FABMS *m/z*: 968 ($[M + H]^+$, calcd for $C_{46}H_{50}NO_{22}$: 968.28245. Found: 968.28460). EIMS *m/z* (rel. int.): 967 $[M]^+$ (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 N_2. Work-up gave 65 mg of residue which was purified by prep. LC (20% Me_2CO 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 $SOCl_2$ (5 ml) in dry benzene (20 ml) was stirred for 2 hr under reflux. Excess $SOCl_2$ 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. prepd above were added to the soln. The reaction mixt. was stirred for 18 hr at room temp. under N_2. Usual work-up gave 43 mg of residue which was purified by prep. LC (25% Me_2CO 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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