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Full Paper

New Diterpenoid Alkaloids from the Roots of *Delphinium tiantaishanense*

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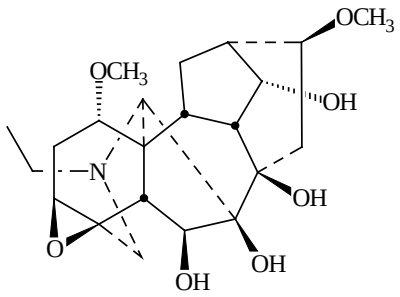
Abstract: Four new diterpenoid alkaloids: tiantaishansine (**1**), tiantaishannine (**2**), tiantaishanmine (**3**), and tiantaishandine (**4**) have been isolated from the roots of *Delphinium tiantaishan*. Their structures were elucidated by chemical evidence and spectral analyses, including ESI-MS, HR-EI-MS, 1D- and 2D-NMR.

Keywords: *Dephinium tiantaishanense*; tiantaishansine; tiantaishannine; tiantaishanmine; tiantaishandine

Introduction

The plant *Dephinium tiantaishanense* W. J. Zhang et G. H. Chen is a new species plant of *Dephinium L.*, mainly distributed around Tiantai Mountain in Pengzhou County, Sichuan Province, China [1]. To our knowledge, no phytochemical investigation of this plant has been undertaken. In the course of our comparative studies of diterpenoid alkaloids from *Aconitum* and *Delphinium* species, four new diterpenoid alkaloids: tiantaishansine (**1**), tiantaishannine (**2**), tiantaishanmine (**3**), and tiantaishandine (**4**), were isolated from the roots of *D. tiantaishanense*. This paper describes the isolation and structure elucidation of these new alkaloids.



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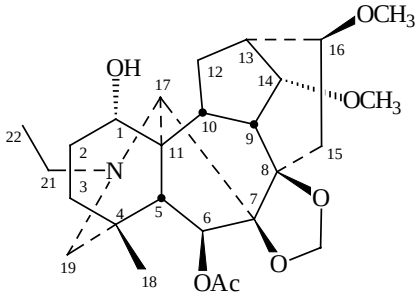

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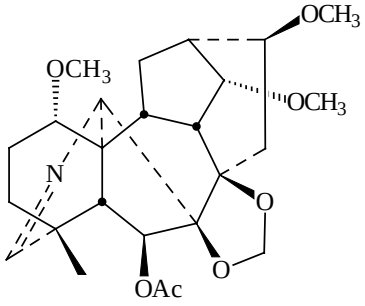

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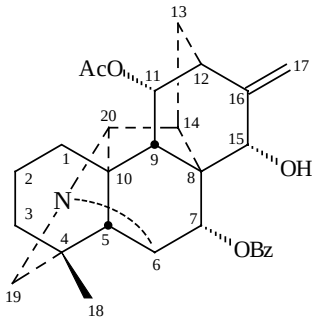

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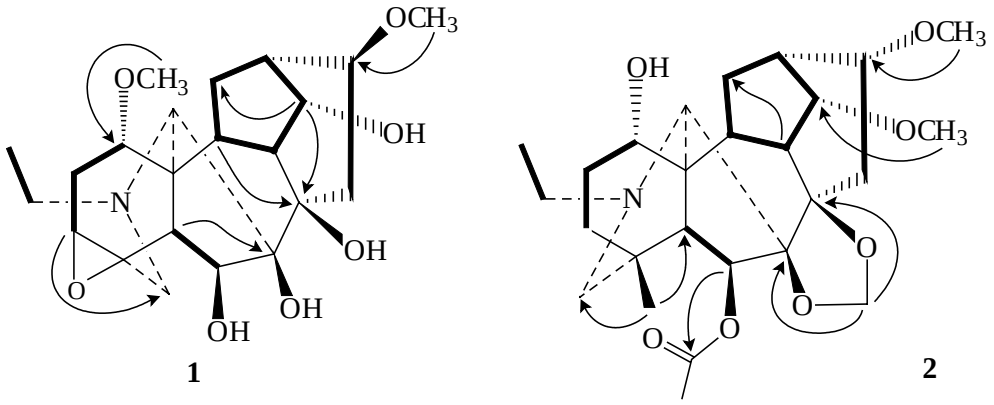
Results and Discussion

Tiantaishansine (**1**) was obtained as an amorphous powder. Its molecular formula $C_{22}H_{33}NO_7$ was inferred from its HR-ESI-MS and ^{13}C-NMR. Its NMR spectra displayed an *N*-ethyl group (δ_H 1.07, 3H, t, J = 7.2 Hz; δ_C 49.9 t, 13.9 q) and two methoxyl groups (δ_H 3.51, 3.41, s, each 3H; δ_C see Table 1). These two methoxyl groups could be assigned at C-1 and C-16, respectively, based on the long-range correlations between 1- OCH_3 (δ_H 3.51)/C-1 (δ_C 77.7) and 16- OCH_3 (δ_H 3.41)/C-16 (δ_C 82.3) in HMBC spectrum (Figure 1). The ^{13}C-NMR spectrum showed the 3,4-epoxy group based on the downfield signal at C-3 (δ_C 57.8, d) and C-4 (δ_C 58.3 s) [2]. The one-proton triplet (J = 4.4 Hz) at δ_H 4.03 in the ^{1}H-NMR spectrum of **1** was assigned to H-14β based on the multiplicity and the coupling constant, resulting in location of the hydroxy group at C-14 [2]. The IR (3425 cm^{-1}) and ^{13}C-NMR (δ_C 91.3 s, 84.4 s, 79.8 d, 74.3 d) spectra showed that there were two tertiary hydroxyl groups and two secondary hydroxyl groups. Except for the secondary hydroxyl group at C-14, the other two tertiary hydroxyl groups could be attributed to C-7 and C-8 based on the HMBC correlations between 5-H/C-7 and 9-H/C-8, respectively. The remaining hydroxyl group could be located at C-6 due to the chemical shift of H-6 (δ_H 4.82 s) and the long-range correlations between H-6/C-5, C-7 in the HMBC spectrum (Figure 1). Thus, on the basis of these observations, the structure of tiantaishansine was established as **1**, which corresponds to a C_{18}-diterpenoid alkaloid.

Tiantaishannine (**2**) was isolated as an amorphous powder, mp 206-208 °C. Its pseudo-molecular formula, $C_{26}H_{40}NO_7$, was derived from HR-ESI-MS (*m/z* 478.2801 $[M+H]^+$, calcd. 478.2799) and ^{13}C-NMR. The NMR data of compound **2** gave distinctive signals at δ_H 0.99 (3H, s), δ_C 26.7 q, for an angular methyl group, δ_H 1.11 (3H, t, J = 7.2 Hz), δ_C 49.6 t, and 13.3 q for the *N*-ethyl group, δ_H 3.36, 3.43 (each 3H, s) for two methoxyl groups, δ_H 2.07 (3H, s), δ_C 21.5 q and 169.7 s for an acetyl group. The signals at δ_H 4.92 and 4.96 (each 1H, s) in ^{1}H- NMR along with signals at δ_C 84.4 s, 90.9 s, and 93.9 t in ^{13}C-NMR showed the presence of 7,8-methylenedioxy group, indicating a lycoctonine-type

C_{19}-diterpenoid alkaloid [2,3]. Two methoxyl groups could be located at C-14 and C-16 due to the long-range correlations between 14-OCH_3 (δ_H 3.43 s) and C-14 (δ_C 83.5 d), 16-OCH_3 (δ_H 3.36, s) and C-16 (δ_C 82.2 d) in the HMBC spectrum of **2** (Figure 1), as well as the acetyl group could be located at C-6 based on the HMBC correlations between H-6 (δ_H 5.44, s)/6-O*C*OCH_3 (δ_C 169.7). The broadened singlet (δ_H 3.81), which could be assigned to H-1*β*, in combination with the C-1 chemical shift at δ_C 71.6 d, suggested a hydroxyl substitution at C-1 [2]. Therefore, the structure of taintaishannine was determined as **2**.

Figure 1 key ^{1}H-^{1}H COSY (▬) and HMBC (↷) correlations of **1** and **2**



The molecular formula ($C_{25}H_{35}NO_7$) of compound **3** was determined by HR-ESI-MS (*m/z* 462.2473, $[M+H]^+$). The NMR spectra strongly suggested that it was a lycoctonine-type diterpenoid alkaloid [2,3], with an angular methyl group (δ_H 1.17, 3H, s; δ_C 22.1 q), three methoxyl groups (δ_H 3.22, 3.34, 3.45, each 3H, s; δ_C see Table 2), an acetyl group (δ_H 2.06, 3H, s; δ_C 21.5 q, 169.5 s), and a methylenedioxy group (δ_H 4.93, 4.97, each 1H, s; δ_C 93.9 t). The significant absence of *N*-methyl, *N*-ethyl showed that there are no substitutions on the nitrogen atom of **3** besides the imine moiety (δ_H 7.46 br s; δ_C 169.9 d). The downfield signal at δ_C 44.4 s can be assigned to C-4 adjacent to a double bond (N=C-19) [4]. Three methoxyl groups could be located at C-1, C-14, and C-16 based on the HMBC correlations of 1- OCH_3 (δ_H 3.22) /C-1 (δ_C 80.4), 14-OCH_3 (δ_H 3.45)/C-14 (δ_C 83.4), 16- OCH_3 (δ_H 3.34)/C-16 (δ_C 81.3) (Figure 2). The one-proton singlet at δ_H 5.25 s was assigned to H-6*α* suggesting an acetyl substitution at C-6 due to the HMBC correlation of H-6/*C*OCH_3. All these evidence supported the structure of compound **3** and named tiantaishanmine.

Figure 2 key ^{1}H-^{1}H COSY (▬) and HMBC (↷) correlations of **3** and **4**

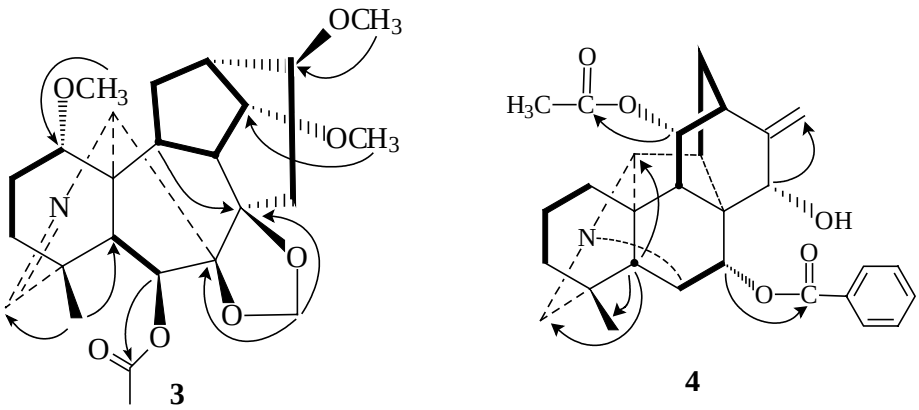


Table 1. NMR data of compounds **1** and **2** in $CDCl_3$ (400 MHz for ^1H, 100 MHz for ^{13}C).

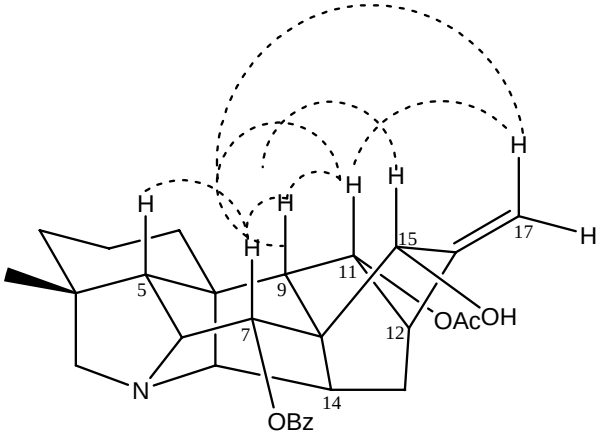
NO.	**1**		**2**	
	δ_C	δ_H Mult (J = Hz)	δ_C	δ_H Mult (J = Hz)
1	77.7 d	3.90 s	71.6 d	3.81 br s
2	31.6 t	1.26 m (α)	29.2 t	1.26 br s (α)
		2.18 m (β)		1.56 br s (β)
3	57.8 d	3.05 m	30.9 t	1.54 m (β)
		-		1.58 m (α)
4	58.3 s	-	32.8 s	-
5	52.2 d	1.56 s	51.1 d	1.39 m
6	79.8 d	4.82 s	78.2 d	5.44 s
7	91.3 s	-	90.9 s	-
8	84.4 s	-	84.4 s	-
9	43.0 d	3.12 t (5.6)	39.5 d	3.44 m
10	43.5 d	2.12 m	45.2 d	2.14 m
11	54.0 s	-	51.3 s	-
12	29.5 t	1.58 m (β)	30.3 t	1.87 m (β)
		2.12 m (α)		2.18 m (α)
13	40.4 d	2.26 m	37.2 d	2.44 m
14	74.3 d	4.03 t (4.4)	83.5 d	3.73 t (4.0)
15	26.7 t	1.97 m (α)	34.2 t	1.88 m (α)
		2.59 m (β)		2.51 m (β)
16	82.3 d	3.39 s	82.2 d	3.28 m
17	67.2 d	2.91 d (1.5)	65.3 d	3.02 s
18	-	-	26.7 q	0.99 s
19	54.3 t	2.49 ABq	61.1 t	2.44 m
	-	3.36 ABq hidden		2.51 m
21	49.9 t	2.98 m	49.6 t	2.74 m
		3.39 m		2.87 m
22	13.9 q	1.07 t (7.2)	13.3 q	1.11 t (7.2)
1-OCH_3	51.7 q	3.51 s	-	-
14-OCH_3	-		57.4 q	3.43 s
16-OCH_3	56.4 q	3.41 s	56.1 q	3.36 s
-OCH_2O-			93.9 t	4.92 s
				4.96 s
OAc			169.7 s	
			21.5 q	2.07 s

Table 2. NMR data of compounds **3** (600 MHz for ^{1}H, 150 MHz for ^{13}C) and **4** (400 MHz for ^{1}H, 100 MHz for ^{13}C) in $CDCl_3$**.**

NO.	**3**		**4**	
	δ_C	δ_H Mult (J = Hz)	δ_C	δ_H Mult (J = Hz)
1	80.4 d	3.23 m	29.0 t	1.55 m (β)
		-		1.81 m (α)
2	25.6 t	1.53 m (α)	19.9 t	0.87 br s (β)
		1.92 m (β)		1.61 m (α)
3	31.9 t	1.31 m (β)	33.1 t	1.25 m
		1.72 m (α)		1.47 m
4	44.4 s	-	37.5 s	-
5	52.5 d	1.41 br s	60.0 d	1.67 s
6	79.9 d	5.25 s	68.0 d	3.33 s
7	90.9 s	-	70.4 d	5.44 d (2.8)
8	82.9 s	-	52.5 s	-
9	39.8 d	3.40 m	47.1 d	2.43 dd (6.6, 1.5)
10	47.9 d	2.20 m	50.9 s	-
11	49.8 s	-	75.4 d	5.28 d (6.4)
12	28.7 t	2.13 m (β)	40.1 d	2.28 br s
		1.88 dd (α) (10, 5.2)		-
13	38.7 d	2.40 m	28.0 t	1.43 m
				2.34 m (hidden)
14	83.4 d	3.73 t (3.2)	39.2 d	2.34 t (9.6)
15	33.4 t	1.85 m (α)	66.0 d	4.06 s
		2.71 m (β)		-
16	81.3 d	3.28 t (5.2)	150.8 s	-
17	63.5 d	4.16 br s	112.2 t	5.04 d (0.9)
18	22.1 q	1.17 s	28.9 q	0.97 s
19	169.9 d	7.46 br s	62.7 t	2.49 s
20	-	-	74.1 d	2.75 s
1-OCH_3	55.4 q	3.22 s	-	-
14-OCH_3	57.7 q	3.45 s	-	-
16-OCH_3	56.4 q	3.34 s	-	-
-OCH_2O-	93.9 t	4.93 s	-	-
		4.97 s		-
OAc	169.5 s	-	170.3 s	-
	21.5 q	2.06 s	21.4 q	2.06 s
ArCO			167.1 s	-
1’			129.7 s	-
2’, 6’			130.1 d	8.14 d (6.8)
3’ ,5’			128.4 d	7.45 m
4’			133.4 d	7.58 m

Tiantaishandine (**4**) mp 248-249 °C, was obtained as an amorphous powder. The HR-ESIMS at *m/z* 476.2439 corresponded to the pseudo-molecular ion $[M+H]^+$ ($C_{29}H_{34}NO_5$ calc. 476.2431). In the NMR spectrum, some characteristic signals of a C_{20}-diterpene alkaloid having a hetisine-type skeleton [C-4 (37.5 s), C-8 (52.5 s), C-10 (50.9, s), C-16 (150.8 s)] [5], together with some important proton signals [H_3-18 (δ_H 0.97, s), H_2-17 (δ_H 5.04, d, J = 0.9 Hz), H-6 (δ_H 3.33, 1H, s), H-20 (δ_H 2.75, 1H, s), and H_2-19 (δ_H 2.49, s)], were observed. In addition to these data, two downfield singlets at δ_C 170.3 s, 167.1 s, and the IR absorptions at 1736 and 1714 cm^{-1}, showed the presence of one acetyl and one benzoyl group in this compound. The one-proton signal (δ_H 5.28, d, J = 6.4 Hz) could be attributed to H-11, based on the HMBC corrections of H-11 (δ_H 5.28) and C-9 (δ_C 47.1), C-10 (δ_C 50.9), C-12 (δ_C 40.1), as well as 1H-doublet at δ_H 5.44, assigned to H-7 due to the correlations of H-7 (δ_H 5.44) and C-8 (δ_C 52.5), C-14 (δ_C 39.2), C-15 (δ_C 66.0). The acetyl and benzyl groups could be located at C-11 and C-7, respectively, based on the correlations between H-11 (δ_H 5.28)/11-O*CO*CH_3 (δ_C 170.3) and H-7 (δ_H 5.44) /7-Ph*CO* (δ_C 167.1). The ^{13}C-NMR spectrum of compound **4** displayed three oxygenated carbon signals (δ_C 66.0 d, 70.4 d, 75.4 d), suggesting the presence of additional hydroxyl group, which could be placed at C-15 based on the HMBC spectrum (Figure 2). The configuration of this hydroxyl group was determined as 15α-OH, based on the NOESY relationships of H-15 (δ_H 4.06) with H-17 (δ_H 5.04), H-9 (δ_H 2.43) and H-12 (δ_H 2.28) (Figure 3). Meanwhile the 11-OAc and 7-OBz were also assigned α-configurations due to the NOESY experiments (Figure 3). Consequently, the structure of tiantaishandine was determined as **4**.

Figure 3. Key NOESY correlations of **4.**



Experimental

General

Melting points were measured on a Thermal Values analytical microscope and were uncorrected. Optical rotations were recorded on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Nicolet FI-IR 200SXY spectrophotomer. ^{1}H- and ^{13}C-NMR spectra were measured in $CDCl_3$ with TMS as the internal standard on a Varian Unity INOVA 400/54 NMR or Bruker AV-600 spectrometers. HR-ESI-MS were measured by a VG AutoSpec 3000 instrument. Silica gel GF254 and H (Qingdao Sea Chemical Factory, China) were used for TLC and column chromatography, respectively. Spots on TLC were detected with modified Dragendorff ’s reagent.

Plant Material

Delphinium tiantaishanense W. J. Zhang et G. H. Chen was collected in the Tiantaishan mountains of Sichuan Province, P.R. China. The plant was identified taxonomically by Wen-Jing Zhang (Disease Prevention and Control Center of Pengzhou City). A voucher speciman (No. 20050818) was deposited in the West China College of Pharmacy, Sichuan University.

Extraction and Isolation

Air-dried powered roots (4.4 kg) of *D. tiantaishan* were percolated with 0.1M HCl (50 L). The filtrate was then made alkaline to pH>9 with 28% aqueous NH_4OH (1.5 L) and extracted five times with ethyl acetate (each 20 L), and the extracts evaporated to give the total crude alkaloids (58 g, yield 1.31%). The crude alkaloids (58 g) were chromatographed over a silica gel (500 g) column eluting with a 7:1→1:1 petroleum ether-acetone gradient to give fractions A (7.5 g), B (9.6 g), C (18.7 g), and D (20.4 g). Fraction A (7.5 g) was rechromatographed on a silica gel column eluting with petroleum ether-acetone (8:1) to afford **2** (120 mg, yield 0.003%). Fraction B (9.6 g) was rechromatographed on a silica gel column eluting with petroleum ether-acetone (5:1) gave **4** (90 mg, yield 0.002%). Fraction C (18.7 g) was rechromatographed on a silica gel column eluting with petroleum-acetone (3:1) to afford **1** (100 mg, yield 0.002%) and **3** (40 mg, yield 0.001%), respectively.

Tiantaishansine (**1**). White amorphous powder, mp 94-96 °C; $[\alpha]_D^{20}$ +35.4° (c=0.84, $CHCl_3$); IR (KBr) cm^{-1}: 3425, 2943, 2879, 1707, 1638, 1460; ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$): see Table 1; HR-ESI-MS: *m/z* $[M+H]^+$ 424.2351, calcd for $C_{22}H_{34}NO_7$, 424.2330.

Tiantaishannine (**2**). White amorphous powder, mp 206-208 °C; $[\alpha]_D^{20}$ -23.9° (c=0.73, $CHCl_3$); IR (KBr) cm^{-1}: 3396, 2932, 1738, 1457, 1367, 1084; ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C- NMR (100 MHz, $CDCl_3$): see Table 1; HR-ESI-MS: *m/z* 478.2801 $[M+H]^+$, calcd for $C_{26}H_{40}NO_7$, 478.2799.

Tiantaishanmine (**3**). White amorphous powder, mp 251-253 °C; $[\alpha]_D^{20}$ +24.2° (c=0.26, $CHCl_3$); IR (KBr) cm^{-1}: 3396, 2926, 1741, 1637, 1461, 1365; ^{1}H-NMR (600 MHz, $CDCl_3$) and ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 2; HR-ESI-MS: *m/z* 462.2473 $[M+H]^+$, calcd for $C_{25}H_{36}NO_7$, 462.2486.

Tiantaishandine (**4**).White amorphous powder, mp 248-249 °C; $[\alpha]_D^{20}$ +35.6° (c=0.85, $CHCl_3$); IR (KBr) cm^{-1}: 3446, 2943, 1736, 1714, 1243, 1108; ^{1}H-NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$): see Table 2; HR-ESI-MS: *m/z* 476.2439 $[M+H]^+$, calcd for $C_{29}H_{34}NO_5$, 476.2431.

Acknowledgements

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Sample Availability: Available from the authors.