Habaenines A and B, Two New Norditerpenoid Alkaloids from *Aconitum habaense*

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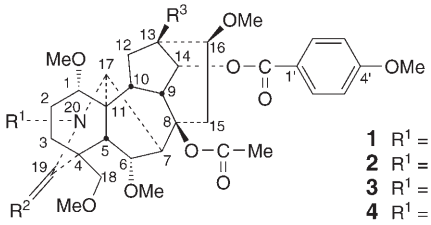
Two new norditerpenoid alkaloids, habaenine A and B (**1** and **2**), together with two known compounds, were isolated from *Aconitum habaense*. The structures of the new compounds were elucidated on the basis of spectral analyses as (1α,6α,16β)-8-(acetyloxy)-20-ethyl-13-hydroxy-1,6,16-trimethoxy-4-(methoxymethyl)-19-oxoaconitan-14-yl 4-methoxybenzoate (**1**) and (1α,6α,16β)-8-(acetyloxy)-13-hydroxy-1,6,16-trimethoxy-4-(methoxymethyl)-aconitan-14-yl 4-methoxybenzoate (**2**).

Introduction. – The genus *Aconitum* (Ranunculaceae) is represented with 208 species in China, mostly growing in the southwestern and northeastern parts of the country on mountains of 1500 meters above sea level or higher [1]. *Aconitum* species produce highly toxic norditerpenoid alkaloids that have attracted considerable interest because of their complex structures, interesting chemistry, and noteworthy physiological effects [2]. *Aconitum habaense* W. T. WANG has long been used in Tibetan folk medicine for the treatment of arthralgia, dysmenorrhea, and colic [3]. As a continuation of our studies on medicinal plants of *Aconitum* species growing on the Yunnan-Tibet Plateau [4–7], *Aconitum habaense* was now examined. To the best of our knowledge, no scientific study on this plant has hitherto been reported. From its roots, two new norditerpenoid alkaloids, named habaenine A and B (**1** and **2**), as well as two known norditerpenoid alkaloids were isolated. The known compounds were identified as vilmorrianine C (**3**) [8] and crassicauline A (**4**) [9]. Here we report on the isolation and structure elucidation of **1** and **2**.



1 R^1 = Et, R^2 = O, R^3 = OH
2 R^1 = H, R^2 = H_2, R^3=OH
3 R^1 = Et, R^2 = H_2, R^3 = H
4 R^1 = Et, R^2 = H_2, R^3 = OH

Results and Discussion. – Habaenine A (**1**) was isolated as an optically active white amorphous solid. Its molecular formula was determined as $C_{35}H_{48}NO_{11}$ by HR-ESI-MS

($[M+1]^+$ at m/z 658.3232). The IR spectrum showed characteristic absorptions for an OH (3433 cm^{-1}, br.), ester (1727 cm^{-1}), and lactam moiety (1640 cm^{-1}) and for an aromatic ring (1607 and 1513 cm^{-1}). The UV absorption at 259 (4.47) nm was consistent with the presence of a 4-methoxybenzoate unit. From the ^{1}H- and ^{13}C-NMR (*Table 1*), HMBC, HMQC, NOESY, and ^{1}H,^{1}H-COSY data (*Fig. 1*), compound **1** was identified as (1α,6α,16β)-8-(acetyloxy)-20-ethyl-13-hydroxy-1,6,16-trimethoxy-4-(methoxymethyl)-19-oxoaconitan-14-yl 4-methoxybenzoate.

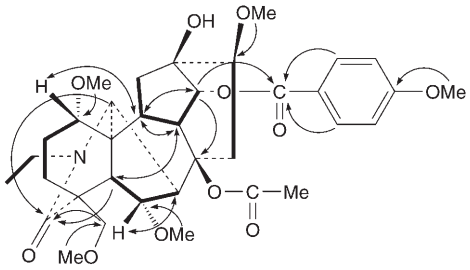


Fig. 1. *Significant ^{1}H,^{1}H-COSY* (—), *HMBC* (→), *and NOESY* (↔) *correlations for* **1**

Table 1. *^{1}H-* (500 MHz) *and ^{13}C-NMR* (125 MHz) *Data of Habaenine A* (**1**) *in CDCl.* δ in ppm, J in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
H–C(1)	3.22 (t, J = 3.8)	83.0	H–C(17)	3.43 (s)	60.0
CH_2(2)	2.04–2.07 (m, H_α), 1.39–1.41 (m, H_β)	25.5	CH_2(18)	4.11 (d, J = 8.4, H_α), 3.52 (d, J = 8.4, H_β)	78.1
CH_2(3)	1.81–1.83 (m, H_α), 1.24–1.26 (m, H_β)	33.0	C(19)	–	172.7
			MeCH_2–N(20)	1.15 (t, J = 7.2)	12.6
C(4)	–	46.5	Me*CH_2*–N(20)	3.79–3.82 (m, H_α), 2.98–3.01 (m, H_β)	40.8
H–C(5)	3.05 (s)	53.8			
H–C(6)	4.02 (d, J = 6.5)	82.0	C(1′)	–	122.1
H–C(7)	2.49 (d, J = 6.5)	48.3	H–C(2′6′)	8.02 (d, J = 8.8, 2 H)	131.3
C(8)	–	76.7	H–C(3′5′)	6.92 (d, J = 8.8, 2 H)	113.3
H–C(9)	2.74 (t, J = 5.5)	43.0	C(4′)	–	163.1
H–C(10)	1.84–1.87 (m)	40.9	*C*(=O)–C(1′)	–	165.7
C(11)	–	48.5	*Me*O–C(1)	3.08 (s)	55.0
CH_2(12)	2.09–2.13 (m, H_α), 2.50–2.54 (m, H_β)	33.8	MeO–C(6)	3.54 (s)	58.3
			MeO–C(16)	3.24 (s)	56.6
C(13)	–	74.7	MeO–C(18)	3.30 (s)	58.6
H–C(14)	4.87 (d, J = 4.7)	77.6	MeO–C(4′)	3.86 (s)	55.0
CH_2(15)	2.62 (dd, J = 16.2, 8.6, H_α), 2.96–2.98 (m, H_β)	36.6	MeCOO–C(8)	–	169.5
			*Me*COO–C(8)	1.33 (s)	21.2
H–C(16)	3.36–3.40 (m)	82.6			

The ^{1}H-NMR spectrum of **1** (*Table 1*) showed signals due to an *AA′BB′* system for four aromatic protons (δ 8.02 and 6.92, each 2 H, d, J = 8.8 Hz), five MeO groups (δ 3.86, 3.54, 3.30, 3.24, and 3.08, each 3 H, s), a strongly shielded MeCO group (δ 1.33, s), and a Me of an *N*-ethyl group (δ 1.15, t, J = 7.2 Hz). The ^{13}C-NMR spectrum (*Table 1*) clearly indicated the presence of a norditerpene moiety (C(1)–C(19)) combined with an anisoyl (= 4-methoxybenzoylunit C(1′) to C (6′), *C*(=O)–C(1′)), five MeO groups, an MeCO group (δ 169.5 and 21.2), an *N*-ethyl group (δ 40.8 and 12.6), and an amide group (δ 172.7). Its

spectral characteristics were similar to those of the known compound, crassicauline A (**4**), except that an amide group (CON, δ 172.7, C(19)) in compound **1** replaced the CH_2N group (δ 53.1, C(19)) in **4**. The ESI-MS of **1**, exhibiting a molecular ion at *m/z* 658 ($[M+1]^+$) compared to *m/z* 644 ($[M+1]^+$) for **4**, is consistent with this contention. In the HMBC plot of **1** (*Fig. 1*) the correlation H–C(14) (δ(H) 4.87)/*C*(=O)–C(1′) (δ(C) 165.7) suggested that an anisoyl group was positioned at C(14), while the correlations H–C(17) (δ(H) 3.43) and H–C(18) (δ(H) 3.52)/C(19) (δ(C) 172.7) suggested that C(19) represents the position of the lactam carbonyl atom. The five MeO groups were assigned as MeO–C(1), MeO–C(6), MeO–C(16), MeO–C(18), and MeO–C(4′), based on the HMQC and HMBC data. The ^{1}H,^{1}H-COSY correlations are shown in *Fig. 1*. The relative configuration of **1** was studied by means of a NOESY experiment (*Fig. 1*). The NOEs H–C(1)/H–C(10), H–C(10)/H–C(14), H–C(14)/H–C(9), and H–C(9)/H–C(10) indicated β-oriented protons at these locations. The coupling constant between H–C(6) and H–C(7) ($J = 6.5$ Hz) confirmed the β-position of H–C(6), and NOE H–C(6)/H–C(7) established the β-orientation of these protons. Further, the NOEs H–C(17)/H_α–C(15) and H_α–C(15)/H–C(16) demonstrated the α-position of H–C(16). The NOEs H–C(16)/H_α–C(15), H–C(17)/H_α–C(12), H–C(5)/H_β–C(2), and H_α–C(2)/H_α–C(3) allowed the steric differentiation of the protons of CH_2(2), CH_2(3), CH_2(12), and CH_2(15).

Habaenine B (**2**) was isolated as an optically active amorphous solid. Its molecular formula was determined as $C_{33}H_{46}NO_{10}$ by HR-ESI-MS ($[M+1]^+$ at *m/z* 616.3099). The IR spectrum showed characteristic absorptions for an OH (3441 cm^{-1}) and ester groups (1733 and 1704 cm^{-1}) and of an aromatic ring (1605 and 1512 cm^{-1}), which was very similar to the IR spectrum of crassicauline A (**4**) but exhibited a band at 3496 cm^{-1} for NH stretching. The UV absorption at 259 (4.57) nm pointed to the presence of a 4-methoxybenzoate unit as in compound **1**. From the ^{1}H- and ^{13}C-NMR (*Table 2*), HMBC, HMQC, ^{1}H,^{1}H-COSY, and NOESY data (*Fig. 2*), compound **2** was identified as (1α,6α,16β)-8-(acetyloxy)-13-hydroxy-1,6,16-trimethoxy-4-(methoxymethyl)aconitan-14-yl 4-methoxybenzoate.

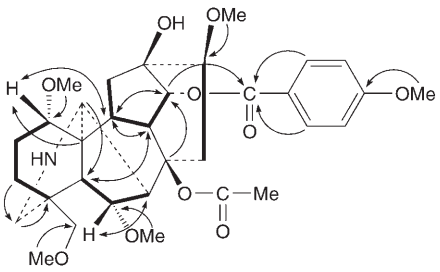


Fig. 2. *Significant ^{1}H,^{1}H-COSY* (▬), *HMBC* (→), *and NOESY* (↔) *correlations for* **2**

The ^{1}H-NMR spectrum of **2** (*Table 2*) showed signals due to an *AA′BB′* system for four aromatic protons (δ 7.98 and 6.90, each 2 H, *d*, $J = 8.9$ Hz), five MeO groups (δ 3.85, 3.54, 3.29, 3.24, and 3.18, each 3 H, *s*) and an MeO group (δ 1.35, *s*). Its spectral characteristics were similar to those of **4**, except for the absence of the *N*-ethyl group in **2**. The ^{13}C-NMR spectrum (*Table 2*) clearly indicated the presence of a norditerpene moiety (C(1)–C(19)) combined with an anisoyl unit (C(1′)–C(6′)), *C*(=O)–C(1′)), five MeO groups, and an MeCO group (δ 169.1 and 21.1). The ^{13}C-NMR data of **2** correlated well with **4**, thus indicating a close structural resemblance between **2** and **4**. Upfield shifts of 5.1 and 5.0 ppm for the C(17) and C(19) signals of **2** were observed, respectively, which was caused by the lacking *N*-ethyl group in **2** [10]. The ESI-MS spectrum of **2** exhibited a molecular ion at *m/z* 616 ($[M+1]^+$), which also confirmed

Table 2. *^1H-* (500 MHz) *and ^{13}C-NMR* (125 MHz) *Data of Habaenine B* (**2**) *in CDCl.* δ in ppm, J in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
H–C(1)	3.25 (t, J = 3.9)	81.6	H–C(16)	3.46 (t, J = 6.8)	81.6
CH_2(2)	1.88–1.91 (m, H_α),	23.0	H–C(17)	3.21 (s)	57.2
	1.41–1.44 (m, H_β)		CH_2(18)	3.53 (d, J = 8.3, H_α),	79.5
CH_2(3)	1.78–1.83 (m, H_α),	28.6		3.06 (d, J = 8.3, H_β)	
	1.37–1.40 (m, H_β)		CH_2(19)	2.18 (d, J = 11.0, H_α),	48.9
C(4)	–	38.7		3.27 (d, J = 11.0, H_β)	
H–C(5)	2.28 (d, J = 6.4)	43.3	C(1′)	–	122.0
H–C(6)	3.99 (d, J = 6.4)	82.1	H–C(2′6′)	7.98 (d, J = 8.9, 2 H)	131.2
H–C(7)	2.98 (s)	53.0	H–C(3′5′)	6.90 (d, J = 8.9, 2 H)	113.3
C(8)	–	85.1	C(4′)	–	163.0
H–C(9)	2.79 (t, J = 5.9)	43.8	*C*(=O)–C(1′)	–	165.4
H–C(10)	2.04–2.07 (m)	39.9	MeO–C(1)	3.18 (s)	55.0
C(11)	–	49.9	MeO–C(6)	3.54 (s)	58.3
CH_2(12)	1.81 (dd, J = 14.3, 4.6, H_α),	34.9	MeO–C(16)	3.24 (s)	57.3
	1.85–1.88 (m, H_β)		MeO–C(18)	3.29 (s)	58.7
C(13)	–	74.2	MeO–C(4′)	3.85 (s)	54.9
H–C(14)	4.89 (d, J = 5.1)	78.3	Me*C*OO–C(8)	–	169.1
CH_2(15)	2.36 (dd, J = 15.3, 8.9, H_α),	39.3	*Me*COO–C(8)	1.35 (s)	21.1
	2.00–2.03 (m, H_β)				

the absence of an *N*-ethyl group in **2** compared to **4** (m/z 644 $[M+1]^+$). In the HMBC experiment of **2** (*Fig. 2*) the correlations H–C(14) (δ(H) 4.89)/*C*(=O)–C(1′) (δ(C) 165.4) suggested that the anisoyl group was positioned at C(14). The five MeO groups were assigned as MeO–C(1), MeO–C(6), MeO–C(16), MeO–C(18), and MeO–C(4′), based on the HMQC and HMBC experiments. The ^{1}H,^{1}H-COSY correlations are shown in *Fig. 2*. The relative configuration of **2** was identical with that of **1**, as can be seen from the NOESY data (*Figs. 1* and *2*).

This work was supported by the *Natural Science Foundation of Yunnan Province* (2005B0001Q) and *Yunnan Education Department* (06Z018A), which are gratefully acknowledged.

Experimental Part

General. M.p.: *XT-4* melting-point apparatus; uncorrected. $[\alpha]_D$: *Jasco 20C* digital polarimeter. UV Spectra: *UV-210A* spectrometer; λ_{max}(log ε) in nm. IR Spectra: *Bio-Rad FTS-135* spectrometer; in cm^{-1}. 1D and 2D NMR Spectra: *Bruker Avance-DRX-500* instrument; $SiMe_4$ as internal reference, δ in ppm, J in Hz. EI-MS: *VG Autospec-3000* mass spectrometer; in m/z (rel. %).

Plant Material. The roots of *A. habaense* W. T. WANG were collected in Shangri-La County, Yunnan Province, P. R. China, in September 2001. The identity of the plant material was verified by Prof. *Zhi-Hao Hu*, Department of Biology, School of Life Science, Yunnan University, P. R. China. A voucher specimen (No. 01-004) was deposited in the Key Laboratory of Medicinal Chemistry for Natural Resources, Yunnan University, Kunming, P. R. China.

Extraction and Isolation. The ground roots (6 kg) of *Aconitum habaense* were extracted with 95% EtOH (5 × 20 l) at r.t. The EtOH extract was evaporated to yield a residue, which was suspended in H_2O and then extracted with petroleum ether (petroleum ether), AcOEt, and BuOH, in this order. The AcOEt extract (76 g) was subjected to column chromatography (CC) (SiO_2, petroleum ether/AcOEt/Et_3N 60 : 1 : 0.1 → 0 : 1 : 0.1): *Fractions 1–10. Fr. 8* was further purified by CC (1. SiO_2, petroleum ether/AcOEt/Et_3N 5 : 1 : 0.1 → 0 : 1 : 0; 2. *Sephadex LH-20*, MeOH): **3** (20 mg) and **4** (38 mg). *Fr. 9* was further

purified by CC (1. SiO_2, petroleum ether/AcOEt/Et_3N 1:1:0.1 → 1:10:0.1; 2. *Sephadex LH-20*, MeOH): **1** (6 mg) and **2** (9 mg).

Habaenine A (=*(1α,6α,16β)-8-(Acetyloxy)-20-ethyl-13-hydroxy-1,6,16-trimethoxy-4-(methoxymethyl)-19-oxoaconitan-14-yl 4-Methoxybenzoate*; **1**): Amorphous solid. $[\alpha]_D^{15} = -44.4$ ($c = 0.045$, $CHCl_3$). UV ($CHCl_3$): 259 (4.47), 313 (3.71), 397 (2.97). IR (KBr): 3433, 2935, 2822, 1727, 1640, 1607, 1513, 1460, 1423, 1376, 1280, 1258, 1170, 1105, 1089, 1022, 984, 858, 772. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS: 657 (1, M^+), 643 (14), 642 (35), 627 (5), 612 (4), 582 (6), 550 (64), 535 (3), 430 (3), 413 (6), 398 (5), 338 (3), 279 (2), 220 (3), 167 (4), 149 (8), 136 (9), 135 (100), 107 (4), 75 (5), 71 (16). HR-ESI-MS: 658.3232 ($[M+1]^+$, $C_{35}H_{48}NO_{11}^+$; calc. 658.3227).

Habaenine B (=*(1α,6α,16β)-8-(Acetyloxy)-13-hydroxy-1,6,16-trimethoxy-4-(methoxymethyl)aconitan-14-yl 4-Methoxybenzoate*; **2**): Amorphous solid. $[\alpha]_D^{15} = -14.9$ ($c = 0.145$, $CHCl_3$). UV ($CHCl_3$): 259 (4.57), 336 (3.82), 387 (3.10). IR (KBr): 3587, 3496, 3441, 2929, 2887, 2823, 1733, 1704, 1605, 1512, 1461, 1374, 1284, 1259, 1170, 1101, 1019, 984, 861, 849, 773. ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS: 616 (1, $[M+1]^+$), 586 (3), 585 (6), 584 (100), 555 (8), 540 (21), 524 (38), 492 (16), 388 (6), 372 (6), 280 (5), 221 (6), 135 (41), 107 (5), 77 (3). HR-ESI-MS: 616.3099 ($[M+1]^+$, $C_{33}H_{46}NO_{10}^+$; calc. 616. 3121).

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Received February 4, 2007