A NEW C_{19}-DITERPENOID ALKALOID, HABAENINE C, FROM *Aconitum habaense*

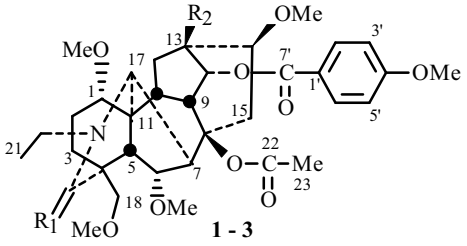
Shu Yang,[1,2] Xiao-dong Yang,[1*] Jing-feng Zhao,[1] Yang Jin,[1] Hong-bin Zhang,[1] and Liang Li[1*]

UDC 547.945

*A new C_{19}-diterpenoid alkaloid, habaenine C (**1**), together with the two known compounds vilmorrianine C and crassicauline A, were isolated from* Aconitum habaense. *The structure of the new compound was elucidated on the basis of spectral analysis, including 2D NMR spectroscopy.*

Key words: *Aconitum habaense*, C_{19}-diterpenoid alkaloid, habaenine C, vilmorrianine C, crassicauline A.

The genus *Aconitum* (Ranunculaceae) is represented by 208 species in China, mostly growing in the southwestern and northeastern parts of the country on mountains 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]. *A. habaense* W. T. Wang has long been used in Tibetan folk medicine for the treatment of arthralgia, dysmenorrhea, and colic [3]. In continuation of our studies on medicinal plants of *Aconitum* species growing on the Yunnan-Tibet Plateau, *A. habaense* has now been examined. In the previous papers [4], we reported two new C_{19}-diterpenoid alkaloids, habaenine A and B, from *A. habaense*. A continuation of our studies on the same plant led to the isolation of a new C_{19}-diterpenoid alkaloid, named habaenine C (**1**), and two known C_{19}-diterpenoid alkaloids, vilmorrianine C (**2**) [5] and crassicauline A (**3**) [6]. The structure of the new compounds was elucidated on the basis of spectral analysis, including 2D NMR spectroscopy.



1 - 3

1: $R_1 = O$, $R_2 = H$
2: $R_1 = H_2$, $R_2 = H$
3: $R_1 = H_2$, $R_2 = OH$

Habaenine C (**1**) was isolated as a white amorphous solid, $[\alpha]_D^{15}$ –7.06° (*c* 0.189, $CHCl_3$). Its molecular formula was determined as $C_{35}H_{48}NO_{10}$ by HR-ESI-MS (found 642.3265, $[M+1]^+$, calc. 642.3278). The IR spectrum showed characteristic absorptions for OH (3432 cm^{-1}, br), ester (1718 cm^{-1}), the lactam moiety (1640 cm^{-1}), and the aromatic ring (1607 and 1512 cm^{-1}). The UV absorption at 260 (4.48) nm is consistent with the presence of a *p*-methoxybenzoate unit.

1) Key Laboratory of Natural Resources and Pharmaceutical Chemistry (Yunnan University), Ministry of Education, School of Chemical Science and Teleology, Yunnan University, Kunming 650091, P. R. China, fax: 86 871 5035538, e-mail: xdyang120@hotmail.com; 2) College of Fundamental and Information Engineering, Yunnan Agricultural University, Kunming 650201, P. R. China. Published in Khimiya Prirodnykh Soedinenii, No. 3, pp. 265-266, May-June, 2008. Original article submitted March 30, 2007.

 0009-3130/08/4403-0334

TABLE 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data of Habaenine C (**1**) ($CDCl_3$, δ, ppm, J/Hz)

C atom	δ_H	δ_C (DEPT)	C atom	δ_H	δ_C (DEPT)
1	3.34 (t, J = 3.8)	81.6 (CH)	17	3.28 (s)	59.9 (CH)
2	1.98-2.03 (m, Hα)	25.3 (CH_2)	18	4.15 (d, J = 8.7, Hα)	78.1 (CH_2)
	1.47-1.52 (m, Hβ)			3.48 (d, J = 8.7, Hβ)	
3	2.17-2.22 (m, Hα)	27.1 (CH_2)	19	-	172.8 (C)
	1.24-1.27 (m, Hβ)		20	3.81-3.84 (m, Hα)	40.7 (CH_2)
4	-	46.5 (C)		2.96-2.99 (m, Hβ)	
5	3.06 (s)	54.0 (CH)	21	1.17 (t, J = 7.2)	12.5 (CH_3)
6	4.08 (d, J = 6.8)	82.9 (CH)	1′	-	122.3 (C)
7	2.49 (d, J = 6.8)	48.3 (CH)	2′/6′	8.01 (d, J = 8.8, 2H)	131.3 (CH)
8	-	76.7 (C)	3′/5′	6.91 (d, J = 8.8, 2H)	113.3 (CH)
9	2.12 (t, J = 5.5)	44.3 (CH)	4′	-	163.0 (C)
10	2.60-2.63 (m)	41.2 (CH)	7′	-	165.6 (C)
11	-	48.8 (C)	MaO (1)	3.24 (s)	54.9 (CH_3)
12	1.76-1.79 (m, Hα)	32.8 (CH_2)	MeO (6)	3.49 (s)	56.6 (CH_3)
	1.81-1.84 (m, Hβ)		MeO (16)	3.31 (s)	56.0 (CH_3)
13	2.52 (m)	37.6 (CH)	MeO (18)	3.40 (s)	58.6 (CH_3)
14	5.04 (t, J = 9.5)	74.2 (CH)	MeO (4)	3.85 (s)	54.8 (CH_3)
15	2.38 (dd, J = 16.2, 8.6, Hα)	35.8 (CH_2)	22	-	169.4 (C)
	2.96-2.98 (m, Hβ)		23	1.40 (s)	21.2 (CH_3)
16	3.21-3.23 (m)	81.6 (CH)			

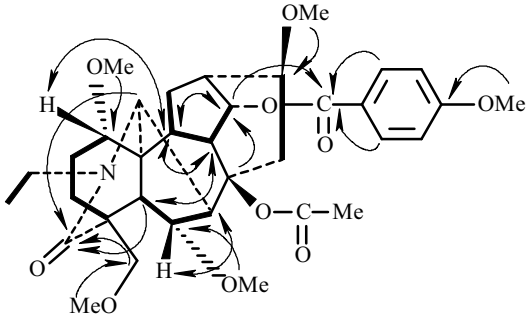


Fig. 1. Significant ^{1}H-^{1}H COSY (—), HMBC (→), and NOESY (←→) correlations for **1**.

The ^{1}H NMR spectrum of **1** (Table 1) showed signals due to an AA′BB′ system for four aromatic protons (δ 8.01, 6.91, each 2H, d, J = 8.8 Hz), five MeO groups (δ 3.85, 3.49, 3.40, 3.31, 3.24, each 3H, s), a strongly shielded acetyl group (δ 1.40, 3H, s), and a methyl of *N*-ethyl group (δ 1.17, 3H, t, J = 7.2 Hz). The ^{13}C NMR spectrum (Table 1) clearly indicated the presence of a C_{19}-diterpenoid moiety (C(1)–C(19)) combined with an anisoyl unit (=4-methoxybenzoyl, C(1′)–C(7′)), five methoxy groups (5×OCH_3), an acetyl group (δ 169.4, 21.2), an *N*-ethyl group (δ 40.7, 12.5), and an amide group (δ 172.8). Its spectral characteristics were similar to those of the known compound vilmorrianine C (**2**), except that an amide group (*N*-CO, δ 172.8, C(19)) in compound **1** replaced the *N*-CH_2 group (δ 53.1, C(19)) in **2**. The ESI-MS spectrum of **1** exhibited a molecular ion at *m*/*z* 641 $[M]^+$ compared to *m*/*z* 627 $[M]^+$ for **2**, which is consistent with this contention.

In the HMBC spectrum (Fig. 1) the correlations of H–C(14) (δd (H) 5.04)/C(7′) (δ(C) 165.6) suggested that an anisoyl group was positioned at the C(14) position, while the correlations of H–C(17) (δ (H) 3.28) and H–C(18) (δ(H) 3.48)/C(19) (δ(C) 172.8) suggested that C(19) represents the position of the lactam carboxyl C-atom. The five methoxy 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 spectra. The ^{1}H-^{1}H COSY correlations are shown in Fig. 1.

The relative configuration of **1** was studied by means of the NOESY spectrum (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(5) and H–C(6) (J = 6.8 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)/Hα–C(15) and Hα–C(15)/H–C(16) demonstrated the α-position

of H–C(16). The NOEs H–C(16)/Hα–C(15), H–C(17)/Hα–C(12), H–C(5)/Hβ–C(2), and Hα–C(2)/Hα–C(3) allowed the steric differentiation of the protons of CH_2(2), CH_2(3), CH_2(12), and CH_2(15).

From the above data, compound **1** was identified as (1α,6α,16β)-8-acetoxy-20-ethyl- 1,6,16-trimethoxy-4-(methoxymethyl)-aconitan-19-on-14-yl-4-methoxybenzoate.

EXPERIMENTAL

General Methods. The $[\alpha]_D$ values was obtained on a JASCO-20C digital polarimeter. UV spectra were determined on a UV 210A spectrometer, and IR spectra on a Bio-Red FTS-135 spectrometer. 1D- and 2D-NMR spectra were taken on a DRX-500 instrument with TMS as internal reference. EIMS were recorded on a VG Auto spec-3000 mass spectrometer.

Plant Material. The plant material was collected in Shangri-La County, Yunnan Province, P. R. China, in September 2001, and was identified as *A. habaense* W. T. Wang. A voucher specimen was deposited in the Key Laboratory of Medicinal Chemistry for Natural Resources, Yunnan University.

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

Habaenine C. Amorphous solid. $[\alpha]_D^{15}$ –7.06° (*c* 0.189, $CHCl_3$). UV spectrum ($CHCl_3$): 260, 312, 395 (log ε 4.48, 3.70, 2.98). IR spectrum (KBr, ν, cm^{-1}): 3432, 2928, 2854, 1718, 1640, 1607, 1513, 1462, 1371, 1345, 1278, 1258, 1169, 1107, 1091, 1021, 991, 850, 772. ^1H and ^{13}C NMR: Table 1. HRESIMS: 642.3265 ($[M+1]^+$, $C_{35}H_{48}NO_{10}$; calc. 642.3278). Mass spectrum (ESI-MS, 70 eV, *m*/*z*): 641 (1, M^+), 627 (18), 626 (72), 611 (8), 581 (6), 566 (11), 534 (8), 519 (8), 455 (4), 414 (3), 402 (5), 374 (7), 360 (6), 234 (3), 162 (4), 149 (5), 136 (9), 135 (100), 107 (4), 85 (6), 71 (22).

ACKNOWLEDGMENT

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

REFERENCES

1. Institute of Botany, Chinese Academy of Sciences, *Flora Reipublicae popularis Sinicae*, Science Press, Beijing, 1979, Vol. 27, 113 pp.
2. S. W. Pelletier, N. V. Mody, B. S. Joshi, and L. C. Schramm, in: *Alkaloids: Chemical and Biological Perspectives*, Ed. S. W. Pelletier, J. Wiley & Sons, New York, 1984, Vol. 2, 205 pp.
3. Yunnan Medicinal Material Company, *Index Chinese Medicines Resources Yunnanensis*, Science Press, Beijing, 1993, 352 pp.
4. S. Yang, X. D. Yang, J. F. Zhao, H. B. Zhang, and L. Li, *Helv. Chim. Acta*, **90**, 1160 (2007).
5. T. R. Yang, D. Z. Wang, and D. G. Wu, *Acta Chim. Sin.* **39**, 445 (1981).
6. F. P. Wang and Q. C. Fang, *Planta Med.,* **42**, 375 (1981).