Three New C_{19}-Diterpenoid Alkaloids from *Aconitum transsectum*

by **Yong Shen**[a]), **Hong-Lian Ai**[a]), **Tuan-Wu Cao**[b]), **Jian-Jun Wang**[a]), **Shu-Hui Zi**[a]), **Xue-Mei Zhang**[b]), and **Ji-Jun Chen***[b])

[a]) School of Agriculture and Biological Technic, Yunnan Agricultural University, Kunming 650201, P. R. China

[b]) State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, P. R. China (phone: +86-871-5223265; fax: +86-871-5223265; e-mail: chenjj@mail.kib.ac.cn)

Three new C_{19}-diterpenoid alkaloids, named aconitramines A (**1**), B (**2**), and C (**3**), were isolated from *Aconitum transsectum*. By UV, IR, 1D- and 2D-NMR, and MS analyses, their structures were elucidated as 18-methoxyvilmoraconitine, 18-demethoxydolichotine A, and 18-demethoxydolichotine B. Compound **1** is the second known C_{19}-diterpenoid alkaloid with a three-membered ring formed by C(8), C(9), and C(10).

Introduction. – *Aconitum transsectum* DIELS (Ranunculaceae), a perennial herb distributed in the north-west of Yunnan Province in China, has long been used as a folk medicine to treat rheumatism and pains [1]. Previous phytochemical investigations on this plant revealed that C_{19}-diterpenoid alkaloids were the main constituents [2–4]. Pharmacological studies demonstrated that the diterpenoid alkaloids were the effective components in the *Aconitum* genus [5]. To find more biologically active substances, the roots of *A. transsectum* were phytochemically investigated to afford three new C_{19}-diterpenoid alkaloids, named aconitramines A (**1**), B (**2**), and C (**3**) (*Fig. 1*). This article reports the isolation and structure elucidation of the three new alkaloids.

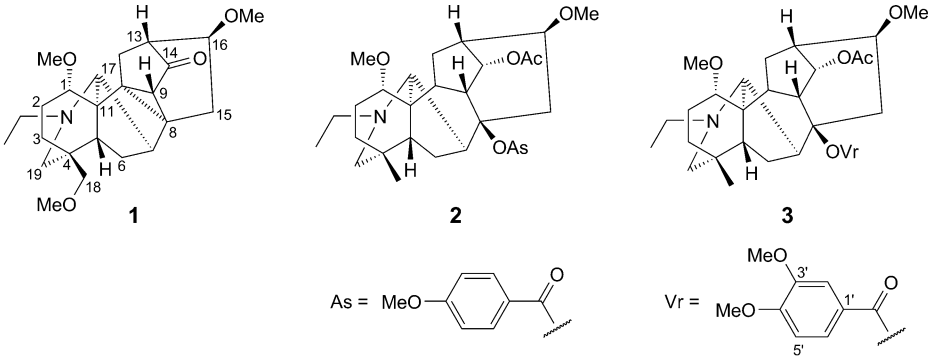

Fig. 1. *Stuctures of compounds* **1**–**3**, *isolated from* Aconitum transsectum

Results and Discussion. – Aconitramine A (**1**) was obtained as a colorless gum and assigned the molecular formula $C_{24}H_{35}NO_4$ by analyses of its EI-MS (*m/z* 401 (M^+)) and HR-EI-MS (*m/z* 402.2638 ($[M+H]^+$). The IR spectrum showed the absorption band for a C=O group (1730 cm^{-1}). The ^{1}H-NMR spectrum (*Table*) displayed one *N*-ethyl (δ(H) 1.03 (*t*, *J* = 7.2 Hz, *Me*CH_2N)) and three MeO groups (δ(H) 3.25, 3.27, and 3.35 (3*s*)), and the ^{13}C-NMR (DEPT) displayed 24 C-atom signals (*Table*) including signals for four Me groups, eight CH_2 groups, seven CH groups, and five quaternary C-atoms. The above spectral data suggested that compound **1** might be an aconitine type C_{19}-diterpenoid alkaloid [6][7]. Comparing the ^{1}H- and ^{13}C-NMR spectral data (*Table*) of compound **1** with those of vilmoraconitine (=(1α,16β)-20-ethyl-1,16-dimethoxy-4-methyl-8,10-cycloaconitan-14-one) [8] showed great similarity, except for the signals of an additional MeO group (δ(H) 3.27 (*s*, MeO–C(18); δ(C) 59.4 (*q*)) and of a CH_2 group (δ(H) 2.97 and 3.08 (2*d*, each *J* = 8.8 Hz, CH_2(18)); δ(C) 79.4 (*t*)) in compound **1**. This CH_2(18) signal of **1**, which was shifted downfield to δ(C) 79.4 (*t*) from δ(C) 26.2 (*q*, Me(18)) in vilmoraconitine, suggested that there was only one MeO group located at C(18). This was confirmed by the HMBC cross-peaks between CH_2(18) (δ(H) 2.97 and 3.08) and C(3) (δ(C) 32.8 (*t*), C(4) (δ(C) 39.2 (*s*)), C(5) (δ(C) 44.0 (*d*)), C(19) (δ(C) 53.2 (*t*)), and *Me*O–C(18) (δ(C) 59.4 (*q*)) (*Fig. 2*). The 2D-NMR spectra including HMQC, HMBC, COSY, and ROESY data resulted in the assignments of all the H- and C-atoms of compound **1** (*Table*). Compound **1** had the same relative configuration as ajabicine (=(1α)-20-ethyl-4-methyl-14-methyleneaconitan-1,8-diol) [9], being supported not only by their almost identical ^{1}H- and ^{13}C-NMR data (*Table*) but also by the ROESY data (*Fig. 3*). Thus, the structure of compound **1** was determined as 18-methoxyvilmoraconitine, named aconitramine A (**1**).

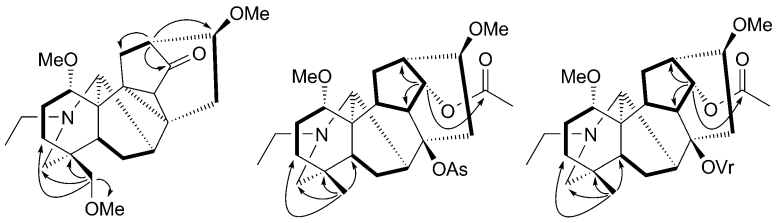

Fig. 2. *Selected ^{1}H,^{1}H-COSY* (—) *and HMBC* (H → C) *features of compounds* **1**–**3**

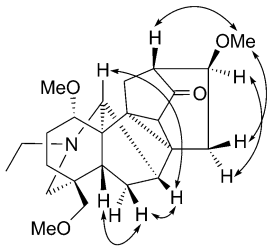

Fig. 3. *Selected ROSEY correlations* (H ↔ H) *of compound* **1**

Table. *[1]H- and [13]C-NMR Data* ($CDCl_3$) *of Compounds* **1**–**3**. δ in ppm, J in Hz.

	1		**2**		**3**	
	δ(H)[a])	δ(C)[b])	δ(H)[c])	δ(C)[d])	δ(H)[a])	δ(C)[b])
H–C(1)	3.43 (*dd*, *J* = 10.3, 6.2)	79.7 (*d*)	3.12 (*dd*, *J* = 8.8, 6.5)	85.7 (*d*)	3.12 (*dd*, *J* = 10.2, 6.6)	85.6 (*d*)
CH_2(2)	2.11 – 2.16, 2.28 – 2.33 (2*m*)	24.3 (*t*)	1.32 – 1.37, 1.77 – 1.83 (2*m*)	25.5 (*t*)	1.40 (*dd*, *J* = 12.6, 6.6), 1.77 – 1.82 (*m*)	25.5 (*t*)
CH_2(3)	1.24 – 1.31, 1.76 – 1.82 (2*m*)	32.8 (*t*)	1.21 – 1.27, 1.75 – 1.81 (2*m*)	37.8 (*t*)	1.18 – 1.23, 1.58 – 1.64 (2*m*)	37.7 (*t*)
C(4)	–	39.2 (*s*)	–	34.4 (*s*)	–	34.3 (*s*)
H–C(5)	1.39 (*d*, *J* = 7.4)	44.0 (*d*)	1.96 – 2.02 (*m*)	45.1 (*d*)	2.89 – 2.93 (*m*)	42.1 (*d*)
CH_2(6)	1.07 (*dd*, *J* = 12.2, 6.8), 1.40 – 1.47 (*m*)	26.2 (*t*)	1.97 – 2.04, 2.33 – 2.39 (2*m*)	26.7 (*t*)	1.93 – 1.98, 2.24 – 2.29 (2*m*)	26.9 (*t*)
H–C(7)	2.19 – 2.26 (*m*)	42.8 (*d*)	1.43 (*d*, *J* = 7.3)	50.9 (*d*)	1.43 (*d*, *J* = 7.2)	50.8 (*d*)
C(8)	–	40.9 (*s*)	–	86.4 (*s*)	–	86.6 (*s*)
H–C(9)	2.12 (*s*)	39.9 (*d*)	2.09 – 2.15 (*m*)	42.4 (*d*)	1.95 – 2.01 (*m*)	45.0 (*d*)
C(10)	–	44.9 (*s*)	3.28 – 3.33 (*m*)	41.7 (*d*)	3.29 – 3.35 (*m*)	41.5 (*d*)
C(11)	–	51.0 (*s*)	–	49.1 (*s*)	–	49.0 (*s*)
CH_2(12)	1.24 – 1.31, 2.15 – 2.19 (2*m*)	29.4 (*t*)	1.94 – 1.99, 2.43 – 2.49 (2*m*)	29.6 (*t*)	1.91 – 1.96, 2.47 – 2.52 (2*m*)	28.7 (*t*)
H–C(13)	2.46 (*t*, *J* = 4.8)	46.7 (*d*)	2.54 – 2.61 (*m*)	39.0 (*d*)	2.27 – 2.32 (*m*)	39.3 (*d*)
C(14)	–	211.1 (*s*)	4.83 (*d*, *J* = 4.7)	75.6 (*d*)	4.79 (*d*, *J* = 4.7)	75.7 (*d*)
CH_2(15)	1.99 – 2.04, 2.11 – 2.16 (2*m*)	30.3 (*t*)	2.15 – 2.19, 2.89 – 2.95 (2*m*)	37.8 (*t*)	2.21 – 2.26, 2.89 – 2.93 (2*m*)	37.8 (*t*)
H–C(16)	3.62 (*dd*, *J* = 9.4, 5.7)	79.6 (*d*)	3.28 – 3.33 (*m*)	83.0 (*d*)	3.26 – 3.30 (*m*)	83.0 (*d*)
H–C(17)	3.54 (br. *s*)	77.6 (*d*)	2.91 (br. *s*)	61.4 (*d*)	2.90 (*s*)	61.6 (*d*)
CH_2(18) or Me(18)	2.97, 3.08 (2*d*, each *J* = 8.8)	79.4 (*t*)	0.70 (s)	26.3 (*q*)	0.69 (s)	26.3 (*q*)
CH_2(19)	2.15, 2.56 (2*d*, each *J* = 11.6)	53.2 (*t*)	1.96, 2.42 (2*d*, each *J* = 11.4)	56.6 (*t*)	1.95, 2.42 (2*d*, each *J* = 11.0)	56.6 (*t*)
MeCH_2N	2.40 – 247, 2.61 – 2.66 (2*m*)	50.2 (*t*)	2.40 – 2.46, 2.46 – 2.53 (2*m*)	49.1 (*t*)	2.40 – 2.46, 2.51 – 2.56 (2*m*)	49.2 (*t*)
MeCH_2N	1.03 (*t*, *J* = 7.2)	13.4 (*q*)	1.07 (*t*, *J* = 7.1)	13.4 (*q*)	1.06 (*t*, *J* = 7.1)	13.4 (*q*)
*Me*O–C(1)	3.35 (*s*)	55.8 (*q*)	3.28 (*s*)	56.1 (*q*)	3.26 (*s*)	56.2 (*q*)
*Me*O–C(16)	3.25 (*s*)	55.6 (*q*)	3.32 (*s*)	56.5 (*q*)	3.32 (*s*)	56.5 (*q*)
*Me*O–C(18)	3.27 (*s*)	59.4 (*q*)	–	–	–	–
*Ac*O–C(14)	–	–	1.78 (*s*)	171.5 (*s*), 21.4 (*q*)	1.77 (*s*)	171.6 (*s*), 21.6 (*q*)
		–	AsO–C(8)[e])	AsO–C(8)[e])	VrO–C(8)[f])	VrO–C(8)[f])
C=O		–	–	164.7 (*s*)	–	164.6 (*s*)
H–C(1′)		–	–	124.0 (*s*)	–	123.8 (*s*)
H–C(2′)	–	–	7.92 (*d*, *J* = 8.8)	131.3 (*d*)	7.48 (*d*, *J* = 1.7)	110.1 (*d*)
H–C(3′)			6.90 (*d*, *J* = 8.8)	113.5 (*d*)	–	148.5 (*s*)
H–C(4′)	–	–	–	163.2 (*s*)	–	152.7 (*s*)
H–C(5′)	–	–	6.90 (*d*, *J* = 8.8)	113.5 (*d*)	6.86 (*d*, *J* = 8.4)	111.5 (*d*)
H–C(6′)	–	–	7.92 (*d*, *J* = 8.8)	131.3 (*d*)	7.59 (*dd*, *J* = 8.4, 1.7)	123.2 (*d*)
*Me*O–C(3′)	–	–	–	–	3.92 (*s*)	55.9 (*q*)
*Me*O–C(4′)	–	–	3.85 (*s*)	55.4 (*q*)	3.94 (*s*)	55.9 (*q*)

[a]) 400 MHz. [b]) 100 MHz. [c]) 500 MHz. [d]) 125 MHz. [e]) As = Anisoyl = 4-methoxybenzoyl. [f]) Vr = Veratroyl = 3,4-dimethoxybenzoyl.

Aconitramine B (**2**) was obtained as a colorless gum and reacted positively to the *Dragendorff* reagent. It was deduced to have a molecular formula $C_{33}H_{45}NO_7$ based on its ESI-MS (*m/z* 568 ($[M+H]^+$)) and HR-ESI-MS (*m/z* 568.3291 ($[M+H]^+$)). The IR spectrum showed the absorptions for a conjugated ester C=O (1738 cm^{-1}) and an aromatic ring (1607, 1511, and 1463 cm^{-1}). The ^{1}H-NMR data (*Table*) displayed the presence of an *N*-ethyl group (δ(H) 1.07 (*t*, $J = 7.1$ Hz, *Me*CH_2N)), two MeO groups (δ(H) 3.28 and 3.32 (2*s*)), a quaternary Me group (δ(H) 0.70 (*s*, Me(18)), and an Ac (δ(H) 1.78 (*s*)) and anisoyl (= 4-methoxybenzoyl; As) group (δ(H) 7.92 and 6.90 (2*d*, each $J = 8.8$ Hz) and 3.85 (*s*)). Careful analyses of the NMR spectra suggested that compound **2** was also an aconitine type C_{19}-diterpenoid alkaloid. The ^{1}H- and ^{13}C-NMR spectra (*Table*) of **2** were identical to those of dolichotine A (= (1α,14α,16β)-20-ethyl-1,16-dimethoxy-4-(methoxymethyl)aconitane-8,11-diol 14-acetate 8-(4-methoxybenzoate)) [10], except for one more quaternary Me group (δ(H) 0.70 (*s*); δ(C) 26.3) in **2**, instead of the the CH_2(18) bearing an MeO group in dolichotine A. The additional quaternary Me group was located at C(4), based on the long-range correlations between Me(18) (δ(H) 0.70) and C(3) (δ(C) 37.8 (*t*)), C(4) (δ(C) 34.4 (*s*)), C(5) (δ(C) 45.1 (*d*)), and C(19) (δ(C) 56. 6 (*t*)) in the HMBC spectrum (*Fig. 2*). Accordingly, compound **2** was established to be 18-demethoxydolichotine A (*Fig. 1*).

Aconitramine C (**3**) had a molecular formula $C_{34}H_{47}NO_8$, in agreement with the ESI-MS (*m/z* 598 ($[M+H]^+$)) and positive-ion mode HR-ESI-MS (*m/z* 598.3373 ($[M+H]^+$). The ^{1}H-NMR data (*Table*) showed the presence of an *N*-ethyl group, a quaternary Me, an Ac, and a veratroyl (= 3,4-dimethoxybenzoyl; Vr) group, which exhibited characteristic features of an aconitine-type C_{19}-diterpenoid alkaloid bearing an *N*-ethyl group. The 1D-NMR spectra (*Table*) of **3** resembled those of dolichotine B [10] except for the presence of one more quaternary Me group (δ(C) 26.3) in **1** at C(4), instead of the CH_2(18) bearing an MeO group in dolichotine B. The quaternary Me group was attached at C(4), as suggested by the HMBC cross-peaks between δ(H) 0.69 (*s*, Me(18)) and C(3), C(4), C(5), and C(19). Hence, compound **3** was defined as 18-demethoxydolichotine B (**3**).

As far as we know, vilmoraconitine [8] is the only known C_{19}-diterpenoid alkaloid with a three-membered ring formed by C(8), C(9), and C(10). Compound **1** is thus the second C_{19}-diterpenoid alkaloid with this three-membered ring isolated from a natural source, providing a new candidate for further pharmacological investigations.

The authors are grateful to the staff of the analytical group of the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, for the measurements of all spectra.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200 – 300 mesh; *Qingdao Meigao Chemical Ltd.*, Qingdao, P. R. China), Al_2O_3 (*Shanghai Wusi Chemical Reagents Co., Ltd.*), and *Sephadex LH-20* (*Pharmacia Fine Chemical Co., Ltd.*, Germany). M.p.: *XRC-1* micro melting point apparatus; uncorrected. Optical rotations: *Horiba SEPA-300* polarimeter. UV Spectra: *Shimadzu-UV-2401A* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad-FTS-135* spectrometer; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker-AM-400* and *-DRX-500* spectrometers; δ in ppm with reference to the solvent signals, *J* in Hz. MS: *VG-Autospec-3000* spectrometer; at 70 eV; in *m/z*. HR-ESI-MS: *API Qstar-Pulsar-1* spectrometer; in *m/z*.

Plant Material. The roots of *Aconitum transsectum* DIELS. were collected in Dali of Yunnan Province, P. R. China, in October 2010, and authenticated by Prof. Dr. *Li-Gong Lei* at the Kunming Institute of Botany. A voucher specimen (No. KIB 2010-10-12) has been deposited with the Group of Anti-Virus and Natural Medicinal Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The roots of *A. transsectum* (10 kg) were powdered and extracted three times with 90% EtOH under reflux for 2 h. After evaporation of the solvent, the crude extract was dissolved with 2% HCl soln. (4 l), and then filtrated. The acidic soln. was basified to pH 9.0 with $NH_3 \cdot H_2O$ (25%) and extracted with $CHCl_3$ to obtain the crude alkaloid extract (115 g) after evaporation of $CHCl_3$. The extract was subjected to CC (SiO_2 (800 g), petroleum ether/acetone/Et_2NH 40:1:1 → 15:8:1): *Fractions A–D. Fr. B* (6.2 g) was subjected to CC (SiO_2, petroleum ether/acetone/Et_2NH 35:1:1), followed by CC (Al_2O_3, petroleum ether/acetone 13:1) and finally purified by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1): **1** (8.2 mg), **2** (20 mg), and **3** (16 mg). *Fr. C* (28.5 g) was subjected to CC (SiO_2, petroleum ether/acetone/Et_2NH 20:3:1) and further purified by CC (Al_2O_3, petroleum ether/acetone 8:1): **3** (24 mg).

Aconitramine A (*=18-Methoxyvilmoraconitine =(1α,16β)-20Ethyl-1,16-dimethoxy-4-(methoxymethyl)-8,10-cycloaconitan-14-one*; **1**). Colorless gum. $[\alpha]_D^{25.1} = -15.77$ ($c = 1.04$, MeOH). UV (MeOH): 206 (3.79). IR (KBr): 1730. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS: 401 (11, M^+), 370 (100, $[M - MeO]^+$). HR-ESI-MS (pos.): 402.2638 ($[M+H]^+$, $C_{24}H_{36}NO_4^+$; calc. 402.2644).

Aconitramine B (*=18-Demethoxydolichotine A =(1α,14α,16β)-20-Ethyl-1,16-dimethoxy-4-methylaconitane-8,14-diol 14-Acetate 8-(4-Methoxybenzoate)*; **2**). Colorless gum. $[\alpha]_D^{27.3} = -13.39$ ($c = 1.14$, MeOH). UV (MeOH): 256 (4.15). IR (KBr): 2929, 1738, 1704, 1607, 1511, 1463, 1367, 1254, 1098, 772. ^{1}H- and ^{13}C-NMR: *Table.* ESI-MS (pos.): 568 ($[M+H]^+$). HR-ESI-MS (pos.): 568.3291 ($[M+H]^+$, $C_{33}H_{46}NO_7^+$; calc. 568.3274).

Aconitramine C (*=18-Demethoxydolichotine B =(1α,14α,16β)-20-Ethyl-1,16-dimethoxy-4-methylaconitane-8,14-diol 14-Acetate 8-(3,4-Dimethoxybenzoate)*; **3**). Colorless gum. $[\alpha]_D^{25.0} = -3.06$ ($c = 2.72$, MeOH). UV (MeOH): 219 (4.36). IR (KBr): 2930, 1737, 1704, 1600, 1515, 1464, 1366, 1246, 1094, 766. ^{1}H- and ^{13}C-NMR: *Table.* ESI-MS (pos.): 598 ($[M+H]^+$). HR-ESI-MS (pos.): 598.3373 ($[M+H]^+$, $C_{34}H_{48}NO_8^+$; calc. 598.3379).

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Received September 8, 2011