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

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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.

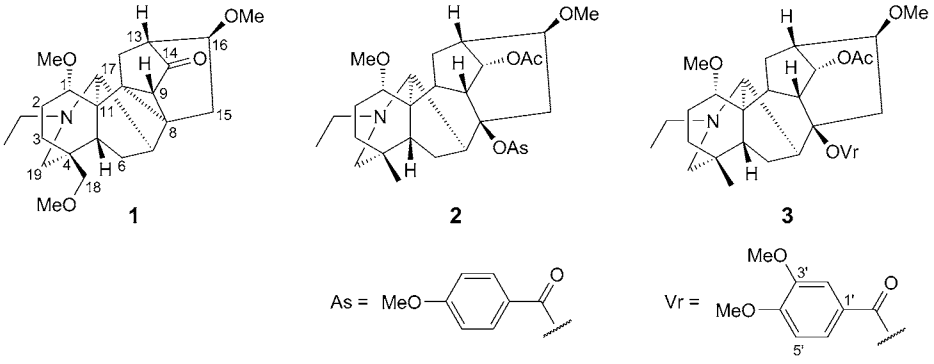

Fig. 1. *Stuctures of compounds* **1**–**3**, *isolated from* Aconitum transsectum

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**).

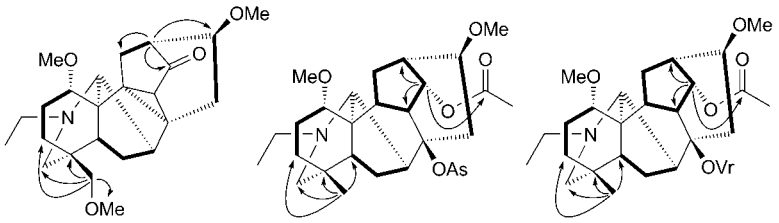

Fig. 2. *Selected ^{1}H,^{1}H-COSY* (—) *and HMBC* (H → C) *features of compounds* **1**–**3**

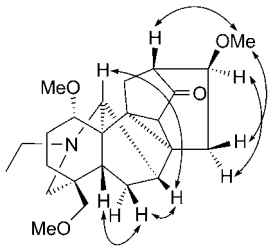

Fig. 3. *Selected ROSEY correlations* (H ↔ H) *of compound* **1**

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*)
Me*C*H_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.

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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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