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

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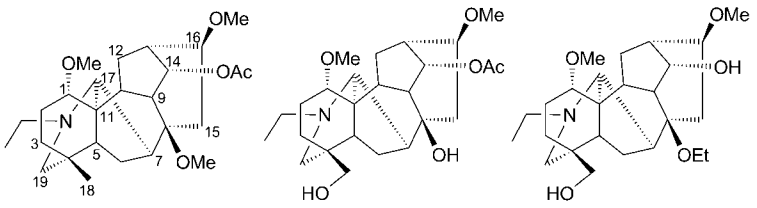
A further study of the alkaloid constituents of *Aconitum forrestii* led to the isolation of three new C_{19}-diterpenoid alkaloids, named 14-acetoxy-8-*O*-methylsachaconitine (**1**), 14-acetoxyscaconine (**2**), and 8-*O*-ethylcammaconine (**3**). Their structures were determined by UV, IR, and MS, 1D- and 2D-NMR analyses.

Introduction. – *Aconitum forrestii* STAPF (Ranunculaceae), a perennial herb distributed in northwestern of Yunnan Province in China, has long been used as a folk medicine to treat rheumatism and pains, and also as an insecticide [1]. Previous phytochemical investigations on this plant revealed that C_{19}-diterpenoid alkaloids were the main constituents [2–6]. Pharmacologically, they can be developed as analgesic, cardiotonic, anti-inflammatory, antirheumatic, and anti-arrhythmic agents [7]. As a part of an ongoing phytochemical investigation of *A. forrestii*, three new C_{19}-diterpenoid alkaloids, named 14-acetoxy-8-*O*-methylsachaconitine (**1**), 14-acetoxyscaconine (**2**), 8-*O*-ethylcammaconine (**3**) (*Fig. 1*) were isolated from the roots of this plant. Herein, we report the isolation and structure elucidation of these three new alkaloids.



1 **2** **3**

Fig. 1. *Structures of compounds* **1**–**3**, *isolated from* Aconitum forrestii

Results and Discussion. – Compound **1** was obtained as a colorless gum, and it gave a positive test with the *Dragendorff*'s reagent. The molecular formula was determined as $C_{26}H_{41}NO_5$ based on ESI-MS (*m*/*z* 448 ($[M + H]^+$)) and HR-ESI-MS (448.3048

($[M+H]^+$); calc. 448.3058). The IR spectrum showed the absorption band for a conjugated ester CO group (1736 cm^{-1}).

The ^{1}H-NMR spectra of **1** exhibited Me signals for one EtN group (δ(H) 1.06 (*t*, $J = 7.0$, *Me*CH_2N)), for an AcO group (δ(H) 1.98 (*s*, MeCOO)), a quaternary Me group (δ(H) 0.76 (*s*, Me(18)), and three MeO groups (δ(H) 3.12 (*s*), 3.27 (*s*), 3.33 (*s*)). Its ^{13}C-NMR (DEPT) displayed 26 C-atom signals (*Table*) including those of six Me, seven CH_2, and nine CH groups, as well as of four quaternary C-atoms. These data indicated that compound **1** might be an aconitine type C_{19}-diterpenoid alkaloid [8][9], bearing an AcO, an EtN, and three MeO groups. In the ^{1}H-NMR spectrum, a *triplet* signal at δ(H) 4.73 (*t*, $J = 4.8$) was assigned to H_β–C(14), implying the presence of an AcO group at C(14) [10]. Notably, the AcO group was attached to C(14) in compound **1**. Comparing the ^{1}H- and ^{13}C-NMR data (*Table*) of compound **1** with those of 8-*O*-methylsachaconitine [11] revealed a great similarity except that signals of an additional AcO group (δ(H) 1.98 (*s*, *Me*COO); δ(C) 171.6 (*s*), 21.4 (*q*)) appeared in the spectra of compound **1**. The signals of AcO at C(14) in compound **1** were confirmed by the cross-peaks between H–C(14) (δ(H) 4.73 (*t*, $J = 4.8$)) and C(9) (δ(C) 43.1 (*d*)), C(13) (δ(C) 38.0 (*d*)), and AcO (δ(C) 171.6 (*s*, Me*C*OO) in the HMBC spectrum (*Fig. 2*). The full NMR assignments of compound **1** were achieved with the aid of the HSQC, HMBC, COSY, and ROESY analyses.

Compound **1** had the same relative configuration as 8-*O*-methylsachaconitine, which was supported not only by their almost identical ^{1}H- and ^{13}C-NMR data (*Table*), but also by a ROESY spectrum (*Fig. 3*). Thus, the structure of compound **1** was elucidated as 14-acetoxy-8-*O*-methylsachaconitine.

Compound **2**, a colorless gum, had the molecular formula $C_{25}H_{39}NO_6$, as established by ESI-MS (*m*/*z* 450 ($[M+H]^+$)) and HR-ESI-MS (450.2823 ($[M+H]^+$); calc. 450.2850), indicating one degree of unsaturation. The IR spectrum showed the absorption for a conjugated ester CO (1737 cm^{-1}) and OH (3466 cm^{-1}). The ^{1}H-NMR data revealed the presence of an EtN group (δ(H) 1.07 (*t*, $J = 7.1$, *Me*CH_2N)), two MeO groups (δ(H) 3.25 (*s*), 3.32 (*s*)), and an AcO group (δ(H) 2.04 (*s*, *Me*COO)).

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 compound **2** were strikingly similar to those of scaconine [12] except the presence of an AcO group (δ(H) 2.04 (*s*), δ(C) 20.9 (*q*), 171.2 (*s*)) in compound **2**, instead of a MeO group at C(14) in scaconine. This finding was supported by the long-range correlations between H–C(14) (δ(H) 4.82 (*t*, $J = 4.7$)) and C(9) (δ(C) 46.8 (*d*)), C(13) (δ(C) 37.6 (*d*)), and AcO (δ(C) 171.2 (*s*, Me*C*OO)) in the HMBC spectrum (*Fig. 2*). Accordingly, compound **2** was established as 14-acetoxyscaconine (*Fig. 1*).

Compound **3** was also obtained as a colorless gum with a molecular formula of $C_{25}H_{41}NO_5$ deduced from its ESI-MS *m*/*z* 436 ($[M+H]^+$) and HR-ESI-MS (positive-ion mode; 436.3045 ($[M+H]^+$); calc. 436.3058). The ^{1}H-NMR data evidenced the presence of an EtN group, an EtO group, and two MeO groups, which exhibited characteristic features of an aconitine type C_{19}-diterpenoid alkaloid bearing an EtN group. The 1D-NMR data (*Table*) of **3** were almost identical to those of cammaconine [13], except that there was one more EtO group (δ(H) 1.02 (*t*, $J = 6.8$, *Me*CH_2O)), 3.27–3.33 (*m*, Me*C*H_2O); δ(C) 55.6 (*t*), 16.1 (*q*)) at C(8) in compound **1**, instead of an OH group in cammaconine. In the HMBC spectrum, long-range correlations, observed

Table. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$, 400 and 100 MHz, resp.) *of Compounds* **1**–**3**. δ in ppm, J in Hz.

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	3.10 (*dd*, *J* = 10.2, 6.8)	85.8 (*d*)	3.07 (*dd*, *J* = 10.0, 6.5)	86.0 (*d*)	3.11 (*dd*, *J* = 10.4, 6.4)	84.9 (*d*)
2a	1.32–1.35 (*m*)	26.8 (*t*)	1.38 (*dd*, *J* = 12.4, 6.5)	25.6 (*t*)	1.35 (*dd*, *J* = 12.1, 6.4)	25.3 (*t*)
2b	1.89–1.95 (*m*)		1.79–1.85 (*m*)		1.74–1.80 (*m*)	
3a	1.22–1.28 (*m*)	37.4 (*t*)	1.25–1.32 (*m*)	32.4 (*t*)	1.18–1.24 (*m*)	32.8 (*t*)
3b	1.82–1.87 (*m*)		1.61–1.66 (*m*)		1.48–1.54 (*m*)	
4	–	34.3 (*s*)	–	37.4 (*s*)	–	38.5 (*s*)
5	1.98 (*d*, *J* = 7.8)	50.1 (*d*)	2.50–2.54 (*m*)	45.9 (*d*)	2.52–2.58 (*m*)	45.8 (*d*)
6a	1.97–2.04 (*m*)	24.4 (*t*)	2.03–2.09 (*m*)	24.8 (*t*)	1.84–1.89 (*m*)	24.6 (*t*)
6b	2.36–2.40 (*m*)		2.39–2.45 (*m*)		2.26–2.31 (*m*)	
7	1.52 (*d*, *J* = 7.2)	39.8 (*d*)	1.43 (*d*, *J* = 7.3)	45.7 (*d*)	1.46 (*d*, *J* = 7.2)	45.6 (*d*)
8	–	77.8 (*s*)	–	72.7 (*s*)	–	78.0 (*s*)
9	1.96–2.01 (*m*)	43.1 (*d*)	1.78–1.83 (*m*)	46.8 (*d*)	1.80–1.85 (*m*)	47.1 (*d*)
10	3.27–3.33 (*m*)	45.1 (*d*)	3.39–3.42 (*m*)	46.0 (*d*)	3.30–3.35 (*m*)	45.3 (*d*)
11	–	51.0 (*s*)	–	48.7 (*s*)	–	48.8 (*s*)
12a	1.86–1.91 (*m*)	29.0 (*t*)	1.95–2.00 (*m*)	27.6 (*t*)	1.76–1.81 (*m*)	27.9 (*t*)
12b	2.40–2.46 (*m*)		2.39–2.45 (*m*)		2.49–2.54 (*m*)	
13	2.38–2.43 (*m*)	38.0 (*d*)	2.51–2.56 (*m*)	37.6 (*d*)	2.34–2.38 (*m*)	37.9 (*d*)
14	4.73 (*t*, *J* = 4.8)	75.8 (*d*)	4.82 (*t*, *J* = 4.7)	75.5 (*d*)	4.03 (*t*, *J* = 4.5)	75.0 (*d*)
15α	2.04–2.11 (*m*)	35.4 (*t*)	2.03–2.08 (*m*)	38.2 (*t*)	2.13–2.19 (*m*)	38.7 (*t*)
15β	2.45–2.52 (*m*)		3.00–3.06 (*m*)		2.47–2.52 (*m*)	
16	3.28–3.33 (*m*)	83.3 (*d*)	3.30–3.35 (*m*)	82.1 (*d*)	3.26–3.31 (*m*)	82.4 (*d*)
17	3.01 (br. *s*)	61.4 (*d*)	2.93 (br. *s*)	62.7 (*d*)	2.93 (*s*)	62.6 (*d*)
18	0.76 (*s*)	26.5 (*q*)	3.76 (*t*, *J* = 9.6), 4.11 (*t*, *J* = 9.6)	70.0 (*t*)	3.53 (*t*, *J* = 9.8), 3.95 (*t*, *J* = 9.8)	68.3 (*t*)
19α	1.98 (*d*, *J* = 11.2)	56.8 (*t*)	1.86 (*d*, *J* = 11.0)	52.6 (*t*)	1.83 (*d*, *J* = 10.8)	52.0 (*t*)
19β	2.46 (*d*, *J* = 11.2)		2.37 (*d*, *J* = 11.0)		2.35 (*d*, *J* = 10.8)	
MeCH_2–N	2.40–2.45 (*m*), 2.64–2.69 (*m*)	50.4 (*t*)	2.38–2.44 (*m*), 2.51–2.56 (*m*)	49.4 (*t*)	2.42–2.47 (*m*), 2.52–2.58 (*m*)	49.3 (*t*)
MeCH_2–N	1.06 (*t*, *J* = 7.0)	13.4 (*q*)	1.07 (*t*, *J* = 7.1)	13.6 (*q*)	1.06 (*t*, *J* = 7.0)	13.2 (*q*)
*Me*O–C(1)	3.27 (*s*)	56.3 (*q*)	3.25 (*s*)	56.4 (*q*)	3.22 (*s*)	56.2 (*q*)
*Me*O–C(8)	3.12 (*s*)	48.1 (*q*)	–	–	–	–
*Me*O–C(16)	3.33 (*s*)	56.3 (*q*)	3.32 (*s*)	56.5 (*q*)	3.29 (*s*)	56.3 (*q*)
*Ac*O–C(14)	1.98 (*s*)	171.6 (*s*), 21.4 (*q*)	2.04 (*s*)	171.2 (*s*), 20.9 (*q*)	–	–
*Et*O–C(8)	–	–	–	–	3.27–3.33 (*m*),	55.6 (*t*),
	–	–	–	–	1.02 (*t*, *J* = 6.8)	16.1 (*q*)

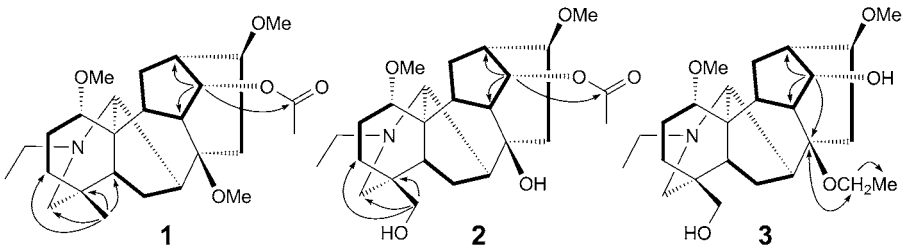


Fig. 2. *Selected $^1H,^1H$-COSY correlations* (—) *and HMBCs* (→) *of compounds* **1**–**3**

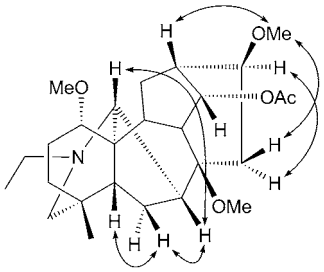


Fig. 3. *Selected ROESY correlations of compound* **1**

from the CH_2 (MeCH_2O) *singlet* (δ(H) 3.27–3.33 (*m*)) to C(8) (δ(C) 78.0 (*s*)), indicated that the EtO group was at C(8). Hence, compound **3** was established as 8-*O*-ethylcammaconine (**3**).

This work was financially supported by the *State Key Laboratory of Phytochemistry and Plant Resources in West China*, KIB, CAS (No. P2012-KF 04), the *National Natural Science Foundation of China* (21362045), *Innovative Team of Yunnan Agricultural University* ([2012]18#), and the project of *Sciences and Technology Department of Yunnan Province* (No. 2012CG007).The authors are grateful to the staff of the analytical group of *State Key Laboratory of Phytochemistry and Plant Resources in West China*, *Kunming Institute of Botany*, *Chinese Academy of Sciences*, for recording the 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 Company, 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. IR Spectra: *Bio-Rad FTS-135* spectrometer. 1D- and 2D-NMR spectra: *Bruker AM-400* and *DRX-500* spectrometers; chemical shifts δ in ppm with reference to the solvent signals. MS: *VG Autospec-3000* spectrometer at 70 eV; in *m*/*z*. HR-ESI-MS: *API Qstar-Pulsar-1* spectrometer.

Plant Material. The roots of *Aconitum forrestii* STAPF were collected in Lijiang of Yunnan Province, P. R. China, in October, 2011, and authenticated by Prof. Dr. *Li-Gong Lei* at Kunming Institute of Botany. A voucher specimen (No. KIB 2011-10-37) had 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. forrestii* (15 kg) were powdered and extracted three times with MeOH under reflux for 2 h. After removal of the solvent under reduced pressure, the crude extract

was dissolved in 10 l of 1.7% HCl soln. and then filtered. The acidic soln. was basified to pH 9.0 with aq. NH_3 (25%) and extracted with AcOEt to obtain crude alkaloidal extract (148 g) after removal of AcOEt *in vacuo*. The alkaloidal extract was separated by CC (SiO_2 (1000 g, 200–300 mesh); petroleum ether (PE)/Et_2N (60:1:1 → 15:10:1) to four fractions, *Frs. A–D. Fr. A* (3.1 g) was subjected to CC (SiO_2; PE/acetone/Et_2N 40:1:1), followed CC (Al_2O_3; PE/ AcOEt 15:1), and finally purified by another CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1) to yield compound **1** (6.8 mg). *Fr. C* (37.4 g) was submitted to CC (SiO_2; PE/acetone/Et_2N 15:2:1, and Al_2O_3; PE/ AcOEt 1:5), and finally purified by CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1) to afford **2** (12 mg) and **3** (11 mg).

*14-Acetoxy-8-*O*-methylsachaconitine* (*=(1α,7β,14α,16β)-20-Ethyl-1,8,16-trimethoxy-4-methylaconitan-14-yl Acetate*; **1**). Colorless gum. $[\alpha]_D^{16.4} = -10.65$ ($c = 0.2$, $CHCl_3$). IR (KBr): 2927, 1736, 1516, 1460, 1362, 1254, 1098. NMR: *Table*. ESI-MS (pos.): 448 ($[M+H]^+$). HR-ESI-MS (pos.): 448.3048 ($[M+H]^+$, $C_{26}H_{42}NO_5^+$; calc. 448.3058).

14-Acetoxyscaconine (*=(1α,7β,14α,16β)-20-Ethyl-8-hydroxy-1,16-dimethoxy-4-methylaconitan-14-yl Acetate*; **2**): Colorless gum. $[\alpha]_D^{15.8} = -10.63$ ($c = 0.2$, $CHCl_3$). IR (KBr): 3466, 2930, 1737, 1511, 1465, 1368, 1253, 1097. NMR: *Table*. ESI-MS (pos.): 450 ($[M+H]^+$). HR-ESI-MS (pos.): 450.2823 ($[M+H]^+$, $C_{25}H_{40}NO_6^+$; calc. 450.2850).

*8-*O*-Ethylcammaconine* (*=(1α,7β,14α,16β)-8-Ethoxy-20-ethyl-4-(hydroxymethyl)-1,16-dimethoxyaconitan-14-ol*; **3**): Colorless gum. $[\alpha]_D^{16.4} = -24.47$ ($c = 0.2$, $CHCl_3$). IR (KBr): 3458, 2927, 1467, 1362, 1244, 1096. NMR: *Table*. ESI-MS (pos.): 436 ($[M+H]^+$). HR-ESI-MS (pos.): 436.3045 ($[M+H]^+$, $C_{25}H_{42}NO_5^+$; calc. 436.3058).

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Received January 17, 2013