Two New C_{20}-Diterpenoid Alkaloids from *Aconitum carmichaelii*

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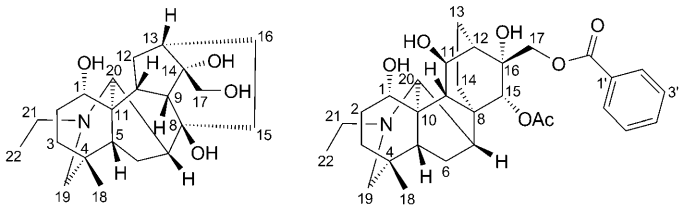
Two new C_{20}-diterpenoid alkaloids, named aconicarchamines A and B (**1** and **2**, resp.), were isolated from *Aconitum carmichaelii*. By UV, IR, MS, and 1D- and 2D-NMR analyses, their structures were elucidated as 14,17-dihydro-14,17-dihydroxyajabicine and 15-*O*-acetyllassiocarpine. Compound **1** is the third C_{20}-diterpenoid alkaloid with the lycoctine skeleton bearing an exocyclic C-atom at C(14).

Introduction. – *Aconitum carmichaelii* DEBEAUX (Ranunculaceae), a traditional Chinese medicine known as 'Fu-Zi' in Chinese, is widely distributed in Yunnan, Shaanxi, and Sichuan Provinces in China, and has long been used as an analgesic and anti-inflammatory agent [1]. Previous phytochemical investigations on this plant revealed that C_{19}- and C_{20}-diterpenoid alkaloids were the main constituents [2–9]. Pharmacological studies demonstrated that diterpenoid alkaloids were the effective components in the *Aconitum* genus [10]. To find more biologically active substances, the roots of *A. carmichaelii* were phytochemically investigated to afford two new C_{20}-diterpenoid alkaloids, named aconicarchamines A[1]) and B[1]) (**1** and **2**, resp.; see *Fig. 1*), together with the nine known alkaloids talatisamine, benzoylmesaconitine, fuziline, karakoline, neoline, songorine, hypaconitine, mesaconitine, and aconitine. We now report on the isolation and structure elucidation of the two new alkaloids.



1[1]) **2**[1])

Fig. 1. *Alkaloids* **1** *and* **2***, isolated from* Aconitum carmichaelii

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

Results and Discussion. – Aconicarchamine A (**1**) was obtained as colorless prisms from $CHCl_3$ and assigned the molecular formula $C_{22}H_{35}NO_4$ by analyses of its FAB-MS (*m*/*z* 378 ($[M + H]^+$)) and HR-ESI-MS (*m*/*z* 378.2635 ($[M + H]^+$)). The IR spectrum showed an absorption band for OH groups (3443 cm^{-1}). In the ^{1}H-NMR spectrum (*Table*), two Me signals at δ(H) 0.84 (*s*, Me(18)) and 1.08 (*t*, $J = 7.0$ Hz, *Me*CH_2–N) were observed, together with an O-bearing CH_2 group (δ(H) 3.42 and 3.45 (2*d*, $J =$ 11.5 Hz, each 1 H, CH_2(17))). Its ^{13}C-NMR (DEPT) revealed the presence of 22 C-atoms (*Table*) including two Me, nine CH_2, and seven CH groups, besides four quaternary C-atoms. Detailed analyses of the MS and 1D-NMR spectra suggested that compound **1** was a C_{20}-diterpenoid alkaloid. Careful comparison of the 1D-NMR data of **1** with those of ajabicine [11] suggested that they share a similar skeleton, except for the O-bearing CH_2(17) group (δ(C) 66.5; δ(H) 3.42 and 3.45) and a different

Table. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$, 400 and 100 MHz, resp.) *of Compounds* **1** *and* **2**. δ in ppm, *J* in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	3.69 (*d*, $J = 5.3$)	72.3 (*d*)	4.75 (*dd*, $J = 10.5, 6.0$)	70.1 (*d*)
CH_2(2)	1.53 – 1.59, 1.59 – 1.66 (2*m*)	29.5 (*t*)	1.85 – 1.92, 1.97 – 2.04 (2*m*)	30.6 (*t*)
CH_2(3)	1.44 – 2.01, 1.66 – 1.71 (2*m*)	31.0 (*t*)	1.25 – 1.32, 1.43 – 1.50 (2*m*)	38.4 (*t*)
C(4)		32.8 (*s*)		33.5 (*s*)
H–C(5)	1.91 (*d*, $J = 7.3$)	46.2 (*d*)	1.42 (*d*, $J = 7.0$)	52.7 (*d*)
CH_2(6)	1.81 (*dd*, $J = 14.2, 7.2$), 2.14 (*dd*, $J = 14.2, 7.3$)	24.9 (*t*)	1.21 – 1.29 (*m*), 3.52 (*dd*, $J = 12.2, 7.0$)	23.0 (*t*)
H–C(7)	1.75 (*d*, $J = 6.8$)	44.0 (*d*)	2.23 (br. *s*)	42.0 (*d*)
C(8)		78.4 (*s*)		42.8 (*s*)
H–C(9)	1.98 (*d*, $J = 7.2$)	48.8 (*d*)	2.41 (*d*, $J = 9.0$)	51.4 (*d*)
H–C(10) or C(10)	1.58 (*dd*, $J = 7.2, 7.0$)	46.7 (*d*)		51.3 (*s*)
C(11) or H–C(11)		48.7 (*s*)	5.46 (*d*, $J = 9.0$)	70.3 (*d*)
CH_2(12) or H–C(12)	1.44 – 1.51, 2.20 – 2.27 (2*m*)	35.4 (*t*)	2.56 (br. *s*)	45.2 (*d*)
H–C(13) or CH_2(13)	2.04 (*d*, $J = 9.5$)	35.7 (*d*)	1.42 – 1.49, 2.29 – 2.36 (2*m*)	20.1 (*t*)
C(14) or CH_2(14)		82.7 (*s*)	0.95 – 0.99, 1.72 – 1.79 (2*m*)	27.4 (*t*)
CH_2(15) or H–C(15)	1.95 (*dd*, $J = 9.5, 3.5$), 1.70 – 1.75 (*m*)	33.1 (*t*)	4.68 (*d*, $J = 4.0$)	85.9 (*d*)
CH_2(16) or C(16)	1.64 – 1.69, 1.77 – 1.84 (2*m*)	32.7 (*t*)		77.3 (*s*)
CH_2(17)	3.42, 3.45 (2*d*, $J = 11.5$)	66.5 (*t*)	5.32, 5.56 (2*d*, $J = 11.5$)	69.3 (*t*)
Me(18)	0.84 (*s*)	27.5 (*q*)	0.68 (*s*)	25.8 (*q*)
CH_2(19)	2.03, 2.23 (2*d*, $J = 11.0$)	60.3 (*t*)	2.17, 2.51 (2*d*, $J = 11.0$)	56.7 (*t*)
H–C(20)	3.03 (*s*)	63.1 (*d*)	4.08 (*s*)	66.9 (*d*)
Me*CH_2*–N	2.48 – 2.56, 2.37 – 2.44 (2*m*)	48.1 (*t*)	2.42 – 2.46, 2.32 – 2.37 (2*m*)	50.7 (*t*)
MeCH_2–N	1.08 (*t*, $J = 7.0$)	13.0 (*q*)	0.99 (*t*, $J = 7.0$)	13.2 (*q*)
AcO–C(15)			1.98 (*s*)	171.8 (*s*), 21.2 (*q*)
Ph*C*OO–C(17)				166.6 (*s*)
C(1′)				131.6 (*s*)
H–C(2′,6′)			8.27 (*d*, $J = 7.4$)	130.1 (*d*)
H–C(3′,5′)			7.28 (*t*, $J = 7.6$)	128.7 (*d*)
H–C(4′)			7.45 (*t*, $J = 7.4$)	133.0 (*d*)

quaternary C(14) (δ(C) 82.7) in **1**, instead of the C(14) bearing an exocyclic CH_2 group in ajabicine; this was further confirmed by the cross-peaks CH_2(17)/C(14), H–C(9)/C(14), and H–C(13)/C(14) in the HMBC spectrum (*Fig. 2*).

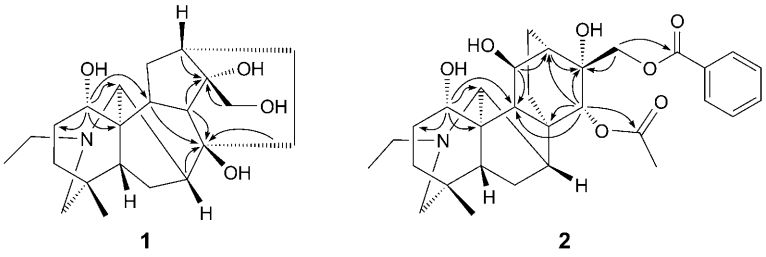


Fig. 2. *Selected HMBCs* (H → C) *of alkaloids* **1** *and* **2**

Compound **1** was shown to possess the same relative configuration as ajabicine, this being supported not only by their almost identical ^{1}H- and ^{13}C-NMR data (*Table*), but also by the ROESY data (*Fig. 3*) showing correlations between H–C(9), H–C(13) (assumed to be β-orientation in ajabicine), and CH_2(17), *i.e.*, β-orientation of the OH–CH_2(17) substituent. Thus, the structure of compound **1** was determined as 14,17-dihydro-14,17-dihydroxyajabicine[1]), named aconicarchamine A (**1**).

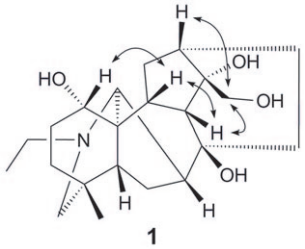


Fig. 3. *Selected ROESY correlations of alkaloid* **1**

Aconicarchamine B (**2**) was isolated as a white powder. The molecular formula was determined to be $C_{31}H_{41}NO_7$ based on the FAB-MS (*m/z* 540 ($[M+H]^+$)) and HR-FAB-MS (*m/z* 540.2965 ($[M+H]^+$)). The IR spectrum showed absorption bands for OH groups (3444 cm^{-1}), a conjugated ester CO group (1721 cm^{-1}), and for an aromatic ring (1603, 1550, and 1451 cm^{-1}). The ^{1}H-NMR data revealed the presence of an EtN group (δ(H) 0.99 (*t*, *J* = 7.0 Hz, *Me*CH_2–N)), a quaternary Me group (δ(H) 0.68 (*s*, Me(18))), an AcO (δ(H) 1.98 (*s*, 3 H)), and a benzoyl group (δ(H) 8.27 (*d*, *J* = 7.4 Hz, 2 H), 7.45 (*t*, *J* = 7.4 Hz, 1 H), 7.28 (*t*, *J* = 7.6 Hz, 2 H)), and the absence of MeO or O–CH_2–O groups. These features are characteristic of an atisine-type C_{20}-diterpenoid alkaloid bearing an EtN group. Comparison of the 1D-NMR spectra (*Table*) of **2** with those of lassiocarpine [12] showed high similarity except that there was an additional AcO group (δ(H) 1.98 (*s*); δ(C) 171.8 (*s*); and 21.2 (*q*)) in compound **2**. The AcO group was determined to be linked to C(15) by the HMBCs between δ(H) 4.68 (*d*, *J* = 4.0 Hz,

H–C(15)) and δ(C) 171.8 (AcO, C=O)), C(8), C(9), C(12), and C(16). The 2D-NMR spectra including HMQC, HMBC, COSY, and ROESY resulted in the assignments of all H- and C-atoms of compound **2** (*Table*). Accordingly, aconicarchamine B (**2**) was established to be 15-*O*-acetyllassiocarpine.

As far as we know, ajabicine [11] and actaline [13] are the only two lycoctine-skeleton C_{20}-diterpenoids reported before. Compound **1** is the third C_{20}-diterpenoid with a lycoctine skeleton bearing an exocyclic C-atom at C(14) isolated from a natural source, providing a new candidate for further pharmacological investigation.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh, *Qingdao Marine Chemical Ltd.*, Qingdao, P. R. China); Al_2O_3 (*Shanghai Wusi Chemical Reagents Company, Ltd.*); *Sephadex LH-20* (*Pharmacia Fine Chemical Co. Ltd.*, Germany). M.p.: *XRC-1* micro melting-point apparatus; uncorrected. Optical rotations: *Jasco-DIP-370* digital 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* (rel. %). HR-ESI-MS: *API-Qstar-Pulsar-1* spectrometer; in *m*/*z*.

Plant Material. The roots of *Aconitum carmichaelii* DEBEAUX were collected in Hanzhong, Shaanxi Province, P. R. China, in October 2003, and authenticated by Prof. Dr. *Li-Gong Lei*, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. KIB 2003-11-03) has been deposited in the Group of anti-Virus and Natural Medicinal Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The roots of *A. carmichaelii* (30 kg) were powdered and extracted three times with 95% EtOH for 2 h under reflux. After evaporation of the solvent, the crude extract was dissolved in 6 l of 1.5% aq. HCl soln. and the soln. then filtrated. The acidic soln. was basified to pH 9.0 with ammonia (25%) and then extracted with $CHCl_3$. The org. phase was concentrated and the crude alkaloid extract (320 g) subjected to CC (SiO_2 (1.6 kg), petroleum ether/Me_2CO/Et_2NH 10:1:1 → 3:1:1): *Fractions 1–6*. *Fr. 5* (15.5 g) was applied to CC (SiO_2 (230 g), petroleum ether/Me_2CO/Et_2NH 15:9:1): *Frs. 5.1–5.3*. *Fr. 5.2* (0.9 g) was further purified by CC (SiO_2 (15 g), benzene/AcOEt/Et_2NH 10:4:1): **1** (18 mg). *Fr. 4* (20.8 g) was subjected to CC (SiO_2 (300 g), petroleum ether/Me_2CO/Et_2NH 15:8:1); then neutral Al_2O_3 (100 g; 200–300 mesh), petroleum ether/Me_2CO 4:1): **2** (20 mg).

Aconicarchamine A (*=14,17-Dihydro-14,17-dihydroxyajabicine = (1α,14α)-20-Ethyl-14-(hydroxymethyl)-4-methylaconitane-1,8,14-triol*; **1**): White prisms ($CHCl_3$). M.p. 210–211°. $[\alpha]_D^{15.8} = +3.60$ (c = 0.36, MeOH). IR (KBr): 3443, 1033. NMR: *Table.* FAB-MS (pos.): 378 ($[M+H]^+$), 361 (8), 148 (22), 122 (33), 117 (21), 105 (33), 91 (52), 79 (36), 58 (100). HR-FAB-MS (pos.): 378.2635 ($[M+H]^+$, $C_{22}H_{36}NO_4^+$; calc. 378.2234).

Aconicarchamine B (*=15*-O-*Acetyllassiocarpine = (1α,7β,11β,15α)-15-(Acetyloxy)-21-ethyl-1,11,16-trihydroxy-4-methyl-7,20-cycloatidan-17-yl Benzoate*; **2**): White power. M.p. 184–185°. $[\alpha]_D^{15.2} = -11.35$ (c = 0.23, MeOH). UV (MeOH): 230 (3.85). IR (KBr): 3444, 1721, 1640, 1603, 1550, 1451, 1247, 1098, 715. NMR: *Table.* FAB-MS (pos.): 540 $[M+H]^+$ (100), 522 (17). HR-FAB-MS (pos.): 540.2965 ($[M+H]^+$, $C_{31}H_{42}NO_7^+$; calc. 540.2234).

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