Macrorhynines A and B, Two Novel Norditerpenoid Alkaloids from *Aconitum macrorhynchum*

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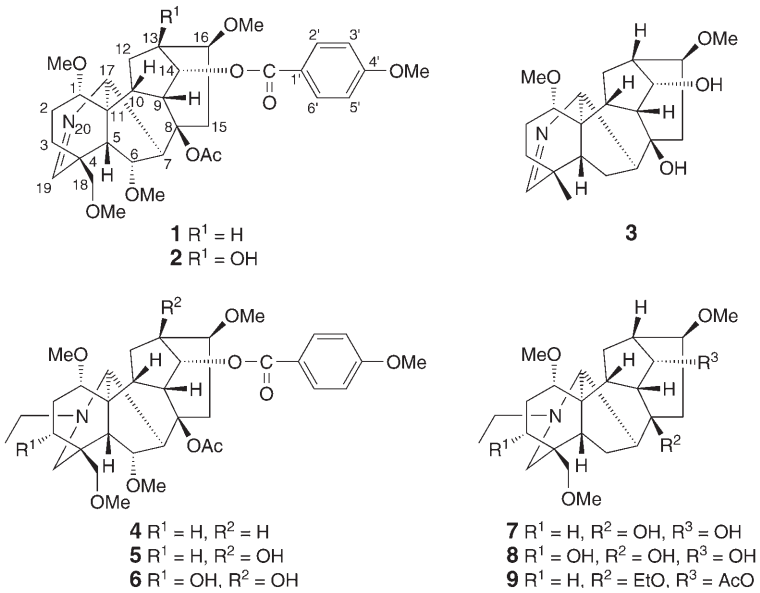
Two novel norditerpenoid alkaloids, macrorhynines A and B (**1** and **2**), together with seven known compounds, were isolated from *Aconitum macrorhynchum*. The structures of the new compounds were elucidated as (1α,6α,14α)-8-acetoxy-1,6,16-trimethoxy-4-(methoxymethyl)aconitan-14-yl 4-methoxybenzoate (**1**) and (1α,6α,14α)-8-acetoxy-13-hydroxy-1,6,16-trimethoxy-4-(methoxymethyl)aconitan-14-yl 4-methoxybenzoate (**2**) on the basis of spectral analyses. The novel compounds were found to contain the rare C(19)=N, azomethine, group.

Introduction. – The genus *Aconitum* (Ranunculaceae) is represented with 208 species in China, mostly growing in the southwestern and northeastern parts of the country on mountains of 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]. *Aconitum macrorhynchum* TURCZ. ex LEDEB has long been used in Tibetan folk medicine for the treatment of arthralgia, dysmenorrhea, and colic [3]. As a continuation of our studies on medicinal plants of *Aconitum* species growing on the Yunnan-Tibet Plateau [4–8], *Aconitum macrorhynchum* was now examined. To the best of our knowledge, no scientific study on this plant has hitherto been reported.

From its roots, two novel norditerpenoid alkaloids, named macrorhynines A and B (**1** and **2**), as well as seven known norditerpenoid alkaloids were isolated. The known compounds were identified as *N*-deethyl-*N*-19-didehydrosachaconitine (**3**) [9], vilmorrianine C (**4**) [10], crassicauline A (**5**) [11], yunaconitine (**6**) [12], talatizamine (**7**) [13], 3-hydroxytalatizamine (**8**) [14], and acoforine (**9**) [15]. Here, we report on the isolation and structure elucidation of **1** and **2**.

Results and Discussion. – Macrorhynine A (**1**) was isolated as an optically active white amorphous solid. Its molecular formula was determined as $C_{33}H_{43}NO_9$ by HR-ESI-MS ($[M+1]^+$ at m/z 598.3285). The IR spectrum showed characteristic absorptions for an OH (3442 cm^{-1}, br.), ester (1718 cm^{-1}), and N=CH (1659 cm^{-1}), and aromatic-ring (1607 and 1513 cm^{-1}). The UV absorption at 259 (4.58) nm is consistent with the presence of a 4-methoxybenzoate unit. From the ^{1}H- and ^{13}C-NMR



(*Table 1*), HMBC, HMQC, NOESY, and ^{1}H,^{1}H-COSY data (*Fig. 1*), compound **1** was elucidated as (1α,6α,14α)-8-acetoxy-1,6,16-trimethoxy-4-(methoxymethyl)aconitan-14-yl 4-methoxybenzoate.

The ^{1}H-NMR spectrum of **1** (*Table 1*) showed signals due to an *AA′BB′* system for four aromatic H-atoms (δ 7.98, 6.91, each 2 H, *d*, J = 8.9 Hz), five MeO groups (δ 3.85, 3.55, 3.30, 3.20, and 3.12, each 3 H, *s*), a strongly shielded MeCO group (δ 1.29, *s*), and a methine of an N=CH group (δ 8.04, *s*). The ^{13}C-NMR spectrum (*Table 1*) clearly indicated the presence of a norditerpene moiety (C(1)–C(19)) combined with an anisoyl (=4-methoxybenzoyl unit; C(1′) to C(6′), C(=O)–C(1′)), five MeO groups, a MeCO group (δ 169.9 and 21.6), and a N=CH group (δ 162.6). Its spectral characteristics were similar to those of the known compound vilmorrianine C (**4**), except for the absence of an N–Et group in **1**. The signals at δ(H) 8.04 (*s*) and δ(C) 162.6 suggested the presence of an N=CH group instead of the N–Et or N–Me group characteristic of many norditerpenoid alkaloids [16]. The ESI-MS of **1** exhibiting a molecular ion at *m*/*z* 597 (M^+) compared to 627 (M^+) for **4** is consistent with this contention.

In the HMBC experiment of **1** (*Fig. 1*) the correlation H–C(14) (δ(H) 4.84)/*C*(=O)–C(1′) (δ(C) 166.1) suggested that an anisoyl group is positioned at C(14), while the correlations H–C(17) (δ(H) 3.72) and H$_\beta$–C(18) (δ(H) 3.64)/C(19) (δ(C) 162.6) suggested that C(19) is involved in the N=CH group. The five MeO 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 data. The ^{1}H,^{1}H-COSY correlations are shown in *Fig. 1*. The relative configuration of **1** was studied by means of a NOESY experiment (*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 H-atoms at these locations. The coupling

Table 1. *^1H-* (500 MHz) *and ^{13}C-NMR* (125 MHz) *Data of Macrorhynine A* (**1**) *in $CDCl_3$.* δ in ppm, J in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
H–C(1)	3.15 (*t*, *J* = 3.9)	81.7	H–C(16)	3.45 (*t*, *J* = 6.8)	83.2
CH_2(2)	2.10–2.12 (*m*, H_α),	24.8	H–C(17)	3.72 (*s*)	69.8
	1.26–1.28 (*m*, H_β)		CH_2(18)	3.81 (*d*, *J* = 8.3, H_α),	74.8
CH_2(3)	2.26–2.28 (*m*, H_α),	34.2		3.64 (*d*, *J* = 8.3, H_β)	
	1.96–1.98 (*m*, H_β)		H–C(19)	8.04 (*s*)	162.6
C(4)	–	48.9	C(1′)	–	122.3
H–C(5)	3.62 (*d*, *J* = 6.4)	37.8	H–C(2′/6′)	7.98 (*d*, *J* = 8.9, 2 H)	131.7
H–C(6)	4.08 (*d*, *J* = 6.4)	82.6	H–C(3′/5′)	6.91 (*d*, *J* = 8.9, 2 H)	113.8
H–C(7)	2.88 (*s*)	43.8	C(4′)	–	163.6
C(8)	–	84.2	*C*(=O)–C(1′)	–	166.1
H–C(9)	2.80 (*t*, *J* = 5.9)	54.1	*Me*O–C(1)	3.12 (*s*)	55.5
H–C(10)	2.10–2.12 (*m*)	40.8	*Me*O–C(6)	3.55 (*s*)	58.9
C(11)	–	51.6	*Me*O–C(16)	3.20 (*s*)	57.4
CH_2(12)	1.96–1.98 (*m*, H_α),	33.0	*Me*O–C(18)	3.30 (*s*)	59.2
	2.12–2.14 (*m*, H_β)		*Me*O–C(4′)	3.85 (*s*)	55.6
C(13)	2.18 (*m*)	74.8	Me*C*OO–C(8)	–	169.9
H–C(14)	4.84 (*d*, *J* = 5.0)	78.0	*Me*COO–C(8)	1.29 (*s*)	21.6
CH_2(15)	2.44 (*dd*, *J* = 15.3, 8.9, H_α),	38.9			
	2.87–2.91 (*m*, H_β)				

constant between H–C(5) and H–C(6) (J = 6.4 Hz) confirmed the β-position of H–C(6), and NOE H–C(6)/H–C(7) established the β-orientation of these H-atoms. Further, the NOEs H–C(17)/H_α–C(15) and H_α–C(15)/H–C(16) demonstrated the α-position of H–C(16). The NOEs H–C(16)/H_α–C(15), H–C(17)/H_α–C(12), H–C(5)/H_β–C(2), and H_α–C(2)/H_α–C(3) allowed the steric differentiation of the H-atoms of CH_2(2), CH_2(3), CH_2(12), and CH_2(15).

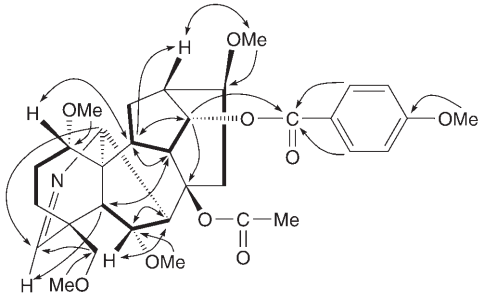


Fig. 1. *Significant $^1H,^1H$-COSY* (—), *HMBC* (→), *and NOESY* (↔) *correlations for* **1**

Macrorhynine B (**2**) was isolated as an optically active amorphous solid. Its molecular formula was determined as $C_{33}H_{43}NO_{10}$ by HR-ESI-MS ($[M + 1]^+$ at m/z 614.3296). The IR spectrum showed characteristic absorptions for an OH (3447 cm^{-1}, br.), ester (1715 cm^{-1}), and N=CH (1634 cm^{-1}) group and an aromatic-ring (1607 and 1513 cm^{-1}). The UV absorption at 257 (4.50) nm is consistent with the presence of a 4-

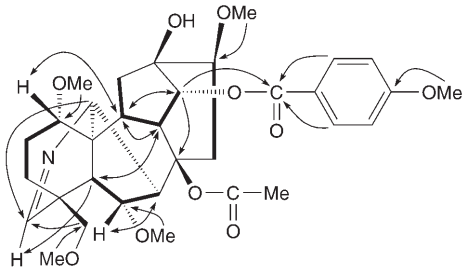


Fig. 2. *Significant $^1H,^1H$-COSY (—), HMBC (→), and NOESY (↔) correlations for* **2**

methoxybenzoate unit. From the ^{1}H- and ^{13}C-NMR (*Table 2*), HMBC, HMQC, NOESY, and ^{1}H,^{1}H-COSY data (*Fig. 2*), compound **2** was elucidated as (1α,6α,14α)-8-acetoxy-13-hydroxy-1,6,16-trimethoxy-4-(methoxymethyl)aconitan-14-yl 4-methoxybenzoate.

The ^{1}H-NMR spectrum of **2** (*Table 2*) showed signals due to an *AA′BB′* system for four aromatic H-atoms (δ 7.97, 6.89, each 2 H, *d*, J = 8.9 Hz), five MeO groups (δ 3.85, 3.53, 3.26, 3.18, and 3.10, each 3 H, *s*), a strongly shielded MeCO group (δ 1.36, *s*), and a methine of an N=CH group (δ 7.30, *s*). The ^{13}C-NMR spectrum (*Table 2*) clearly indicated the presence of a norditerpene moiety (C(1)–C(19)) combined with an anisoyl (=4-methoxybenzoyl unit; C(1′) to C(6′), C(=O)–C(1′)), five MeO groups, an MeCO group (δ 169.7 and 21.5), and an N=CH group (δ 162.3). Its spectral characteristics were similar to those of the known compound crassicauline A (**5**),

Table 2. *^{1}H-* (500 MHz) *and ^{13}C-NMR* (125 MHz) *Data of Macrorhynine B* (**2**) *in $CDCl_3$*. δ in ppm, J in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
H–C(1)	3.88 (*t*, *J* = 3.9)	81.7	H–C(16)	3.48 (*t*, *J* = 6.8)	83.4
CH_2(2)	2.08–2.10 (*m*, H_α),	24.8	H–C(17)	3.77 (*s*)	59.1
	1.76–1.78 (*m*, H_β)		CH_2(18)	3.80 (*d*, *J* = 8.3, H_α),	79.6
CH_2(3)	2.24–2.26 (*m*, H_α),	34.2		3.63 (*d*, *J* = 8.3, H_β)	
	1.91–1.93 (*m*, H_β)		H–C(19)	7.30 (*s*)	162.3
C(4)	–	48.9	C(1′)	–	122.5
H–C(5)	3.60 (*d*, *J* = 6.4)	37.8	H–C(2′/6′)	7.97 (*d*, *J* = 8.9, 2 H)	131.7
H–C(6)	4.04 (*d*, *J* = 6.4)	82.6	H–C(3′/5′)	6.89 (*d*, *J* = 8.9, 2 H)	113.6
H–C(7)	2.78 (*t*, *J* = 8.2)	43.8	C(4′)	–	163.3
C(8)	–	84.2	*C*(=O)–C(1′)	–	165.9
H–C(9)	3.16 (*t*, *J* = 5.9)	54.1	*Me*O–C(1)	3.10 (*s*)	55.4
H–C(10)	2.18–2.20 (*m*)	40.8	*Me*O–C(6)	3.53 (*s*)	58.7
C(11)	–	51.6	*Me*O–C(16)	3.18 (*s*)	57.6
CH_2(12)	1.81 (*d*, *J* = 14.3, H_α),	33.0	*Me*O–C(18)	3.26 (*s*)	59.1
	2.34–2.38 (*m*, H_β)		*Me*O–C(4′)	3.85 (*s*)	55.6
C(13)	–	74.8	MeCOO–C(8)	–	169.7
H–C(14)	4.85 (*d*, *J* = 4.5)	78.0	*Me*COO–C(8)	1.36 (*s*)	21.5
CH_2(15)	2.44 (*dd*, *J* = 15.3, 8.9, H_α),	38.9			
	3.20–3.23 (*m*, H_β)				

except for the absence of an N–Et group in **2**. The ESI-MS of **2** exhibiting a molecular ion at *m/z* 613 (M^+) compared to 643 (M^+) for **5** is consistent with this contention.

In the HMBC plot of **2** (*Fig. 2*), the correlation H–C(14) (δ(H) 4.85)/ C(=O)–C(1′) (δ(C) 165.9) suggested that an anisoyl group was positioned at C(14), while the correlations H–C(17) (δ(H) 3.77) and H–C(18) (δ(H) 3.63)/C(19) (δ(C) 162.3) suggested that C(19) is involved in the N=CH group. The five MeO 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 data. ^{1}H,^{1}H-COSY Correlations of **2** are shown in *Fig. 2*. The relative configuration of **2** was identical with that of **1**, as can be seen from the NOESY data (*Figs. 1* and *2*).

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Experimental Part

General. M.p.: *XT-4* melting-point apparatus, uncorrected. $[\alpha]_D$: *Jasco-20C* digital polarimeter. UV spectra: *UV 210A* spectrometer; λ_{max}(log ε) in nm. IR Spectra: *Bio-Rad FTS-135* spectrometer; in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker Avance-DRX-500* instrument; Me_4Si as internal reference, δ in ppm, *J* in Hz. EI-MS: *VG Autospec-3000* mass spectrometer; in *m/z* (rel. %).

Plant Material. The roots of *A. macrorhynchum* TURCZ. ex LEDEB were collected in Deqin County, Yunnan Province, P. R. China, in September 2001. The identity of the plant material was verified by Prof. *Zhi-Hao Hu*, Department of Biology, School of Life Science, Yunnan University, P. R. China. A voucher specimen (No. 01-005) was deposited in the Key Laboratory of Medicinal Chemistry for Natural Resources, Yunnan University, Kunming, P. R. China.

Extraction and Isolation. The ground roots (5 kg) of *Aconitum macrorhynchum* were extracted with 95% EtOH (5 × 20 l) at r.t. The EtOH extract was evaporated to yield a residue, which was suspended in H_2O and then extracted with petroleum ether, AcOEt, and BuOH, in this order. The AcOEt extract (62 g) was subjected to column chromatography (CC; SiO_2; petroleum ether/AcOEt/Et_3N 60:1:0.1 → 0:1:0.1): *Fractions 1–6*. *Fr. 3* was further purified by CC (1. SiO_2; petroleum ether/AcOEt/Et_3N 5:1:0.1 → 0:1:0; 2. *Sephadex LH-20*, MeOH): **4** (15 mg), **5** (38 mg), and **9** (12 mg). *Fr. 5* was further purified by CC (1. SiO_2; petroleum ether/AcOEt/Et_3N 1:1:0.1 → 1:10:0.1; 2. *Sephadex LH-20*, MeOH): **1** (4 mg) and **2** (6 mg). The BuOH extract (76 g) was subjected to CC (SiO_2; petroleum ether/AcOEt/ Et_3N 30:1:0.1 → 0:1:0.1): *Fractions 1–8*. *Fr. 4* was further purified by CC (SiO_2; petroleum ether/ AcOEt/Et_3N 3:1:0.1 → 0:1:0): **6** (10 mg) and **7** (14 mg). *Fr. 7* was further purified by CC (1. SiO_2; petroleum ether/AcOEt/Et_3N 0:1:0.1 → 1:20:0.1; 2. *Sephadex LH-20*, MeOH): **3** (7 mg) and **8** (11 mg).

Macrorhynine A (*=(1α,6α,14α)-8-Acetoxy-1,6,16-trimethoxy-4-(methoxymethyl)aconitan-14-yl 4-Methoxybenzoate*; **1**): Amorphous solid. $[\alpha]_D^{25} = -9.09$ ($c = 0.110$, $CHCl_3$). UV ($CHCl_3$): 232 (4.48), 259 (4.58). IR (KBr): 3442, 2955, 2925, 2854, 1718, 1659, 1607, 1513, 1461, 1373, 1317, 1281, 1260, 1230, 1169, 1093, 1024, 983, 923, 850, 803, 771. ^{1}H- and ^{13}C-NMR: *Table 1*. ESI-MS: 597 (5, M^+), 566 (3), 538 (2), 506 (2), 446 (2), 420 (1), 385 (1), 370 (1), 354 (2), 298 (1), 236 (2), 180 (2), 149 (3), 136 (9), 135 (100), 107 (4), 92 (4), 77 (6). HR-ESI-MS: 598.3285 ($[M + 1]^+$, $C_{33}H_{44}NO_9^+$; calc. 598.3298).

Macrorhynine B (*=(1α,6α,14α)-8-Acetoxy-13-hydroxy-1,6,16-trimethoxy-4-(methoxymethyl)aconitan-14-yl 4-Methoxybenzoate*; **2**): Amorphous solid. $[\alpha]_D^{25} = -50.75$ ($c = 0.116$, $CHCl_3$). UV ($CHCl_3$): 234 (4.40), 257 (4.50). IR (KBr): 3447, 2964, 2822, 1715, 1634, 1607, 1513, 1460, 1369, 1280, 1231, 1170, 1097, 1020, 944, 911, 849, 801, 772, 760. ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS: 613 (4, M^+), 595 (3), 566 (2), 538 (3), 506 (3), 446 (1), 420 (2), 385 (2), 370 (1), 354 (1), 298 (2), 236 (3), 180 (1), 149 (4), 136 (9), 135 (100), 107 (5), 92 (5), 77 (7). HR-ESI-MS: 614.3296 ($[M + 1]^+$, $C_{33}H_{44}NO_{10}^+$; calc. 614.3311).

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