New Diterpenoid Alkaloids from *Aconitum recemulosum* FRANCH.

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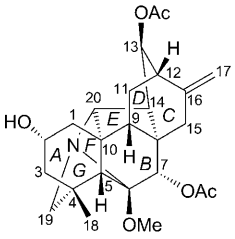
Two new diterpenoid alkaloids, racemulosines A (**1**) and B (**2**), were isolated from the roots of *Aconitum racemulosum* FRANCH. The structures of the new alkaloids were elucidated by analysis of physical and spectroscopic data, and the structure of **1** was further confirmed by a single-crystal X-ray diffraction analysis. Furthermore, compound **1**, at $2.25 \cdot 10^{-4}$ mol/l, showed moderate activity against platelet aggregation induced by PAF (platelet-activation factor).

Introduction. – Plants of the genus *Aconitum* have been used as a traditional Chinese medicinal herb having an analgesic effect. *Aconitum racemulosum* FRANCH. is a species endemic to Qianxi County of Guizhou Province. It is mainly used as a folk medicine to treat fever and rheumatism [1]. To the best of our knowledge, no phytochemical study on this plant had been undertaken [2][3]. In the course of searching bioactive diterpenoid alkaloids, two new alkaloids were isolated from the roots of *Aconitum racemulosum* FRANCH., including one hetisine-type C_{20}-diterpenoid alkaloid, racemulosine A (**1**), and one aconitine-type C_{19}-diterpenoid alkaloid, racemulosine B[1]) (**2**). In this article, we describe the isolation and structural elucidation of **1** and **2**, and their activities against platelet aggregation induced by PAF (platelet-activation factor).



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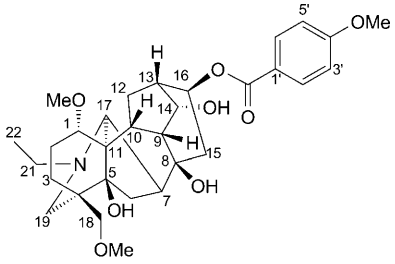

2[1])

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

Results and Discussion. – Racemulosine A (**1**) was isolated as colorless needles. Its molecular formula was determined to be $C_{25}H_{33}NO_6$ from the HR-ESI-MS (*m/z* 444.2395 ($[M+H]^+$)), suggesting 10 degrees of unsaturation. The IR absorptions at 3445 and 1736 cm^{-1} indicated the presence of OH and C=O groups. The ^{1}H- and ^{13}C-NMR data (*Table 1*) of **1** showed two AcO groups (δ(H) 2.07, δ(C) 20.8 and 171.0; δ(H) 2.12, δ(C) 20.7 and 171.2) and one MeO group (δ(H) 3.17, δ(C) 52.6). The remaining 20 C-atoms including one exocyclic C=C bond, one tertiary Me group, four quaternary C-atoms, and eight CH and five CH_2 groups made up its basic heptacyclic skeleton, which strongly suggested that compound **1** is a hetisine-type C_{20}-diterpenoid alkaloid [4–6] (hetisine = (2α,11α,13*R*)-hetisane-2,11,13-triol). Two broad *s* at δ(H) 4.66 and 4.81 (br. *s*, each 1 H) were ascribed to the exocyclic CH_2(17)=C(16) moiety, while the four characteristic H-atom signals of hetisine-type alkaloids [7] appeared at δ(H) 2.92 and 3.19 (*d*, *J* = 11.6, H_α– and H_β–C(19), resp.), 3.55 (br. *s*, H–C(20)), and 1.42 (br. *s*, H–C(12)), which was confirmed by the HMBC spectrum of **1**. Moreover, C(6) at δ(C) 102.0 was attached to both an O-atom and an N-atom, which is a structural character of some hetisine-type alkaloids [8], and the HMBC of the H-atoms of the MeO group with C(6) indicated that the MeO group was linked to C(6) (*Fig. 1*). The HMBCs H–C(7) (δ(H) 5.24)/C(6) and Me*C*=O (δ(C) 171.0) suggested that this AcO

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

	δ(H)	δ(C)		δ(H)	δ(C)
H_α–C(1)	2.26 (br.)	31.3 (*t*)	H–C(12)	1.42 (br.)	47.1 (*d*)
H_β–C(1)	1.33 (*d*, *J* = 3.2)		H–C(13)	4.88 (*t*, *J* = 2.0)	72.3 (*d*)
H–C(2)	4.13 (*t*, *J* = 2.0)	65.5 (*d*)	H–C(14)	2.61 (*d*, *J* = 9.6)	44.9 (*d*)
H_α–C(3)	1.78 (br.)	42.6 (*t*)	CH_2(15)	2.15–2.17 (*m*)	29.6 (*t*)
H_β–C(3)	1.54 (*d*, *J* = 4.0)		C(16)		145.7 (*s*)
C(4)		35.9 (*s*)	H_α–C(17)	4.66 (*s*)	108.3 (*t*)
H–C(5)	1.75 (*s*)	57.8 (*d*)	H_β–C(17)	4.81 (*s*)	
C(6)		102.0 (*s*)	Me(18)	1.14 (*s*)	30.4 (*q*)
H–C(7)	5.24 (*s*)	71.0 (*d*)	H_α–C(19)	2.92 (*d*, *J* = 11.6)	61.4 (*t*)
C(8)		47.7 (*s*)	H_β–C(19)	3.19 (*d*, *J* = 11.6)	
H–C(9)	2.21 (br.)	39.1 (*d*)	H–C(20)	3.55 (*s*)	67.8 (*d*)
C(10)		46.5 (*s*)	*Me*O–C(6)	3.17 (*s*)	52.6 (*q*)
H_α–C(11)	1.61 (br.)	22.1 (*t*)	*Ac*O–C(7)	2.07 (*s*)	20.8 (*q*), 171.0 (*s*)
H_β–C(11)	2.09–2.10 (*m*)		*Ac*O–C(13)	2.12 (*s*)	20.7 (*q*), 171.2 (*s*)

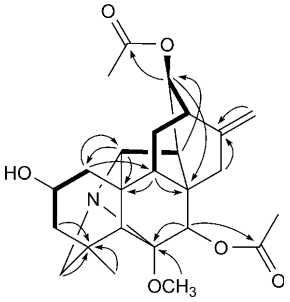

Fig. 1. *Key ^{1}H,^{1}H-COSY* (—) *and HMBCs* (H → C) *of* **1**

group was located at C(7). The position of the other AcO group was determined to be C(13) by the HMBCs H–C(13) (δ(H) 4.88)/C(12), C(14), and Me*C*=O (δ(C) 171.2). The OH group of **1** was ascribed to C(2) by ^{1}H,^{1}H-COSY and HMBC data (*Fig. 1*), which were similar to those of orochrine (=(2α,21*S*)-2,6-dihydroxy-21-methyl-13-oxohetisanium) [7]. Thus, the planar structure of **1** was established.

The relative configuration of **1** was established by analysis of a single-crystal X-ray diffraction study. In the crystal structure (*Fig. 2*), the rings *A* (C(1)–C(2)–C(3)–C(4)–C(5)–C(10)) and *B* (C(5)–C(6)–C(7)–C(8)–C(9)–C(10)) took a chair conformation; the ring *C* (C(8)–C(9)–C(11)–C(12)–C(16)–C(15)) took a twist-boat confirmation; the rings *D* (C(8)–C(9)–C(11)–C(12)–C(13)–C(14)) and *G* (C(4)–C(5)–C(10)–C(20)–N(1)–C(19)) took a boat conformation; and the rings *E* (C(8)–C(9)–C(10)–C(20)–C(14)) and *F* (C(5)–C(6)–C(10)–C(20)–N(1)) took an envelope conformation. H–C(2), H–C(5), H–C(7), H–C(9), H–C(12), H–C(14), H–C(20), MeO–C(6), and Me–C(4) all took the β-configuration, and H–C(13) was α-orientated.

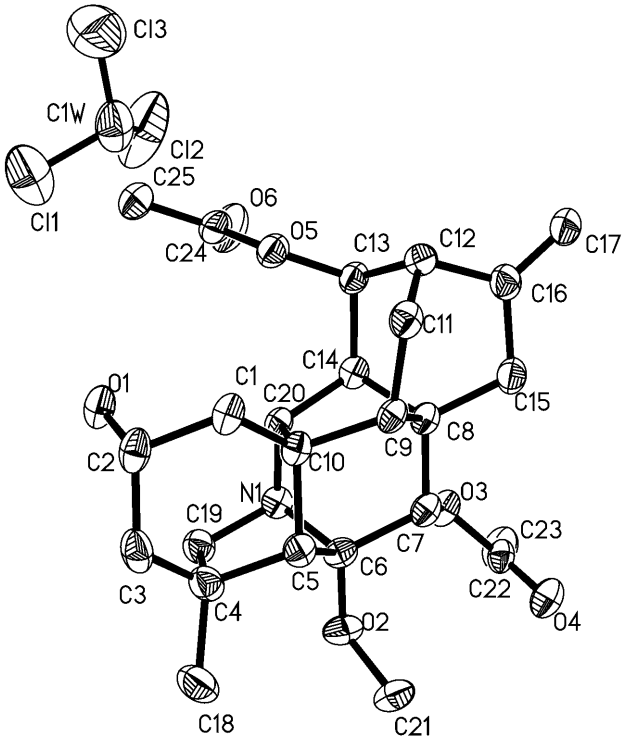

Fig. 2. *X-Ray crystal structure of* **1** (ORTEP drawing)

Racemulosine B (**2**) was isolated as an orange oil. Its molecular formula was determined to be $C_{31}H_{44}NO_8$ on the basis of the HR-ESI-MS (*m*/*z* 558.3069 ([*M* + H]$^+$)). The IR spectrum of **2** showed absorptions for an OH group (3449 cm^{-1}), a C=O group (1705 cm^{-1}), and an aromatic moiety (1607, 1512, and 1490 cm^{-1}). The

NMR spectra (*Table 2*) indicated the presence of one $MeCH_2N$ group (δ(H) 1.06 (*t*, $J = 7.2$ Hz, 3 H); δ(C) 13.6 (*q*) and 49.1 (*t*)), an anisoyl group (δ(H) 7.94 and 6.90 (*AA′BB′*, $J = 8.0$ Hz, each 2 H); δ(C) 165.3 (*s*), 131.5 (2*d*), 122.7 (*s*), 113.7 (2*d*), and 163.4 (*s*)), and three MeO groups (δ(H) 3.27, 3.34, and 3.86 (*s*, each 3 H); δ(C) 56.5 (*q*), 59.6 (*q*), and 55.5 (*q*)) including one of the anisoyl group. The remaining 19 C-signals (*Table 2*) exhibited characteristic data of aconitine-type C_{19}-diterpenoid alkaloids, including seven CH_2 and eight CH groups and four quaternary C-atoms. The ^{1}H- and ^{13}C-NMR data of **2** were very closely related to those of the known compound circinasine D (=(1α,14α,16β)-20-ethyl-1-methoxy-4-(methoxymethyl)aconitane-5,8,14,16-tetrol 14-(4-methoxybenzoate)) [9]. Comparison of the NMR spectra of these two alkaloids showed that H–C(14) (*t*, $J = 4.8$ Hz) of **2** was upfield-shifted from δ(H) 5.29 to 4.34, while H–C(16) (*d*, $J = 9.6$ Hz) of **2** was downfield-shifted from δ(H) 3.80 to 5.09, which revealed the locations of the OH group at C(14) and the anisoyl ester group at C(16) in **2**, which were inverted in comparison with those of circinasine D. The coupling constant of H–C(16) of **2**, similar to that of circinasine D, suggested the β-orientation for anisoyl ester group in **2**. A detailed analysis of the 2D-NMR data including HMQC and HMBC spectra (*Fig. 3*), further confirmed this assumption, especially the HMBCs C(14)/H–C(10) and H–C(9), and C(16)/H–C(13) and H–C(15). Thus, the structure of **2** was determined as shown in *Fig. 3* and named racemulosine B (**2**).

The effects against platelet aggregation induced by PAF were evaluated for compounds **1** and **2**. Compound **1**, at $2.25 \cdot 10^{-4}$ mol/l, showed a significant inhibitory activity (inhibition [%]: 26.73 ± 8.55 for **1** and 39.33 ± 16.53 for aspirin at $1 \cdot 10^{-3}$ mol/l; $n = 4$, $X^- \pm$ s.d.) of *in vitro* platelet aggregation induced by PAF, and compound **2** at

Table 2. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *of* **2**[1]). δ in ppm, J in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
H–C(1)	3.15–3.17 (*m*)	83.8 (*d*)	H_β–C(15)	2.16–2.18 (*m*)	
H_α–C(2)	2.32–2.35 (*m*)	26.1 (*t*)	H–C(16)	5.09 (*d*, $J = 9.6$)	74.9 (*d*)
H_β–C(2)	2.01–2.04 (*m*)		H–C(17)	3.13 (*s*)	63.4 (*d*)
H_α–C(3)	2.21–2.22 (*m*)	28.3 (*t*)	H_α–C(18)	2.97 (*d*, $J = 9.2$)	78.9 (*t*)
H_β–C(3)	1.39–1.41 (*m*)		H_β–C(18)	3.66 (*d*, $J = 9.2$)	
C(4)		41.0 (*s*)	H_α–C(19)	1.82 (*d*, $J = 11.2$)	55.4 (*t*)
C(5)		84.6 (*s*)	H_β–C(19)	2.56 (hidden)	
H_α–C(6)	2.13 (br.)	34.6 (*t*)	H_α–C(21)	2.35–2.38 (*m*)	49.1 (*t*)
H_β–C(6)	1.97–1.99 (*m*)		H_β–C(21)	2.53–2.55 (*m*)	
H–C(7)	2.03 (*s*)	44.8 (*d*)	Me(22)	1.06 (*t*, $J = 7.2$)	13.6 (*q*)
C(8)		73.5 (*s*)	*Me*O–C(1)	3.27 (*s*)	56.5 (*q*)
H–C(9)	2.56 (*t*, $J = 4.8$)	47.0 (*d*)	*Me*O–C(18)	3.34 (*s*)	59.6 (*q*)
H–C(10)	2.23–2.25 (*m*)	40.9 (*d*)	Ar*C*O		165.3 (*s*)
C(11)		50.5 (*s*)	C(1′)		122.7 (*s*)
H_α–C(12)	1.36 (*d*, $J = 4.0$)	28.2 (*t*)	H–C(2′, 6′)	7.94 (*d*, $J = 8.0$)	131.5 (*d*)
H_β–C(12)	2.21–2.23 (*m*)		H–C(3′, 5′)	6.90 (*d*, $J = 8.0$)	113.7 (*d*)
H–C(13)	2.26–2.29 (*m*)	39.3 (*d*)	C(4′)		163.4 (*s*)
H–C(14)	4.34 (*t*, $J = 4.8$)	75.1 (*d*)	*Me*O–C(4′)	3.86 (*s*)	55.5 (*q*)
H_α–C(15)	2.73–2.75 (*m*)	40.2 (*t*)			

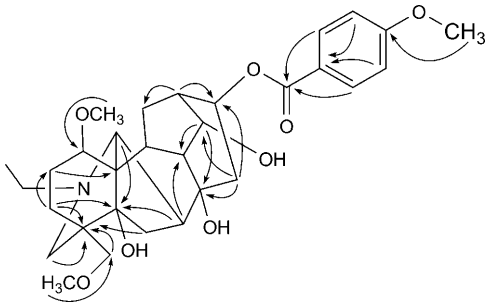

Fig. 3. *Key HMBCs* (H ⌒ C) *of* **2**

$1.79 \cdot 10^{-4}$ mol/l showed a weak inhibitory activity (inhibition [%]: 17.12 ± 8.55 for **2** and 39.33 ± 16.53 for aspirin at $1 \cdot 10^{-3}$ mol/l; $n = 4$, $X^- \pm$ s.d.).

Financial support from the *Modernization of Traditional Chinese Medicine of Guizhou Province* (No. 20065041) and the *Ministry of Science and Technology* (No. 2007CB516813) are gratefully acknowledged.

Experimental Part

General. All solvents used for extraction and isolation were distilled prior to use. Petroleum ether for chromatography had a b.p. range of 60–90°. Column chromatography (CC): silica gel (SiO_2; 200–300 and 300–400 mesh, resp.; *Qingdao Haiyang Chem. Ind. Co. Ltd.*, China), SiO_2 *H* (10–40 μm; *Qingdao*); monitoring by TLC, detection by spraying with *Dragendrorff*'s reagent. Optical rotations: *Jasco-DIP-370* digital polarimeter. IR Spectra: *Bio-Rad-FTS-135* spectrometer; KBr discs; in cm^{-1}. 1D- and 2D-NMR Spectra: *Inova-400* MHz NMR spectrometer with Me_4Si as an internal standard; chemical shifts δ in ppm rel. to residual solvent signals, *J* in Hz. ESI- and HR-ESI-MS: *VG-Autospec-3000* spectrometers; in *m*/*z* (rel. %).

Plant Material. Roots of *Aconitum racemulosum* FRANCH. were collected in Qianxi of Guizhou Province, P. R. China, in November 2007, and identified by Prof. *De-Yuan Chen*, Guiyang College of Traditional Chinese Medicine.

Extraction and Isolation. The air-dried roots of *Aconitum racemulosum* FRANCH. (3.5 kg) were percolated three times with 95% EtOH to give a crude extract. The extract was concentrated and the residue partitioned between AcOEt and 5% HCl soln. The aq. phase was adjusted to pH *ca.* 9 with sat. NH_3/H_2O soln. and extracted with $CHCl_3$ to give crude alkaloids (22.5 g). The crude alkaloids were subjected to CC (SiO_2, $CHCl_3$/MeOH 50:1 → 1:1): *Fractions A–K*. *Fr. B* was further subjected to CC (SiO_2, petroleum ether/acetone/Et_2NH 5:2:0.1). *Fr. C* (3.4 g), eluted with $CHCl_3$/MeOH 50:1, was separated and purified by repeated CC (SiO_2, $CHCl_3$/MeOH 40:1 and petroleum ether/Et_2NH 20:1 → 4:1): **1** (203 mg). *Fr. D* was subjected to repeated CC (SiO_2 *H*, petroleum ether/acetone/Et_2NH 15:3:1 → 15:5:1) and CC (SiO_2, petroleum ether/Et_2NH 100:1 → 20:1): **2** (41 mg).

Racemulosine A (=*(2α,7α,13*S*)-6-Methoxyhetisane-2,7,13-triol 7,13-Diacetate*; **1**): Colorless needles (petroleum ether/acetone). M.p. 286–288°. $[\alpha]_D^{28} = -22.8$ ($c = 0.11$, $CHCl_3$). IR (KBr): 3445, 2923, 1736, 1657, 1428, 1370, 1236, 962, 880. ^{1}H- and ^{13}C-NMR (400 and 100 MHz, resp., $CDCl_3$): *Table 1*. ESI-MS: 444.2 ($[M+H]^+$). HR-ESI-MS: 444.2395 ($[M+H]^+$, $C_{25}H_{34}NO_6^+$; calc. 444.2386).

Racemulosine B (=*(1α,14α,16β)-20-Ethyl-1-methoxy-4-(methoxymethyl)aconitane-5,8,14,16-tetrol 16-(4-Methoxybenzoate)*; **2**): Orange oil. $[\alpha]_D^{28} = 0.00$ ($c = 0.08$, $CHCl_3$). IR (KBr): 3449, 2925, 1705, 1607, 1512, 1490, 1465, 1258, 1170, 1102. ^{1}H- and ^{13}C-NMR (400 and 100 MHz, resp., $CDCl_3$): *Table 2*. ESI-MS: 558.2 ($[M+H]^+$). HR-ESI-MS: 558.3069 ($[M+H]^+$, $C_{31}H_{44}NO_8^+$; calc. 558.3066).

X-Ray Crystal Data of **1**. A colorless crystal (0.20 × 0.30 × 0.30 mm) obtained from petroleum ether/acetone was selected for X-ray analysis. The crystallographic data was collected with a *MAC-DIP-2030*K

diffractometer and graphite-monochromated CuK_α radiation. Structure analysis was made with the SHELXS97 program on a PC. The compound crystallized in the space group $P2_1$; $a = 10.5004(2)$, $b = 11.5990(2)$, $c = 11.6645(2)$ Å; $\beta = 106.14(1)^\circ$; $V = 1364.7$ (1) Å^3, $Z = 2$, $D_{calc.} = 1.370$ g/cm^3. The final R indexes were $R_1 = 0.0490$, $wR_2 = 0.1379$, and $S = 1.039$. CCDC-716901 contains the crystallographic data for compound **1**. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/data_request/cif.

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Received February 23, 2009