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TWO NEW C_{19}-DITERPENOID ALKALOIDS FROM *ACONITUM RACEMULOSUM* FRANCH VAR. *PENGZHOUENSE*

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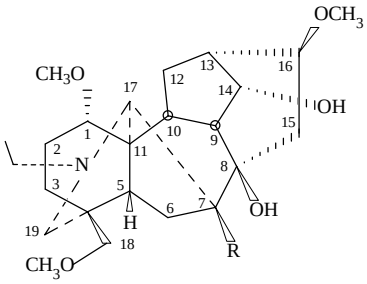
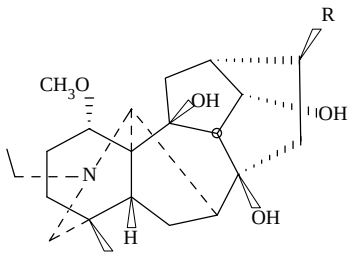
Abstract- Two new C_{19}-ditepenoid alkaloids, racemulolines A (**1**) and B (**2**), were isolated from the whole plants of *Aconitum racemulosum* Franch var. *pengzhounense,* and their structures were elucidated by spectral analysis.

In continuation of our research in *Aconitum racemulosum* Franch var. *pengzhonense* W. J. Zhang et G. H. Chen,[1] two C_{19}-diterpenoid alkaloids, racemulolines A (**1**) and B (**2**), were isolated. In this paper, we report separation and structural elucidation of the new alkaloid (**1**~**2**). The new bases, racemulolines A (**1**) and B (**2**), were isolated as amorphous powder substances. Their molecular formulae, $C_{24}H_{39}NO_6$ and $C_{22}H_{35}NO_4$, respectively, inferred from their HREIMS and ^{13}C NMR spectra. Their NMR and MS spectra showed that they were the C_{19}-diterpenoid alkaloids.[2,3]



1 R= OH
3 R= H



2 R=H
4 R=OCH_3

The NMR spectra of recemuloline A (**1**) gave the signals at δ_H 1.06 (3H, t, *J*=7.1 Hz) and δ_C 51.4 t, 14.4 q for an *N*-ethyl group, δ_H 3.23, 3.28, 3.33 (each 3H, s), δ_C 55.8 q, 56.3 q and 59.2 q for three aliphatic methoxyl groups. Its IR and ^{13}C NMR spectra also showed distinctive signals at

3360 cm^{-1}, δ_C 74.3 s and 87.6 s for two tertiary hydroxyl groups, and δ_C 75.6 d for a secondary hydroxyl group. The presence of the base peak at *m/z* 406 (M^+-31) in the MS spectrum of **1** showed that it had the methoxyl group at C-1.[4] Another methoxyl group in **1** may be located at C-18 by showing the oxygenated methylene signal at δ_C 78.9 t,[2,3] and the remained methoxyl group at C-16 only according to biogenetical consideration for the C_{19}-diterpenoid alkaloids. The 1H triplet (*J*=4.8 Hz) signal at δ 4.10 in the ^{1}H NMR spectrum of **1** is assigned to the H-14β, indicating the presence of 14-hydroxyl group. Possibilities of the 5- or 10-hydoxyl group substitutions may be ruled out because of the absence of the down-shifted of C-5 or C-11, as in bonvalol[5] or 10-hydroxy isotalatizidine,[6] in the ^{13}C NMR spectrum of **1**. Its ^{13}C NMR spectrum (Table 1) is very similar to that of talatisamine (**3**)[2] except for the signals belonging to C-6, C-7, C-8, C-15 and C-17, indicating that it had an additional tertiary hydroxyl group at C-7. The structure of racemuloline A, thus, was determined as **1**.

Table **1** ^{13}C NMR data of compounds (**1**, **2**, **3** and **4**) (50 MHz; $CDCl_3$)

Carbon	**1**	**3**	**2**	**4**	Carbon	**1**	**3**	**2**	**4**
1	85.6 *d*	86.1	78.8 *d*	78.6	13	37.2 *d*	37.7	34.8 *d*	37.5
2	25.3 *t*	25.7	26.0 *t*	26.0	14	75.6 *d*	75.7	73.0 *d*	73.9
3	32.2 t	32.5	37.5 *t*	37.5	15	34.0* *t*	39.2	30.3 *t*	39.5
4	38.3 *s*	38.6	34.4 *s*	34.3	16	81.7 *d*	82.2	25.7 *t*	81.5
5	46.1 *d*	38.6	47.0 *d*	36.6	17	64.0 *d*	62.8	63.8 *d*	63.5
6	33.2* *t*	24.8	22.7 *t*	23.7	18	78.9 *t*	79.4	26.3 *q*	26.2
7	87.6 *s*	45.7	45.9 *s*	44.8	19	52.0 *t*	53.1	56.6 *t*	56.3
8	74.3 *s*	72.7	74.3 *s*	72.0	$N-CH_2$	51.4 *t*	53.1	49.4 *t*	49.3
9	47.8 *d*	46.9	55.2 *d*	55.6	CH_3	14.4 *q*	13.6	13.5 *q*	13.6
10	45.3 *d*	45.7	80.7 *s*	80.8	1'	55.8 *q*	56.1	56.0 *q*	56.3
11	48.7 *s*	48.6	54.7 *s*	54.0	16'	56.3 *q*	56.3	—	55.9
12	27.2 *t*	28.6	40.5 *t*	37.5	18'	59.2 *q*	59.3	—	—

* exchangeable.

The NMR spectral data of racemuloline B (**2**) revealed signals at δ_H 1.05 (3H, t, *J*=7.1 Hz), δ_C 49.4 t, 13.5 q for an *N*-ethyl group; δ_H 3.28 (3H, s), δ_C 56.0 q for one methoxyl group, and δ_H 0.78 (3H, s), δ_C 26.3 q for the methyl group. Its IR and ^{13}C NMR spectra showed the presence of two tertiary hydroxyl groups (3366 cm^{-1}, δ_C 74.3 s and 80.7 s) and the secondary hydroxyl group (3366 cm^{-1}, δ_C 73.0 d). The presence of the lower-field 1H triplet (*J*=5.2 Hz) signal at δ 4.65 attributable to a proton attracted to C-14 carrying a hydroxyl group in the ^{1}H NMR spectrum of **2** indicated that the compound had a hydroxyl group at C-10.[7] Only one methoxyl group in **2** was located at C-1 because of showing the base peak at m/z 346 (M^+-31) in the MS spectrum of racemuloline B.[4] Comparison of the ^{13}C NMR spectra of **2** with genicunine C (**4**)[8] showed that it lacked the 16-OMe group. The ^{13}C NMR data of both the alkaloids are very similar except for

C-8, C-13, C-15 and C-16 (Table 1), finally leading to determine the structure of racemuloline B as **2**.

EXPERIMENTAL

General Experimental Procedure. IR spectrum was recorded on a Nicolet 200 SXV spectrophotometer. Optical rotation was measured by a Perkin-Elmer 241 polarimeter, $CHCl_3$, 1cm cell. HREIMS was taken on a VG Auto spec 3000 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC-E 200 spectrometer, TMS as internal standard. Silica gel GF_{254} and H (Qindao Sea Chemical Factory, China) were used for TLC, Chromatotron and CC, respectively. Spots on chromatograms were detected under UV light (254 nm) and with modified Dragendorff's reagent. A polyvinyl sulfonic ion exchange resin (H-form, cross linking 1×3, Nankai University Chemical Factory, China) was used in the extraction of total alkaloids.

Plant Material. Plants were collected in Peng country of Sichuan province, China, and authenticated by Professor W. T. Wang, Institute of Botany, Chinese Academy of Sciences, where a voucher specimen has been deposited.

Extraction. According to the literature method[9], 4.0 kg of dried powdered whole plants of *Aconitum racemulosum* Franch var. *pengzhouense* were percolated at room temperature with 0.2% HCl until 50 L were collected. A column of 9 kg wet resin (dry weight 0.9 kg) was used to treat the percolates. After exchange, the resin was washed repeatedly on a section filter with deionized water, spread out and air dried overnight. The resin, now weight 1.1 kg, was well mixed with 1,800 mL of 10% ammonia and continuously extracted in a specially designed extractor[9] with several portions of ether (total amount: 2,500 mL) under reflux until a negative detection to modified Dragendorff's reagent. White powder (18.6 g) of the crude alkaloid Ⅰ from the ethereal extracts were collected by evaporation of ether on reduced pressure. Then, extraction of the resin with 95% EtOH (5,000 mL) for 10 h furnished 38.6 g of the brownish residue of the crude alkaloid Ⅱ.

Isolation. Using a pH gradient method, the crude alkaloids Ⅰ (18 g) was separated in four parts, part A (pH 2, 1.7 *g*), part B (pH 7, 5.7 *g*), part C (pH 9, 9.5 *g*) and part D (pH 11, 910 mg). Part A was chromatographed successively on silica gel column eluting with $CHCl_3$-MeOH (93:7 →95:5) to give racemuloline A (**1**). Part D was chromatographed on silica gel Chromatotron eluting with $CHCl_3$-MeOH (95:5)→cyclohexane-acetone (2:1) to afford racemulodine B (**2**) (20

mg).

Identification of compounds (1) and (2). Racemuloline A (**1**): white amorphous powder substance, $[\alpha]_D^{17}$ +11.2 ° (c 0.5). IR_{max}^{KBr} cm^{-1}: 3360, 1093; EIMS: *m/z* (%) 437 (3, M^+), 406 (100, M-31), 390 (14), 407 (30), 71 (13), 45 (19); ^1H NMR (200 MHz) δ 1.06 (3H, t, *J*=7.1 Hz, *N*CH_2*CH*$_3$), 3.23, 3.28, 3.33 (each 3H, s, 3×OCH_3), 4.10(1H, t, *J*=4.8 Hz, H-14β); ^{13}C NMR (50 MHz) see Table 1; HREIMS *m/z* 437.2759, calcd for $C_{24}H_{39}NO_6$ 437.2777.

Racemulonine B (**2**): white amorphous powder substance, $[\alpha]_D^{17}$ +20.5 ° (c 0.55). IR_{max}^{KBr} cm^{-1}: 3366, 1094; EIMS *m/z* (%) 377 (10, M^+), 346 (100, M^+-31), 362 (5, M^+-15), 330 (9); ^1H NMR (200 MHz): δ 0.78 (3H, s, H-18), 1.05 (3H, t, *J*=7.1 Hz, *N*CH_2*CH*$_3$), 3.28 (3H, s, OCH_3), 3.76 (1H, dd, *J*=12.6, 6.8 Hz, H-1β), 3.56 (1H, br s, W1/2=10.9 Hz, H-1α), 3.69 (1H, d, *J*=8.4 Hz, H-6β), 4.65 (1H, t, *J*=4.8 Hz, H-14β); ^{13}C NMR (50 MHz) see Table 1; HREIMS *m/z* 377.2561, calcd for $C_{22}H_{35}NO_4$ 377.2566.

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