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Racemulosine, a Novel Skeletal C_{20}-Diterpenoid Alkaloid from *Aconitum racemulosum* Franch var. *pengzhouense*

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Abstract—The structure of racemulosine (**1**), a novel skeletal C_{20}-diterpenoid alkaloid isolated from *Aconitum racemulosum* Franch var. *pengzhouense*, was confirmed on the basis of spectroscopic data including 2D NMR (HMQC, ^{1}H COSY, HMBC, NOESY) and especially X-ray crystallographic analysis. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

In the course of our studies on the alkaloids of *Aconitum* and *Delphinium*,[1–8] we investigated the alkaloids of *Aconitum racemulosum* var. *pengzhouense* (*Ranunculaceae*),[9] a species endemic to Peng county, Sichuan province in China. The total alkaloid (11.3 g) was obtained by using an ion-exchange resin method[10] from the dry whole plant (2.2 kg) and subjected to pH gradient extraction followed by silica gel chromatography to yield a novel skeletal C_{20}-diterpenoid alkaloid racemulosine (**1**), in addition to the known norditerpenoid alkaloids, 1-*epi*-chasmanine, talatisamine, isotalatizidine, vilmorrianine D, nevadenine, pseudaconine, viresenine, lycoctonine, hordenine and the diterpenoid alkaloid (**2**).[11] We describe here the structure elucidation of this new minor alkaloid by applying 2D NMR techniques and X-ray crystallographic analysis.

Results and Discussion

Racemulosine (**1**) was obtained as colorless orthorhombic crystals ($CHCl_3$–MeOH), mp 228–230°C; $[\alpha]_D^{17}$ −19.2 (*c* 0.5, $CHCl_3$–MeOH). The formula $C_{22}H_{32}N_2O_3$ was confirmed by its HRMS, 2D NMR data and X-ray crystallographic analysis. The 1D NMR (^{1}H, ^{13}C) and 2D NMR (^{1}H–^{1}H COSY, HMQC, HMBC, NOESY) spectra in $CDCl_3$–CD_3OD were measured on a 400 MHz spectrometer (Table 1). The IR and ^{1}H (^{13}C) NMR spectra of racemulosine (**1**) showed the presence of one methine carbon bearing the hydroxyl group (3328 cm^{-1}; δ_H 3.86, d, J=8.8 Hz; δ_C 76.0d), one quaternary carbon bearing the hydroxyl group (3328 cm^{-1}; δ_C76.0s), one *N*-ethyl group (δ_H 1.11, 3H, t, J=7.2 Hz; 2.58, 2H, q, J=7.0 Hz; δ_C 48.3t, 13.4q), and one primary amide group (3411, 3183, 1652, 1602, 1450 cm^{-1}; δ_C 179.5s). Its ^{1}H (^{13}C) NMR spectra also displayed one vinyl group (δ_H 5.63, 1H, dd, J=17.6, 11.2 Hz; 4.90, 1H, dd, J=17.2, 1.2 Hz; 4.95, 1H, dd, J=11.0, 1.2 Hz; δ_C 142.3d, 113.2t). Unsuccessful attempts to deduce the structure of racemulosine led us to use X-ray diffraction analysis to determine unambiguously its structure as **1** (Fig. 1).

*Crystal structure for racemulosine (**1**):* a colorless orthorhombic crystal from $CHCl_3$–MeOH was mounted on a P_4 four circle diffractometer and exposed to graphite-monochromated MoKα irradiation. The unit cell parameters are a=10.9720(10) Å, b=12.3650(10) Å, c=13.867(2) Å in space group $P2_12_12_1$ (Z=4). Of the 3131 measured with $1.5 \leq \theta \leq 27$ scan, 2824 were independently observed at the level of $F > 4\sigma(F)$. The structure was solved by the direct method using the program SHELXTL and the atomic parameters were refined by the full-matrix least squares on F^2 method. The final R indexes [$I \geq 2\sigma(I)$] was R^1=0.0364, wR^2=0.0797.

The ORTEP diagram of racemulosine (**1**) and the labeling of all nonhydrogen atoms are shown in Fig. 1. The X-ray crystallographic studies of racemulosine (**1**) had shown that the ring A adopts an envelope comformation, stabilized by the intramolecular N(1)···H–O–C(1) hydrogen bond with a dihedral angle of 140.4°. This is similar to the norditerpenoid alkaloids possessing an α-hydroxyl group at C-1.[12] While its ring D exists in a boat conformation, like all of the norditerpenoid alkaloids, which is flattened at C-15. In addition, another unusual intramolecular hydrogen bond with a dihedral angle of 164.4° between the C(17)═O and OH group at C-8 was also observed.

Keywords: *Aconitum racemulosum* Franch var. *pengzhouense*; diterpenoid alkaloid; racemulosine.

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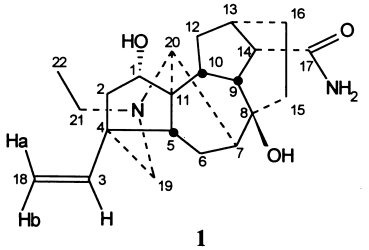
Table 1. ^{1}H and ^{13}C NMR data of racemulosine (**1**) ($CDCl_3$–CD_3OD)

No	^{1}H	Mult (J=Hz)	^{13}C	HMBC (H→C)	NOESY
1	3.86	d (8.8)	76.0d	2, 4, 10, 20	H-5, H-10, H-12, H-17
2	1.63 (a)/2.24 (e)	m/dd(14.4,9.6)	49.9t	1, 4/4, 5	H-21/H-3, H-19
3	5.63	dd (17.6, 11.2)	142.3d	2, 5, 19	H-2, H-5
4	–	–	58.0s	–	–
5	1.93	t (7.8)	54.1d	1, 3, 4, 20	H-1, H-3
6	1.50 (a)/2.00 (e)	m/m	25.5t	4, 5, 7, 8/ 4, 5, 7, 8	H-9, H-19
7	2.10	m	47.6d	5, 6, 8, 9, 15	
8	–	–	76.0s	–	–
9	2.38	m	44.7d	7, 8, 10	H-6
10	2.08	m	46.6d	1, 8, 9, 12, 13	H-1, H-14
11	–	–	47.8s	–	–
12	1.30 (a)/2.15 (e)	m/m	27.4t	9	H-11, H-14, H-17
13	2.54	m	35.9d	9, 10, 15, 16	
14	2.66	t (4.0)	51.3d	8, 9, 12, 13, 20	H-10
15	1.72	m	32.5t	8	
16	1.70 (a)/2.10 (e)	m/m	34.6t	8.14	
17	–	–	179.5s	–	–
18	4.90 (a)/4.95 (b)	dd (17.2, 1.2)/ dd (11.0, 1.2)	113.2t	3/3	
19	2.37/2.61	ABq (10.8)	56.0t	2, 3, 21/20	H-2 (e)/H-6 (a)
20	3.00	br. s	63.1d	5, 8, 10, 21	H-1
21	2.58	q (7.0)	48.3t	19	H-1
22	1.11	t (7.2)	13.4q		H-17, H_2-19

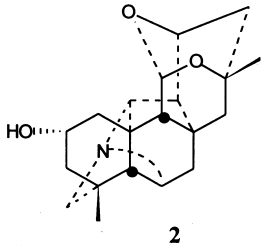
It is noteworthy that the new type diterpenoid alkaloid racemulosine (**1**) is derived biogenetically from the denudatine-type diterpenoid alkaloids, such as **3**, both rings A and C undergoing Wagner–Meerwein rearrangement followed by functionalization of the *exo* methylene group (Fig. 2). In this case, the rearrangement process for the ring A in **3** is similar to that from **4** to **5** (Fig. 3).[13] While the B-homo-C-nor rearrangement in **3** was also observed in the total synthesis of the norditerpenoid alkaloids, such as delphinine[14] and (±)-13-desoxydelphonine[15] or conversion from the atisane→lycoctonine skeleton[16] and, as well, the naturally occurring diterpenoid alkaloids, such as actaline (**6**)[17] and ajabicine (**7**).[18]

Finally, unambiguous assignments of the ^{1}H and ^{13}C chemical shifts for racemulosine (**1**) were accomplished using the 2D NMR techniques (^{1}H–^{1}H COSY, HMQC HMBC, NOESY).

It is of interest to note that racemulosine (**1**) is the first novel skeletal C_{20}-diterpenoid alkaloid with the rearrangements of both the A and C rings.



1



2

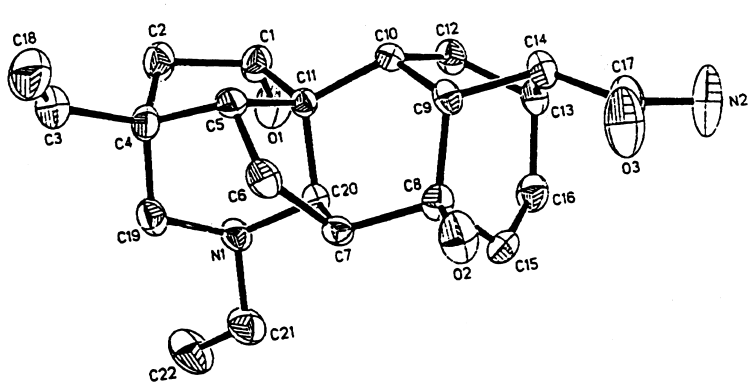


Figure 1. ORTEP stereodrawing of racemulosine (**1**).

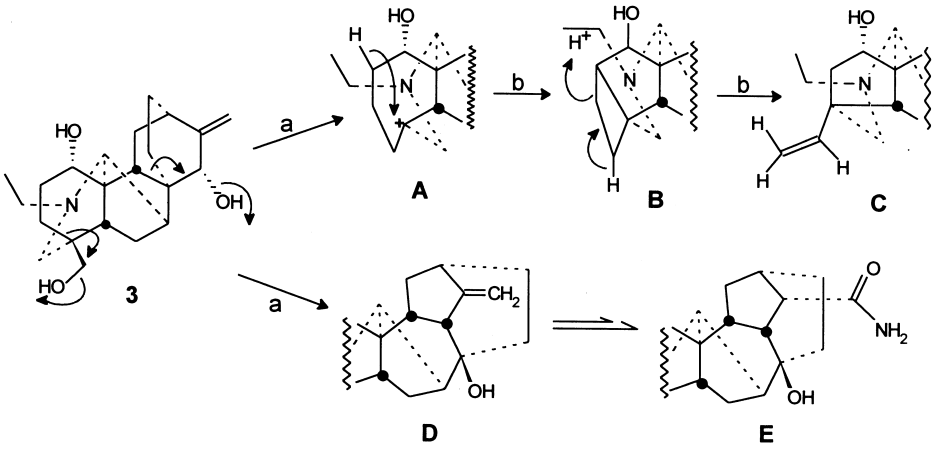


Figure 2. A plausible biogenetic pathway to formation of the A and C rings for racemulosine (**1**), (a) Wagner–Meerwein rearrangement; (b) hydrogen elimination.

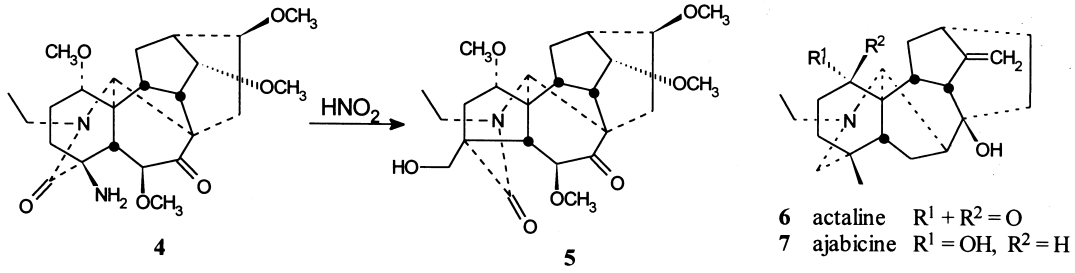


Figure 3. Acid-catalyzed rearrangement of the A ring for compound **4**.

Experimental

General

IR spectrum: Nicolet 200 SXV; OR: Perkin–Elmer 241, $CHCl_3$, 1 cm cell; MS: VG 7070E GC/MS/DES and VG Auto spec 3000; ^1H and ^{13}C NMR: Varian INOVA-400/54, TMS as internal standard; Melting point (uncorrected): RD-1 apparatus; Silica gel (GF_{254} and H) (Qindao Sea Chemical Factory, China) were used for TLC, Chromatodron and CC; A polyvinyl sulphonic 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 county 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 and isolation of alkaloids

According to the literature method,[10] 2.2 kg of dried powdered whole plants of *A. racemulosum* Franch var. *pengzhouense* was percolated with 0.2% HCl until 40 L was collected. A column of resin (dry weight 0.9 kg) was used to treat the percolates, the resin was then washed repeatedly with deionized water, spread out and air dried overnight. The resin was well mixed with 2.3 L of aqueous ammonia (10%) and continuously extracted in a specially designed extractor[10] with several portions of ether under reflux until a negative detection to Dragendorff's reagent. White powder (10.26 g) of the crude alkaloid I from the ethereal extracts were collected by evaporation of ether, then, extraction of the resin with 95% EtOH for 3 h followed by evaporation of EtOH furnished 1.04 g of the crude alkaloid II. This was chromatographed on Si gel (50 g) eluting $CHCl_3$–MeOH (93:7) to give fractions (183 mg), which was chromatographed on a Chromatotron (Si gel GF_{254}, 1 mm thick) eluting with cyclohexane–acetone (3:1) to give racemulosine (**1**) (63 mg). Separation and identification (TLC, mp, MS, ^1H and ^{13}C NMR) of the known alkaloids see Ref. 11.

Racemulosine. IR (KBr) 3411, 3328, 3183, 3081, 1652, 1650, 1602, 1450, 1424, 1303, 1013, 911 cm^{-1}; ^1H and ^{13}C NMR see Table 1. EIMS *m/z* (rel. int.): 372 (M^+, 20), 357 (M−15, 100), 539 (10); HRMS (EI) calcd for $C_{22}H_{32}N_2O_3$: (M^+) *m/z* 372.2411. Found: 372.2401.

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