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Two New Diterpenoid Alkaloids from *Aconitum brachypodum*

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Two new diterpenoid alkaloids, N(19)-en-denudatine (**1**) and N(4)-butanone-flavaconitine (**2**), were isolated from *Aconitum brachypodum* Diels.. Their structures were elucidated by comprehensive spectroscopic analyses including UV, IR, MS, 1D- and 2D-NMR.

Key Words: *Aconitum brachypodum*, Diterpenoid alkaloids, N(19)-en-denudatine and N(4)-butanone-flavaconitine

Introduction

Aconitum brachypodum Diels., a folk herb, is mainly distributed in Yunnan and Sichuan provinces in China.[1] Its dried roots, named "Xue-Shang-Yi-Zhi-Hao" in the Chinese Pharmacopoeia,[2] were widely used in traditional Chinese medicine for the treatment of rheumatism and pains.[3] As part of our ongoing phytochemical investigation on *A. brachypodum*, two new diterpenoid alkaloids, named N(19)-en-denudatine (**1**) and N(4)-butanone-flavaconitine (**2**), were isolated from the 90% EtOH extract of its roots. Herein, we describe the isolation and structural elucidation of the two new compounds.

Compound **1**, $[\alpha]_D^{23.6}$ +39.32 (*c* 1.28, MeOH), was obtained as a white amorphous powder. Its molecular formula was determined to be $C_{20}H_{27}NO_2$ based on EI-MS ($[M]^+$; *m/z* 313) and HR-ESI-MS(+) [*m/z* 314.2129 ($[M+H]^+$, calc. 314.2120)] analyses, indicating 8 degrees of unsaturation. The IR spectrum showed the absorption bands for hydroxyl (3405 cm^{-1}) and olefinic carbon (1650 cm^{-1}). In the 1D NMR spectrum, a methyl [δ_H 0.81 (s, H-18); δ_C 22.0 (q, C-18)] was observed, together with a N=CH [δ_H 7.29 (br.s, H-19); δ_C 168.6 (d, C-19)] and an olefinic group [δ_H 5.27 (br.s, Ha-17), 5.76 (br.s, Hb-17); δ_C 155.3 (s, C-16), 109.5 (t, C-17)]. Its ^{13}C-NMR (DEPT) spectrum displayed 20 carbon signals including 1 methyl, 7 methylenes, 8 methines and 4 quaternary carbons, suggesting compound **1** might be an atisine-type C_{20}-diterpenoid alkaloid.[4,5] The 1D NMR spectral data (Table 1) were similar to those of denudatine[6] except that there was an N=CH group in compound **1**, instead of the NCH_2CH_3 in denudatine. The existence of a double bond between the N and C-19 was finally verified by the HMBC correlations (Figure 2) between the olefinic proton of H-19 (δ_H 7.29, br.s) and C-3, C-4, C -5, C-18 and C-20. All the proton and carbon signals were assigned as Table 1 by 1D NMR, HMQC, HMBC, ^{1}H-^{1}H COSY and ROESY analyses.

Compound **1** was presumed to possess a similar relative configuration as denudatine (H-5β, H-9β and H-12β), based on their almost identical ^{1}H- and ^{13}C-NMR data (Table 1). Thus, compound **1** had the orientation of H-5β, H-7β, H-9β, OH-11 β, H-12β, OH-5β, CH_3-18β, H-19β, H-20β, which was determined by the ROESY correlations of CH_3-18 with H-5, H-19, and H-9 with OH-11, OH-15, and OH-11 with H-10, H-12, OH-15, H-20, and H-7 with H-20 (Figure 3). Therefore, the structure of compound **1** was characterized as shown in Figure 1, named as N(19)-en-denudatine [=(11β,15β)-11,15 -dihydroxyl atisine].

Compound **2** was obtained as a white amorpous powder and had a molecular formula of $C_{35}H_{47}NO_{12}$ based on ESI-MS

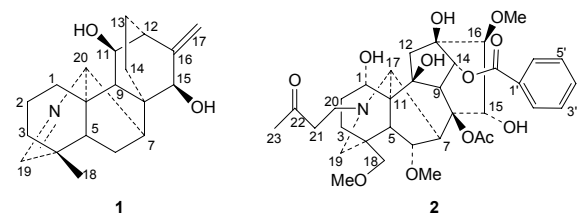

Figure 1. Structures of compounds **1** and **2**.

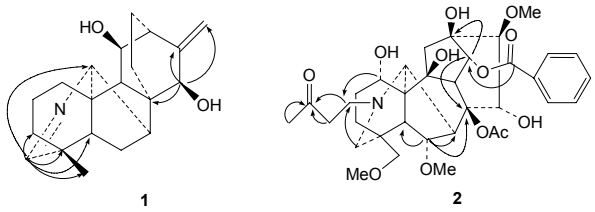

Figure 2. Key HMBC correlations of compounds **1** and **2**.

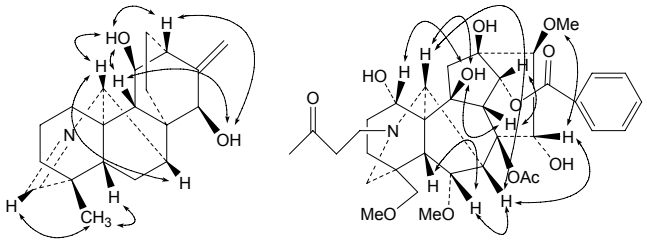

Figure 3. Key ROESY correlations of compounds **1** and **2**.

Table 1. ^{1}H- and ^{13}C-NMR data of compound **1** and **2** in $CDCl_3$

No	**1**		**2**	
	δ_H [a]	δ_C [b]	δ_H [c]	δ_C [d]
1	2.13 (m), 2.16 (m)	27.2 t	4.06 (dd, 8.5, 6.2)	69.2 d
2	1.43 (m), 1.54 (m)	21.2 t	0.81 (m), 1.72 (m)	29.5 t
3	0.97 (dd, 13.3, 4.1), 1.27 (dd, 13.3, 4.0)	35.0 t	1.18 (m), 1.52 (m)	30.8 t
4		44.8 s		37.8 s
5	1.11 (d, 7.2)	49.4 d	2.53 (d, 6.5)	40.3 d
6	1.54 (m), 1.73 (dd, 12.2, 7.2)	24.9 t	4.03 (d, 6.5)	83.1 d
7	2.53 (br.s)	49.1d	2.83 (s)	48.3 d
8		45.5 s		89.4 s
9	2.02 (d, 9.6)	55.9 d	2.70 (d, 5.0)	52.7 d
10		45.8 s		78.7 s
11	4.10 (dd, 9.2, 5.0)	73.1 d		54.2 s
12	2.25 (m)	48.3 d	2.23 (d, 10.8), 2.77 (d, 10.8)	47.2 t
13	1.43 (m), 3.51 (ddd, 13.2, 7.7, 5.5)	25.5 t		74.6 s
14	1.21 (m), 1.55 (m)	28.4 t	5.36 (d, 5.2)	78.3 d
15	4.65 (d, 5.6)	77.3 d	4.51 (d, 5.4)	79.6 d
16		155.3 s	3.33 (d, 5.4)	89.3 d
17	5.27 (br.s), 5.76 (br.s)	109.5 t	2.74 (s)	62.8 d
18	0.81 (s)	22.0 q	3.31 (d, 8.2), 3.54 (d, 8.2)	79.7 t
19	7.29 (br.s)	168.6 d	2.26 (d, 10.4), 2.67 (d, 10.4)	57.2 t
20	4.58 (br.s)	72.2 d	2.67 (m), 3.02 (m)	48.7 t
21			2.63 (m), 2.86 (m)	41.4 t
22				207.2 s
23			2.17 (s)	30.3 q
OAc-8				172.3 s
			1.25 (s)	21.3 q
OMe-6			3.16 (s)	61.5 q
OMe-16			3.30 (s)	58.1 q
OMe-18			3.75 (s)	59.1 q
OBz-14				165.9 s
1'				129.6 s
2', 6'			8.02 (d, 7.2)	129.6 d
3', 5'			7.46 (t, 7.2)	128.7 d
4'			7.59 (t, 7.2)	133.4 d
OH-11	4.98 (s)			
OH-15	6.70 (br.d, 6.2)			

[a]recorded at 400 MHz, [b]recorded at 100 MHz, [c]recorded at 500 MHz, [d]recorded at 125 MHz.

($[M+H]^+$ *m/z* 674) and HR-ESI-MS (674.3193 $[M+H]^+$; calc. 674.3176). The IR bands exhibited hydroxyl (3484 cm^{-1}), ester carbonyl (1720 cm^{-1}) and aromatic ring (1603, 1548, 1452 cm^{-1}) functions. The ^{1}H-NMR spectra of compound **2** displayed one N(4)-butanone (δ_H 2.67, 3.02, each 1H, m, H-20; 2.63, 2.86, each 1H, m, H-21; 2.17, 3H, s, H-23), three methoxyls (δ_H 3.75, 3.20, 3.29, 9H, s×3), one acetyl (δ_H 1.25, 3H, s) and a benzoyl group (δ_H 7.46, t, J = 7.2, 2H; 7.59, t, J = 7.2, 1H; 8.02, d, J = 7.2, 2H). Its ^{13}C-NMR (DEPT) spectrum revealed the presence of 35 carbon signals including 5 methyls, 7 methylenes, 14 methines and 9 quaternary carbons. The above spectral data suggested that compound **2** might be an aconitine type C_{19}-diterpenoid alkaloid.[7-8] The NMR data of compound **2** were identical with those of flavaconitine[9] except that compound **2** had one N(4)-butanone as the NMR spectra displayed (Table 1). This was further confirmed by the cross-peaks between H-17 (δ_H 2.74, 1H, s), H-19 (δ_H 2.26, 2.67, each 1H, d, J = 10.4 Hz) and C-20 in the HMBC spectrum (Figure 2).

Compound **2** had the same relative configuration as flavaconitine, not only being supported by their almost same ^{1}H and ^{13}C-NMR data, but also being verified by the ROESY experiment. As shown in Figure 3, the correlations between H-7 (assumed to be β-orientation with reference to the aconitine type C_{19}-diterpenoid alkaloids[10-12]) and H-6, H-15, H-17, H-17 and OH-10, OH-10 and H-1, H-9, H-9 and H-14, H-15 and OMe-16 were observed by the ROESY spectrum, indicating the β-orientations of H-1, H-5, H-6, H-7, H-9, OH-10, H-14, H-15, OMe-16 and H-17. Thus, the structure of compound **1** was determined as 18-dehydroxylgeniculatine D [=(1α,6α,14α,15α,16β)-6,16-dimethoxy-4-(methoxymethyl) aconitane-1,10,13,14,15- hexol 8-acetate 14-benzoate N(4)-butanone].

Experimental

General experimental procedures. Optical rotations were determined on a Horiba SEPA-300 polarimeter. IR (KBr) spectra were recorded on a Bio-Rad FTS-135 spectrometer. 1D and 2D NMR spectra were measured on Bruker AM-400 and DRX-500 spectrometers with TMS as the internal standard. MS were recorded on a VG Auto Spec-3000 mass spectrometer. Silica gel (200 - 300 mesh) and Al_2O_3 for column chromatography were obtained from the Qingdao Meigao Chemical Company, Ltd., and Shanghai Wusi Chemical Reagents Company, Ltd., respectively. Sephadex LH-20 was purchased from Pharmacia Fine Chemical Co. Ltd., Germany.

Plant material. The roots of *Aconitum brachypodum* Diels. were collected in Dongchuan of Yunnan Province, P. R. China, in November, 2006, and authenticated by Prof. Dr. Li-Gong Lei from Kunming Institute of Botany. A voucher specimen (No. KIB 2006-11-03) had 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. brachypodum* (50 kg) were powdered and extracted three times with 90% EtOH under reflux for 2 hr. After being removed solvent under reduced pressure, the crude extract was dissolved with 20 L of 2% HCl solution, then filtrated. The acidic solution was basified to pH 9.0 with ammonia (25%) and extracted with $CHCl_3$ to obtain crude alkaloidal extract (520 g) after removal of $CHCl_3$ in vacuum. The extract was chromatographed over silica gel (5.2 kg, 200 - 300 mesh) CC and eluted with petroleum ether/acetone/diethylamine (15 : 1 : 1 → 3 : 1 : 1) to provide five fractions Frs. 1-5. The Fr. 3 (75.0 g) was subjected to silica gel CC (petroleum

ether/acetone/diethylamine, 15 : 2 : 1), followed by Al_2O_3 CC (petroleum ether/acetone, 6 : 1) and finally purified through Sephadex LH - 20 ($CHCl_3$/MeOH, 1 : 1) to yield compound **1** (12 mg) and **2** (10 mg).

Compound 1: a white amorphous powder. $[\alpha]_D^{23.6}$ +39.32 (*c* 1.28, MeOH). IR (KBr) cm^{-1}: 3405, 1650, 1459, 1097. ^{1}H- and ^{13}C-NMR data: Table 1. EI-MS: 313 ($[M]^+$). HR-ESI-MS (pos.): 314.2129 ($[M+H]^+$, $C_{20}H_{28}NO_2^+$; calc. 314.2120).

Compound 2: a white amorphous powder. mp 153 - 154 °C. $[\alpha]_D^{20.8}$ −16.39 (*c* 0.14, MeOH). UV: λ_{max}^{MeOH} (log ε) 230 (4.41). IR (KBr) cm^{-1}: 3484, 2934, 1720, 1603, 1548, 1452, 1279, 1010, 716. ^{1}H- and ^{13}C-NMR data: Table 1. ESI-MS (pos.): 674 ($[M+H]^+$). HR-ESI-MS (pos.): 674.3193 ($[M+H]^+$, $C_{35}H_{48}NO_{12}^+$; calc. 674.3176).

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