Three New Diterpenoid Alkaloids from Roots of *Aconitum ouvrardianum* Hand.-Mazz.

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A new C_{19}-diterpenoid alkaloid, ouvrardiantine (1) and two new C_{20}-diterpenoid alkaloids, ouvrardiandines A (2) and B (3) were isolated from the root of *Aconitum ouvrardianum* Hand.-Mazz. The structure of the new alkaloids was established on the basis of spectral data (1D- and 2D-NMR, HR-MS).

Key words *Aconitum ouvrardianum*; Ranunuclaceae; diterpenoid alkaloid; ouvrardiantine; ouvrardiandine A; ouvrardiandine B

The plant *Aconitum ouvrardianum* Hand.-Mazz. grows in Diqing country of Yunnan province in China at an elevation of 3000 m. Biological activity studies performed on diterpenoid alkaloids of "CaoWu" species have shown analgesia, anti-imflammatory and antiarrhythmic action.[1,2] To our knowledge, no phytochemical study of this plant has previously been undertaken. These reasons and our interest in the species prompted us to work with the *A. ouvrardianum*. In the course of our studies on the alkaloids of this plant, a new C_{19}-diterpenoid alkaloid, ouvrardiantine (**1**) and two new C_{20}-diterpenoid alkaloids, ouvrardiandines A (**2**) and B (**3**) have been isolated from the root of *A. ouvrardianum*. This paper describes the separation and structural elucidation of these new alkaloids.

Results and Discussion

Ouvrardiantine (**1**) was obtained as a white amorphous powder, $[\alpha]_D^{20}$ +25° (c=0.8, $CHCl_3$), whose molecular formula was determined as $C_{35}H_{49}NO_{11}$ by HR-ESI-MS (m/z 660.3388 $[M+H]^+$). The NMR spectral of **1** exhibited characteristic features of an aconitine-type C_{19}-diterpenoid alkaloid[3] bearing an *N*-ethyl group (δ_H 1.16, 3H, t, J=7.2 Hz; δ_C 12.2 q, 48.8 t), four methoxyl groups (δ_H 3.18, 3.27, 3.31, 3.52, each 3H, s; δ_C 58.1 q, 59.0 q, 56.0 q, 58.8 q), an acetyl group (δ_H 1.36, 3H, s; δ_C 21.6 q, 169.8 s), and an anisoyl group (δ_H 6.91, 8.01, each 2H, AA′BB′ system, J=8.8 Hz; δ_H 3.87, 3H, s; Ar-OCH_3). The four methoxyl groups were located at C-1, C-6, C-16, and C-18, respectively, on the basis of the HMBC correlations between 1-OCH_3 (δ_H 3.31) and C-1 (δ_C 85.6 d), 6-OCH_3 (δ_H 3.18) and C-6 (δ_C 82.2 d), 16-OCH_3 (δ_H 3.52) and C-16 (δ_C 83.7 d), and 18-OCH_3 (δ_H 3.27) and C-18 (δ_C 79.1 t). The one-proton doublet signal at δ_H 4.88 (1H, d, J=5.2 Hz) was assigned to H-14β,[3] as well as the upfield proton signal of the acetyl at δ_H 1.36 due to the shielding effect of the 14α-OAs, suggesting the presence of 14-anisoyl group and the acetyl group at C-8.[3] In the ^{1}H-NMR, the downfield chemical shift of 16-OCH_3 (δ_H 3.52, 3H, s) in addition to the doublet signal of H-14 indicated the presence of hydroxyl group at C-13.[3] Meanwhile, another hydroxyl group was located at C-2, based on the chemical shifts (δ_C 62.3 d) of this carbon and the HMBC correlations of H-2 (δ_H 4.01) with C-4 (δ_C 38.9 s) and C-11 (δ_C 52.7 s). The configuration of 2-OH was α-orientation, based on the downfield shift of C-19 (δ_C 51.8 t) in the ^{13}C-NMR spectra of compound **1**. In this case, there was a hydrogen bond formed between N atom and 2α-OH group (Fig. 2).[4] Finally, all the evidence given above led to assignment of the structure of ouvrardiantine as **1**.

Ouvrardiandine A (**2**) was isolated as a white amorphous powder, $[\alpha]_D^{20}$ −62° (c=0.3, $CHCl_3$). The HR-ESI-MS of **2** exhibited a protonated molecular ion peak at m/z 500.2647 $[M+H]^+$ (Calcd 500.2643) corresponding to the pseudo-molecular formula of $C_{28}H_{37}NO_7$. The ^{1}H- and ^{13}C-NMR spectra of **2** exhibited characteristic features of an atisine-type alkaloid.[5] Its NMR data showed the presence of an *N*-methyl group (δ_H 2.36, 3H, s; δ_C 41.8 q), two ketonic carbons (δ_C 202.6 s, 209.5 s), an exo-methylene (δ_H 5.10, 5.24, each 1H, s; δ_H 115.9 t), a methyl group (δ_H 1.29, 3H, s; δ_C 22.3 q), an acetyl (δ_H 2.14, 3H, s; δ_C 170.7 s, 21.1 q), and a 2-methylbutyryl ester group (δ_H 0.92, 3H, t, J=7.3 Hz; 1.16, 3H, d, J=7.1 Hz; 1.48, 1.72, 2H, m; 2.41, 1H, m; δ_C 11.7 q, 16.7 q, 26.5 t, 41.4 d, 176.5 s). Its ^{1}H- and ^{13}C-NMR spectra also showed the distinctive *N*,*O*-mixed acetal moiety (δ_H 4.33, 1H, s; δ_C 93.4 d). The two ketonic ^{13}C-signals at δ_C 209.5 s and δ_C 202.6 s were attributable to C-13 and C-2, respec-

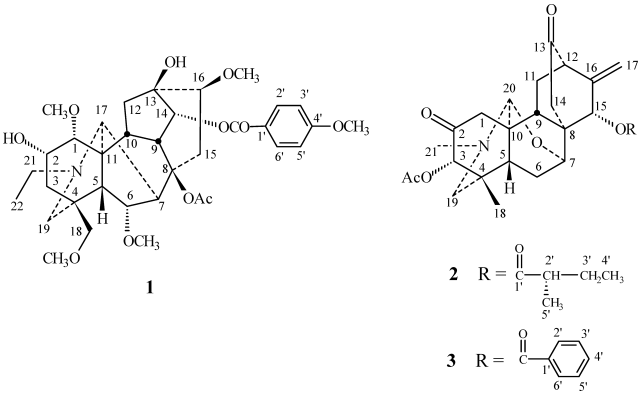

Fig. 1. The Structure of Compounds **1**—**3**

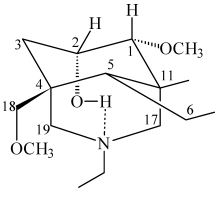

Fig. 2. The Hydrogen Bond Formation between N Atom and 2α-OH of **1**

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Compound **1**

Carbon	δ_C	δ_H mult. (J=Hz)	Carbon	δ_C	δ_H mult. (J=Hz)
1	85.6 d	3.12 d (4.8)	17	60.7 d	2.80 br s
2	62.3 d	4.01 m	18	79.1 t	3.01 d (8.4) (α)
3	42.1 t	1.74 dd (14.8, 3.6) (α)			3.65 d (8.4) (β)
		1.96 dd (15.0, 2.2) (β)	19	51.8 t	2.53, 2.58 ABq (11.4)
4	38.9 s		21	48.8 t	2.61, 2.68 m
5	49.4 d	2.23 d (6.4)	22	12.2 q	1.16 t (7.2)
6	82.2 d	4.01 d (6.0)	1-OCH_3	56.0 q	3.31 s
7	49.7 d	3.10 br s	6-OCH_3	58.1 q	3.18 s
8	85.3 s		16-OCH_3	58.8 q	3.52 s
9	45.6 d	2.97 m	18-OCH_3	59.0 q	3.27 s
10	40.8 d	2.20 m	8-OAc	169.8 s	
11	52.7 s			21.6 q	1.36 s
12	37.7 t	2.18 m (α)	14-OAs		
		2.68 m (β)	CO	166.1 s	
13	74.7 s		1′	122.6 s	
14	78.4 d	4.88 d (5.2)	2′, 6′	131.7 d	8.01 d (8.8)
15	39.5 t	2.46 dd (14.2, 5.6) (α)	3′, 5′	113.8 d	6.91 d (8.8)
		2.97 m (β)	4′	163.5 s	
16	83.7 d	3.30 m	4′-OCH_3	55.4 q	3.87 s

tively, based on HMBC correlations of C-13 with H-14 (δ_H 2.40, 2.92) and C-2 with H-1 (δ_H 2.28), respectively. The acetoxyl group and the 2-methylbutyryl ester group could be assigned at C-3 and C-15, respectively, as a result of the HMQC and the HMBC correlations of H-3 (δ_H 4.54) with $COCH_3$ (δ_C 170.7, s), C-4 (δ_C 39.4, s), C-5 (δ_C 56.8, d), and H-15 (δ_H 5.56) with CO-methylbutyryl (δ_C 176.5, s), C-16 (δ_C 142.4, s), C-17 (δ_C 115.9, t), respectively. In the NOE spectrum of **2**, the irradiation of H-3 (δ_H 4.54) led to the enhancement of the signals of H-5 (δ_H 2.02) and H-18 (δ_H 1.29), indicating that the 3-acetoxyl group was α-oriented. Meanwhile, the positive correlation of H-15 (δ_H 5.56) with H-9 (δ_H 2.41) also confirmed the α-configuration of the 15-(2-methylbutyryl) ester group. In addition, the stereochemistry of this group may be assigned as "*S*" based on references, which reported some other natural products bearing the same moiety, [6—9] and the absolute configuration of C-2′ was in agreement with the L-isoleucine pathway in the biosynthesis of the secondary metabolites.[10] The structure of ouvrardiandine A thus was deduced as **2** by careful analysis of the 1D-NMR and 2D-NMR (^{1}H–^{1}H COSY, HMQC, HMBC and NOE) spectra.

Ouvrardiandine B (**3**) was a white amorphous powder, $[\alpha]_D^{20}$ −17.6° (c=0.2, $CHCl_3$). The pseudo-molecular formula $C_{30}H_{33}NO_7$ was inferred from its HR-ESI-MS (m/z 542.2133 $[M+Na]^+$, Calcd 542.2149). The ^{1}H- and ^{13}C-NMR spectra of **3** were very similar to that of **2** except for the lack of a 2-methylbutyryl ester group, but gave the distinctive signals of a benzoyl group (δ_H 7.47, 7.62, 8.03; δ_C see Table 2). Comparison of the ^{13}C-NMR and MS data of **2** and **3** determined the structure of ouvrardiandine B to be **3**.

Table 2. ^{1}H- and ^{13}C-NMR Spectral Data of Compounds **2** and **3**

Carbon	**2**		**3**	
	δ_C	δ_H mult. (J=Hz)	δ_C	δ_H mult. (J=Hz)
1	47.7 t	2.28 br s (α)	47.7 t	2.37 br s (α)
		2.29 br s (β)		2.37 br s (β)
2	202.6 s		202.5 s	
3	79.7 d	4.54 s	79.8 d	4.57 s
4	39.4 s		39.5 s	
5	56.8 d	2.02 br s	56.8 d	2.07 br s
6	43.2 t	1.62 d (12.0) (α)	43.2 t	1.67 d (12.0) (α)
		2.05 dd (12.0, 6.0) (β)		2.10 dd (12.0, 6.0) (β)
7	74.1 d	4.03 d (6.0)	74.1 d	4.05 dd (6.0)
8	52.9 s		53.0 s	
9	39.0 d	2.41 m	39.0 d	2.50 m
10	42.4 s		42.9 s	
11	26.4 t	1.83 m (α)	26.4 t	1.88 m (α)
		2.20 m (β)		2.27 m (β)
12	53.8 d	3.10 t (2.9)	53.8 d	3.19 t (2.8)
13	209.5 s		209.6 s	
14	39.4 t	2.40 d (hidden) (α)	39.6 t	2.59 d (19.6) (α)
		2.92 d (19.0) (β)		3.04 d (19.6) (β)
15	75.0 d	5.56 s	76.0 d	5.85 s
16	142.4 s		141.9 s	
17	115.9 t	5.10 s	116.5 t	5.24 s
		5.24 s		5.32 s
18	22.3 q	1.29 s	22.3 q	1.31 s
19	49.7 t	2.77, 2.84 ABq (10.2)	49.7 t	2.79, 2.85 ABq (10.2)
20	93.4 d	4.33 s	93.4 d	4.35 s
21	41.8 q	2.36 s	41.9 q	2.39 s
3-OAc	170.7 s		170.8 s	
	21.1 q	2.14 s	21.2 q	2.14 s
15-Ester				
CO	176.5 s			
2′	41.4 d	2.41 m		
3′	26.5 t	1.48, 1.71 m		
4′	11.7 q	0.92 t (7.3)		
5′	16.7 q	1.16 d (7.1)		
15-OBz				
CO			166.4 s	
1′			129.2 s	
2′, 6′			129.8 d	8.03 d (8.0)
3′, 5′			128.6 d	7.47 t (8.0)
4′			133.6 d	7.62 t (8.0)

Experimental

General Silica gel H and GF_{254} (Qingdao Haiyang Chemical Group Co., China) were used for column chromatography and TLC, respectively. The spots were detected by Dragendorff reagent. Optical rotation measurements were made using a Perkin-Elmer 341 polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Unity INOVA 400/45 and Bruker Avance 600 spectrometer in $CDCl_3$ with TMS as internal standard. HR-ESI-MS was measured on a VG Auto Spec 3000 mass spectrometer. IR was measured on a Nicolet FI-IR 200SXY spectrophotometer.

Plant Material *Aconitum ouvrardianum* HAND.-MAZZ. was collected in

the Diqing country of Yunnan province, China. The plant was identified taxonomically by Professor Qin-Er Yang (Institute of Botany, Chinese Academy of Sciences, Beijing). A voucher specimen was deposited in the herbarium of the West China College of Pharmacy, Sichuan University.

Extraction and Isolation of Alkaloids Powdered roots (1.6 kg) of *Aconitum ouvrardianum* were percolated with 0.2% HCl until 40 l was collected. The filtrate was then alkalized with NH_4OH to pH >9, extracted with ethyl acetate three times, and evaporated to give the total crude alkaloids (18.0 g). The crude alkaloids (8.0 g) were chromatographed on silica gel columns using gradient elution with petroleum ether–acetone (10 : 1→4 : 1) to give fractions I—IV. Fraction II (300 mg) was separated on a silica gel column with cyclohexane–acetone (20 : 1) to give compound **1** (8 mg). Silica gel column chromagraphy of Fraction III (500 mg) was eluted with ether–acetone (10 : 1) to give **2** (5 mg) and **3** (4.5 mg).

Ouvrardiantine (**1**): White amorphous powder, $[\alpha]_D^{20}$ +25° (*c*=0.8, $CHCl_3$), IR (KBr) cm^{-1}: 3491, 1718, 1607, 1512, 1459, 1258, 1099; ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz): see Table 1; HR-ESI-MS: *m*/*z* 660.3388 $[M+H]^+$, Calcd for $C_{35}H_{50}NO_{11}$ 660.3378.

Ouvrardiandine A (**2**): White amorphous powder, $[\alpha]_D^{20}$ −62° (*c*=0.3, $CHCl_3$), IR (KBr) cm^{-1}: 3445, 1731, 1462, 1364, 1234, 1045; ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz): see Table 2; HR-ESI-MS: *m*/*z* 500.2647 $[M+H]^+$, Calcd for $C_{28}H_{38}NO_7$ 500.2643

Ouvrardiandine B (**3**): White amorphous powder, $[\alpha]_D^{20}$ −17.6° (*c*=0.2, $CHCl_3$), IR (KBr) cm^{-1}: 3445, 1718, 1602, 1515, 1454, 1269, 1108, 1045; ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz): see Table 2; HR-ESI-MS: *m*/*z* 542.2133 $[M+Na]^+$, Calcd for $C_{30}H_{33}NNaO_7$ 542.2149.

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