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THREE NEW C_{19}-DITERPENOID ALKALOIDS FROM *ACONITUM APETALUM*

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Abstract – Three new C_{19}-diterpenoid alkaloids, named apetaldine H (**1**), apetaldine I (**2**) and apetaldine J (**3**), were isolated from the root of *Aconitum apetalum*. Their structures were elucidated by extensive spectroscopic analyses including 1D, 2D NMR, and HR-ESI-MS. Compounds (**1–3**) were evaluated for their cytotoxicity against the MCF-7, HepG2 and H460 human cancer cell lines.

Diterpenoid alkaloids, the largest and most complex group of terpenoid alkaloid, have many biological activities such as anti-inflammatory, analgesic, anti-arrhythmia, antifungal, and cytotoxic properties.[1] In our extensively phytochemical researches on the pharmacologically interesting plants of the genera *Aconitum* and *Delphinium*, we obtained a series of structurally and chemotaxonomically diverse diterpenoid alkaloids.[2] Based on the results of previous investigation, a series of diterpenoid alkaloids including seven new C_{19}-diterpenoid alkaloids apetaldines A-G, and ten known alkaloids were isolated from *Aconitum apetalum*, a traditional herb and mainly distributed in Xinjiang of China.[3]

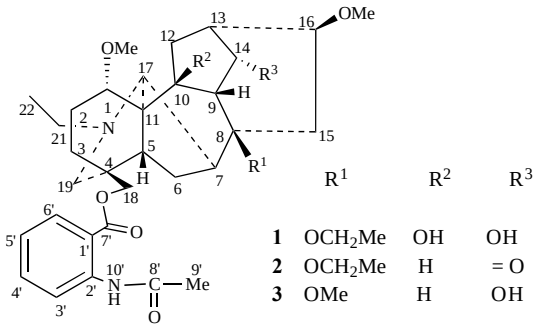

Figure 1. Structures of compounds **1-3**

To find more biologically active secondary metabolites, three new C_{19}-diterpenoid alkaloid, named apetaldine H (**1**), apetaldine I (**2**) and apetaldine J (**3**) were isolated from this plant. All of these compounds were tested for their cytotoxicity against the MCF-7, HepG2 and H460 human cancer cell lines. Herein, isolation, structural elucidation, as well as cytotoxicity of these diterpenoid alkaloids are reported.

The 95% EtOH extract of roots of *A. apetalum* was separated into alkaloid and non-alkaloid fractions. The alkaloid fraction was chromatographed over silica gel to afford three new C_{19}-diterpenoid alkaloids.

Compound **1** was isolated as a white amorphous powder, its molecular formula, $C_{34}H_{48}N_2O_8$ was deduced from the HR-ESI-MS *m/z* 613.3488 $[M + H]^+$ (calcd. for $C_{34}H_{49}N_2O_8$, 613.3489) and ^{13}C NMR spectroscopic data. The IR (KBr) spectrum showed absorption bands for hydroxy group (3316 cm^{-1}), amide carbonyl group (1688 cm^{-1}) and ester carbonyl group (1741 cm^{-1}). Compound **1** exhibited characteristic NMR spectral features of an aconitine-type alkaloid [4] bearing an *N*-ethyl group [δ_H 1.09 (3H, t, *J* = 7.2 Hz), 2.36 (1H, m) and 2.55 (1H, m); δ_C 49.4 t, 13.6 q], two methoxy groups [δ_H 3.29 and 3.35 (s, each 3H); δ_C 56.2 q, 56.6 q], and an ethoxy group [δ_H 1.12 (3H, t, *J* = 7.2 Hz), 3.37 (1H, m) and 3.49 (1H, m); δ_C 56.1 t, 16.2 q]. In addition, the 1D NMR and 2D NMR data (Table) indicated the presence of one NH group [δ_H 11.01 (1H, s)], an *ortho*-disubstituted benzene unit [δ_H 8.70 (1H, d, *J* = 8.4 Hz), 7.56 (1H, t, *J* = 8.4Hz), 7.10 (1H, t, *J* = 8.4 Hz), 7.97 (1H, d, *J* = 8.4 Hz), δ_C 114.9 s, 141.9 s, 120.6 d, 135.0 d, 122.7 d, 130.7 d, 168.4 s], and an acetyl group (δ_H 2.24 s; δ_C 169.2 s, 25.7 q), which were assigned to an *o*-acetamidobenzoate moiety (-OCOC_6H_4-*o*-NHAc). [5] The two methoxy groups were attributed to C-1 and C-16, respectively, based on the HMBC correlations of 1-OCH_3 (δ_H 3.29) with C-1 (δ_C 78.4) and 16-OCH_3 (δ_H 3.35) with C-16 (δ_C 82.1) (Figure 2). The *o*-acetamidobenzoate moiety was located at C-18 by the long-range correlations in the HMBC experiment from H-18 (δ_H 4.03 and 4.07) and H-6′ (δ_H 7.97) to C-7′ (δ_C 168.4) as well as the amido proton with C-1′ (δ_C 114.9), C-2′ (δ_C 141.9), and C-8′ (δ_C 169.2). Besides, the existence of eight oxygenated carbons deduced from its HR-ESI-MS and ^{13}C NMR spectrum suggested **1** possess two hydroxy groups. One hydroxy group was positioned at C-14, due to the HMBC correlations from H-9 (δ_H 2.04), H-13 (δ_H 2.51), H-16 (δ_H 3.30) to C-14 (δ_C 73.5). The ^1H-^1H COSY correlations of H-13/H-14 also supported the location of the hydroxy group. Comparison of the NMR data of **1** with those of the known alkaloid talassicumine A [6] indicated that there was a hydroxy group at C-10 in **1**.

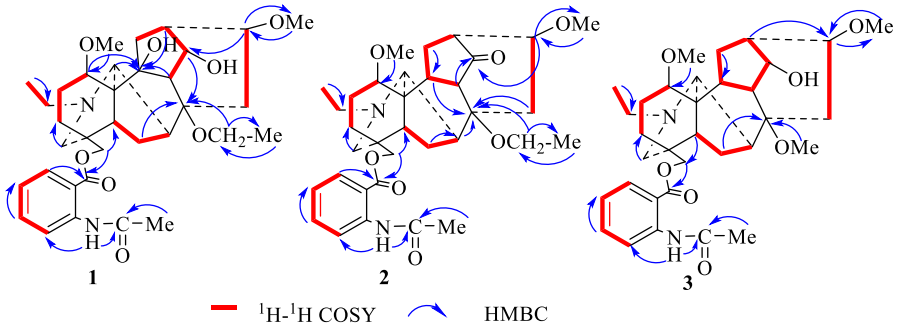

Figure 2. Key HMBC and ^1H-^1H COSY correlations of compounds **1**-**3**

This conclusion was supported by the appearance of a new quaternary carbon signal at 81.1 ppm and the absence of a methine signal at 38.6 ppm (compared the ^{13}C NMR data with talassicumine A). Moreover, the existence of hydroxy group at C-10 was confirmed by the long-range correlations from four protons

signals at H-1 (δ_H 3.78), H-9 (δ_H 2.04), H-13 (δ_H 2.51), H-17 (δ_H 2.89) to C-10 (δc 81.1) in the HMBC spectrum. Accordingly, the substitution pattern and assigned planar structure of **1** was confirmed by complete ^{1}H-^{1}H COSY, HMQC, and HMBC spectroscopic analyses.

The relative configuration of compound **1** was deduced from the vicinal coupling constants (Table) and NOESY experiment (Figure 3). The NOESY cross-peak between H-16/H-17 showed that H-16 was α-oriented. Furthermore, the characteristic proton signals (δ_H 3.78, 1H, dd, J = 10.2, 6.6 Hz; δ_H 4.58, 1H, dd, J = 10.2, 5.4 Hz) could be assigned to H-1β and H-14β,[7] respectively. Since the absolute configuration of the aconitine-type skeleton was repeatedly confirmed by the X-ray crystallographic analysis of analogues isolated from species of the same genus,[8] it is proposed that the absolute configuration of this skeleton was retained in **1**. Thus, the structure and absolute configuration of apetaldine H (**1**) was determined as shown in Figure 1, and the full assignment of its spectroscopic data was achieved based on 1D and 2D NMR analyses.

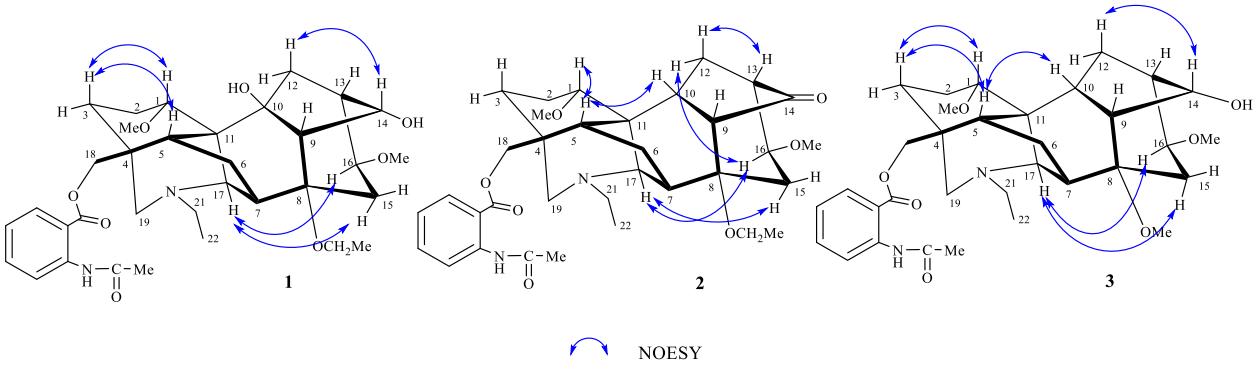

Figure 3. Key NOESY correlations of compounds **1**-**3**

Compound **2** showed a molecular formula of $C_{34}H_{46}N_2O_7$ which was determined by HR-ESI-MS. Analysis of the NMR spectra suggested **2** was also an aconitine-type alkaloid, bearing an *N*-ethyl group (δ_H 1.07, 3H, t, J = 7.2 Hz; δ_C 49.0 t, 13.4 q), two methoxy groups (δ_H 3.31, 6H, s; δ_C 55.9 q, 56.1 q), an ethoxy group (δ_H 1.09 t, J = 7.2 Hz; δ_C 58.2 t, 15.8 q), and an *o*-acetamidobenzoate moiety as compound **1**. Further analysis of the HMBC correlations of H-9, H-12, H-13, H-16 with C-14 confirmed the 14-keto group (δ_C 216.8). In addition, the locations of the methoxy groups at C-1 and C-16 and the ethoxy group at C-8 were confirmed by analysis of the HMBC correlations. Accordingly, the observation of HMBC cross-peak between H-18 and C-7′ suggested the location of the *o*-acetamidobenzoate group at C-18. The vicinal coupling constants (Table) and the NOESY experiment (Figure 3) showed the similar stereochemistry of **2** as **1**. The NOE correlations between H-1/H-5 and H-5/H-10 indicated that these hydrogens were β-oriented.

Table. ^{1}H and ^{13}C NMR Spectroscopic Data[a,b] for Compounds **1-3** in $CDCl_3$ **(**δ in ppm, J in Hz**)**

NO.	**1**[a]		**2**[a]		**3**[b]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	3.78 dd (10.2, 6.6)	78.4	3.23 dd (10.2, 6.6)	85.5	3.12 t (6.4)	85.7
2	a 2.10 m b 2.34 m	26.1	a 2.07 m b 2.31 m	25.5	a 1.87 m b 2.13 m	28.5
3	a 1.48 dd (13.2, 4.2) b 1.88 m	32.6	a 1.45 br.t (7.2) b 1.87 m	32.4	a 1.44 m b 1.88 m	32.9
4	—	38.0	—	38.0	—	38.3
5	1.97 d (7.8)	42.3	1.71 d (7.2)	45.1	1.69 d (7.2)	46.4
6	a 1.50 m b 1.96 dd (15.0, 7.8)	24.7	a 1.54 dd (15.0, 7.8) b 1.90 dd (15.0, 7.8)	25.1	a 1.42 m b 1.97 m	24.1
7	2.43 m	40.5	1.92 m	43.5	2.45 m	40.3
8	—	77.3	—	86.2	—	77.9
9	2.04 d (4.8)	55.5	2.39 d (6.0)	52.6	2.23 m	45.7
10	—	81.1	2.33 m	45.4	2.35 t (5.2)	38.0
11	—	54.4	—	48.7	—	49.1
12	a 1.71 dd (16.2, 8.4) b b 2.77 d (16.2)	40.2	a 2.02 m b 2.13 dd (14.4, 7.2)	24.4	a 1.81 m b 2.14 m	28.5
13	2.51 m	39.4	2.48 m	46.1	1.84 m	46.1
14	4.58 dd (10.2, 5.4)	73.5	—	216.8	3.99 t (7.2)	75.1
15	a 2.12 m b 2.26 m	36.7	a 1.86 m b 2.37 m	33.0	a 2.05 m b 2.15 m	33.2
16	3.30 m	82.1	3.78 t (5.4)	84.7	3.37 m	82.3
17	2.89 br. s	63.0	3.55 br. s	62.0	3.06 br. s	62.6
18	a 4.03 ABq (10.8) b 4.07 ABq (10.8)	71.0	a 3.99 ABq (11.4) b 4.04 ABq (11.4)	70.1	a 3.98 ABq (11.2) b 4.08 ABq (11.2)	70.9
19	a 2.16 d (11.4) b 2.65 d (11.4)	52.8	a 2.20 d (11.4) b 2.65 d (11.4)	52.5	a 2.10 d (11.2) b 2.64 d (11.2)	52.8
21	a 2.36 m b 2.55 m	49.4	a 2.45 m b 2.58 m	49.0	a 2.42 m b 2.53 m	49.4
22	1.09 t (7.2)	13.6	1.07 t (7.2)	13.4	1.08 t (7.2)	13.7
1-OMe	3.29 s	56.2	3.31 s	55.9	3.36 s	56.5
8-OMe	—	—	—	—	3.14 s	48.4
8-OEt	a 3.37 m b 3.49 m	56.1	a 3.42 m b 3.47 m	58.2	—	—
	1.12 t (7.2)	16.2	1.09 t (7.2)	15.8	—	—
16-OMe	3.35 s	56.6	3.31 s	56.1	3.27 s	56.5
1′	—	114.9	—	114.5	—	114.9
2′	—	141.9	—	141.5	—	141.8
3′	8.70 d (8.4)	120.6	8.70 d (8.4)	120.2	8.68 d (8.4)	120.6
4′	7.56 t (8.4)	135.0	7.55 t (8.4)	134.6	7.55 t (8.4)	134.9
5′	7.10 t (8.4)	122.7	7.09 t (8.4)	122.3	7.09 t (8.4)	122.6
6′	7.97 d (8.4)	130.7	7.95 d (8.4)	130.2	7.96 d (8.4)	130.6
7′	—	168.4	—	167.9	—	168.3
8′	—	169.2	—	168.9	—	169.2
9′	2.24 s	25.7	2.23 s	25.3	2.23 s	25.6
10′	11.01 s	—	11.02 s	—	11.02 s	—

a ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz). **b** ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz).

The cross-peak between H-16 with H-12b and H-17 in the NOESY experiment revealed that these hydrogens were *α*-orientation. Therefore, the structure of apetaldine I (**2**) was determined as shown in Figure 1.

HR-ESI-MS of compound **3** showed a molecular formula of $C_{33}H_{46}N_2O_7$. From its NMR data (Table), an *N*-ethyl group, three methoxy groups, and an *o*-acetamidobenzoate moiety could be easily recognized. The NMR spectroscopic data of **3** were comparable to **1**, meaning it was also a C_{19}-diterpenoid alkaloid. The methoxy groups were assigned at C-1 (δ_C 85.7), C-8 (δ_C 77.9), and C-16 (δ_C 82.3) based on the HMBC correlations of the methoxy proton signals with C-1, C-8, and C-16. In addition, compound **3** possessed a similar configuration to that of **1,** according to the deduction from its NOESY experiment (Figure 3). Accordingly, the structure of apetaldine J (**3**) was confirmed by extensive analysis of its NMR spectra.

Due to their structural complexity and bioactive diversity, the diterpenoid alkaloids attracted much attention of researchers in recent years.[9] As far as we know, there were only a few aconitine-type diterpenoid alkaloid bearing a hydroxy group at C-10 position. Apetaldine H (**1**) is another aconitine-type diterpenoid alkaloid including a hydroxy group at C-10 and an *o*-acetamidobenzoate moiety at C-18, providing a new candidate for further pharmacological investigation.

To evaluate the biological activities of these compounds isolated from the roots of *A. apetalum* for future applications, alkaloids **1** - **3** were tested for their *in vitro* cytotoxicity against the MCF-7, HepG2 and H460 human cancer cell lines. Unfortunately, all of the compounds were inactive (IC_{50} > 50 μM, n = 3).

EXPERIMENTAL

General experimental procedure. Optical rotations were measured in $CHCl_3$ using a PerkinElmer polarimeter with a sodium lamp operating at 598 nm and 20 °C. The IR spectra were obtained using a Thermo Fisher Nicolet 6700 spectrometer. The HR-ESI-MS data were measured using a Q-TOF micro mass spectrometer (Waters). The NMR spectra were recorded on Bruker AV 600 and Bruker AV 400 spectrometer. The TLC plates were precoated with silica gel GF_{254} (Qingdao Haiyang Chemical Co., Ltd., China), and it was visualized under a UV lamp at 254 nm or by spraying with Dragendorff's reagent or iodine.

Plant materials. See ref. 3.

Extraction and isolation. The total alkaloid extracts (40 g) were chromatographed on a silica gel column, eluting with a CH_2Cl_2−MeOH gradient system (80:1 to 0:1, v/v), to afford Frs. A_1−A_8 (in ref. 3). Fraction A_3 (3.2 g) was submitted to silica gel CC eluting with light petroleum: acetone: Et_2NH (50:1:0.1 to 0:1:0.1, v/v/v) to afford fractions B_1 (500 mg), B_2 (130 mg), B_3 (200 mg), and B_4 (830 mg) respectively. Compounds **1** (6 mg) and **2** (8 mg) were obtained by purifying fractions Fr. B_2 by silica gel column (light

petroleum: acetone: Et_2NH, 18:1:0.1 to 0:1:0.1, v/v/v). Fraction B_4 was submitted to CC eluting with light petroleum: acetone: Et_2NH (13:1:0.1 to 0:1:0.1, v/v/v) to yield compound **3** (2 mg).

Apetaldine H (**1**)

White amorphous powder; $[\alpha]_D^{20}$ +5.6 (*c* 0.25, $CHCl_3$); IR (KBr) ν_{max} 3316, 2923, 2853, 1741, 1688, 1605, 1589, 1455, 1377, 1296, 1163, 1089, 758; ^{1}H NMR (600 MHz, $CDCl_3$) data and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table; HR-ESI-MS (*m/z*): 613.3488 $[M + H]^+$, calcd. for $C_{34}H_{49}N_2O_8$, 613.3489.

Apetaldine I (**2**)

White amorphous powder; $[\alpha]_D^{20}$ +14.6 (*c* 0.35, $CHCl_3$); IR (KBr) ν_{max} 3473, 3318, 2967, 2925, 2820, 1748, 1702, 1686, 1589, 1526, 1448, 1261, 1240, 1091, 758; ^{1}H NMR (600 MHz, $CDCl_3$) data and ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table; HR-ESI-MS (*m/z*): 595.3389 $[M + H]^+$, calcd. for $C_{34}H_{47}N_2O_7$, 595.3383.

Apetaldine J (**3**)

White amorphous powder; $[\alpha]_D^{20}$ +4.9 (*c* 0.80, $CHCl_3$); IR (KBr) ν_{max} 3317, 3282, 2926, 1704, 1686, 1605, 1589, 1526, 1448, 1367, 1295, 1261, 1240, 1089, 757; ^{1}H NMR (400 MHz, $CDCl_3$) data and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Table; HR-ESI-MS (*m/z*): 583.3383 $[M + H]^+$, calcd. for $C_{33}H_{47}N_2O_7$, 583.3383.

Cell lines and cell culture and Cytotoxicity assay

The *in-vitro* growth inhibitory activities of compounds **1**-**3** were assayed by the MTT method.[10] The HepG2 (human hepatic carcinoma), MCF-7 (human breast cancer) and H460 (human lung adenocarcinoma) cell lines were obtained from ATCC. Cells were plated in 96-well plates 24 h before treatment and continuously exposed to different concentrations of compounds for 72 h. DMSO (0.1% *v/v*) was used as the negative controls and adriamycin (≥ 98%; Sigma Chemical Co., Ltd., Shanghai, China) was used as the positive control.

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