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### THREE NEW C<sub>19</sub>-DITERPENOID ALKALOIDS FROM *ACONITUM APETALUM*

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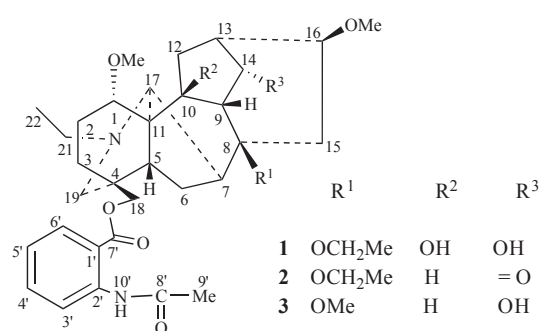
**Abstract** – Three new C<sub>19</sub>-diterpenoid alkaloids, named apetalidine H (**1**), apetalidine I (**2**) and apetalidine J (**3**), were isolated from the root of *Aconitum apetalum*. Their structures were elucidated by extensive spectroscopic analyses including 1D, 2D NMR, and HR-ESI-MS. Compounds (**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.<sup>1</sup> 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.<sup>2</sup> Based on the results of previous investigation, a series of diterpenoid alkaloids including seven new C<sub>19</sub>-diterpenoid alkaloids apetalidines A-G, and ten known alkaloids were isolated from *Aconitum apetalum*, a traditional herb and mainly distributed in Xinjiang of China.<sup>3</sup>

To find more biologically active secondary metabolites, three new C<sub>19</sub>-diterpenoid alkaloid, named apetalidine H (**1**), apetalidine I (**2**) and apetalidine J (**3**) were isolated from this plant. All of these compounds were tested for their

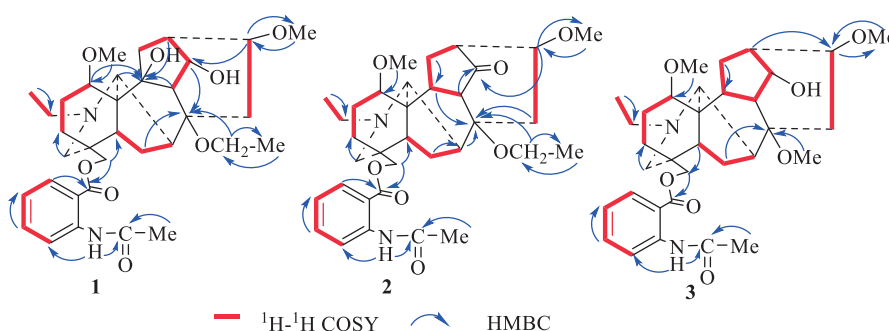
cytotoxicity against the MCF-7, HepG2 and H460 human cancer cell lines. Herein, isolation, structural elucidation, as well as cytotoxicity of these diterpenoid alkaloids are reported.

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<sub>19</sub>-diterpenoid alkaloids.



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

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 ( $3316\text{ cm}^{-1}$ ), amide carbonyl group ( $1688\text{ cm}^{-1}$ ) and ester carbonyl group ( $1741\text{ cm}^{-1}$ ). Compound **1** exhibited characteristic NMR spectral features of an aconitine-type alkaloid<sup>4</sup> bearing an *N*-ethyl group [ $\delta_H$  1.09 (3H, t,  $J = 7.2$  Hz), 2.36 (1H, m) and 2.55 (1H, m);  $\delta_C$  49.4 t, 13.6 q], two methoxy groups [ $\delta_H$  3.29 and 3.35 (s, each 3H);  $\delta_C$  56.2 q, 56.6 q], and an ethoxy group [ $\delta_H$  1.12 (3H, t,  $J = 7.2$  Hz), 3.37 (1H, m) and 3.49 (1H, m);  $\delta_C$  56.1 t, 16.2 q]. In addition, the 1D NMR and 2D NMR data (Table) indicated the presence of one NH group [ $\delta_H$  11.01 (1H, s)], an *ortho*-disubstituted benzene unit [ $\delta_H$  8.70 (1H, d,  $J = 8.4$  Hz), 7.56 (1H, t,  $J = 8.4$  Hz), 7.10 (1H, t,  $J = 8.4$  Hz), 7.97 (1H, d,  $J = 8.4$  Hz),  $\delta_C$  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 ( $\delta_H$  2.24 s;  $\delta_C$  169.2 s, 25.7 q), which were assigned to an *o*-acetamidobenzoate moiety ( $-OCOC_6H_4-o-NHAc$ ).<sup>5</sup> The two methoxy groups were attributed to C-1 and C-16, respectively, based on the HMBC correlations of 1-OCH<sub>3</sub> ( $\delta_H$  3.29) with C-1 ( $\delta_C$  78.4) and 16-OCH<sub>3</sub> ( $\delta_H$  3.35) with C-16 ( $\delta_C$  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 ( $\delta_H$  4.03 and 4.07) and H-6' ( $\delta_H$  7.97) to C-7' ( $\delta_C$  168.4) as well as the amido proton with C-1' ( $\delta_C$  114.9), C-2' ( $\delta_C$  141.9), and C-8' ( $\delta_C$  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 ( $\delta_H$  2.04), H-13 ( $\delta_H$  2.51), H-16 ( $\delta_H$  3.30) to C-14 ( $\delta_C$  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<sup>6</sup> indicated that there was a hydroxy group at C-10 in **1**.

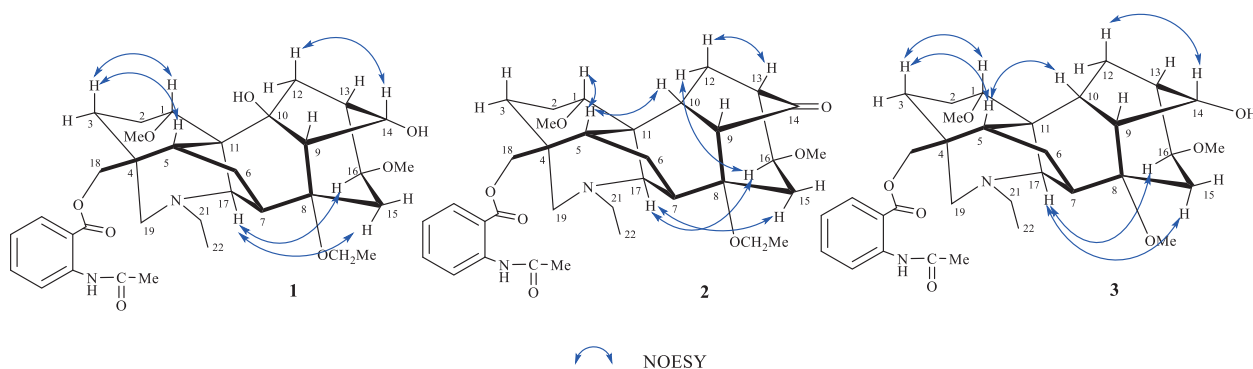


**Figure 2.** Key HMBC and  $^1H$ - $^1H$  COSY correlations of compounds **1-3**

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 ( $\delta_{\text{H}}$  3.78), H-9 ( $\delta_{\text{H}}$  2.04), H-13 ( $\delta_{\text{H}}$  2.51), H-17 ( $\delta_{\text{H}}$  2.89) to C-10 ( $\delta_{\text{C}}$  81.1) in the HMBC spectrum. Accordingly, the substitution pattern and assigned planar structure of **1** was confirmed by complete  $^1\text{H}$ - $^1\text{H}$  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  $\alpha$ -oriented. Furthermore, the characteristic proton signals ( $\delta_{\text{H}}$  3.78, 1H, dd,  $J = 10.2, 6.6$  Hz;  $\delta_{\text{H}}$  4.58, 1H, dd,  $J = 10.2, 5.4$  Hz) could be assigned to H-1 $\beta$  and H-14 $\beta$ ,<sup>7</sup> 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,<sup>8</sup> it is proposed that the absolute configuration of this skeleton was retained in **1**. Thus, the structure and absolute configuration of apetalidine 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  $\text{C}_{34}\text{H}_{46}\text{N}_2\text{O}_7$  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 ( $\delta_{\text{H}}$  1.07, 3H, t,  $J = 7.2$  Hz;  $\delta_{\text{C}}$  49.0 t, 13.4 q), two methoxy groups ( $\delta_{\text{H}}$  3.31, 6H, s;  $\delta_{\text{C}}$  55.9 q, 56.1 q), an ethoxy group ( $\delta_{\text{H}}$  1.09 t,  $J = 7.2$  Hz;  $\delta_{\text{C}}$  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 ( $\delta_{\text{C}}$  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  $\beta$ -oriented.

**Table.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR Spectroscopic Data<sup>a,b</sup> for Compounds **1-3** in  $\text{CDCl}_3$  ( $\delta$  in ppm,  $J$  in Hz)

NO.	<b>1<sup>a</sup></b>		<b>2<sup>a</sup></b>		<b>3<sup>b</sup></b>	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$
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	—	—
16-OMe	1.12 t (7.2) 3.35 s	16.2 56.6	1.09 t (7.2) 3.31 s	15.8 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	—

<sup>a</sup>  $^1\text{H}$  NMR (600 MHz),  $^{13}\text{C}$  NMR (150 MHz). <sup>b</sup>  $^1\text{H}$  NMR (400 MHz),  $^{13}\text{C}$  NMR (100 MHz).

The cross-peak between H-16 with H-12b and H-17 in the NOESY experiment revealed that these hydrogens were  $\alpha$ -orientation. Therefore, the structure of apetalidine 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 ( $\delta_C$  85.7), C-8 ( $\delta_C$  77.9), and C-16 ( $\delta_C$  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 apetalidine 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.<sup>9</sup> As far as we know, there were only a few aconitine-type diterpenoid alkaloid bearing a hydroxy group at C-10 position. Apetalidine 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 ( $IC_{50} > 50 \mu M$ ,  $n = 3$ ).

## 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<sub>254</sub> (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<sub>1</sub>-A<sub>8</sub> (in ref. 3). Fraction A<sub>3</sub> (3.2 g) was submitted to silica gel CC eluting with light petroleum: acetone: Et<sub>2</sub>NH (50:1:0.1 to 0:1:0.1, v/v/v) to afford fractions B<sub>1</sub> (500 mg), B<sub>2</sub> (130 mg), B<sub>3</sub> (200 mg), and B<sub>4</sub> (830 mg) respectively. Compounds **1** (6 mg) and **2** (8 mg) were obtained by purifying fractions Fr. B<sub>2</sub> by silica gel column (light

petroleum: acetone: Et<sub>2</sub>NH, 18:1:0.1 to 0:1:0.1, v/v/v). Fraction B<sub>4</sub> was submitted to CC eluting with light petroleum: acetone: Et<sub>2</sub>NH (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<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3316, 2923, 2853, 1741, 1688, 1605, 1589, 1455, 1377, 1296, 1163, 1089, 758; <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) data and <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) data, see Table; HR-ESI-MS (*m/z*): 613.3488 [M + H]<sup>+</sup>, calcd. for C<sub>34</sub>H<sub>49</sub>N<sub>2</sub>O<sub>8</sub>, 613.3489.

#### **Apetaldine I (2)**

White amorphous powder;  $[\alpha]_D^{20} +14.6$  (*c* 0.35, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3473, 3318, 2967, 2925, 2820, 1748, 1702, 1686, 1589, 1526, 1448, 1261, 1240, 1091, 758; <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) data and <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) data, see Table; HR-ESI-MS (*m/z*): 595.3389 [M + H]<sup>+</sup>, calcd. for C<sub>34</sub>H<sub>47</sub>N<sub>2</sub>O<sub>7</sub>, 595.3383.

#### **Apetaldine J (3)**

White amorphous powder;  $[\alpha]_D^{20} +4.9$  (*c* 0.80, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3317, 3282, 2926, 1704, 1686, 1605, 1589, 1526, 1448, 1367, 1295, 1261, 1240, 1089, 757; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) data and <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) data, see Table; HR-ESI-MS (*m/z*): 583.3383 [M + H]<sup>+</sup>, calcd. for C<sub>33</sub>H<sub>47</sub>N<sub>2</sub>O<sub>7</sub>, 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.<sup>10</sup> 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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