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短距乌头中三个新的 C_{19}-二萜生物碱

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摘要 从短距乌头中分离得到 3 个新的 C_{19}-二萜生物碱和 7 个已知化合物, 通过高分辨率质谱(HR-ESI-MS)、红外吸收光谱法(IR)、一维核磁共振(1D NMR)、二维核磁共振(2D NMR)和 X 单晶衍射等波谱技术鉴定了其结构. Brevicalcarine A (**1**)是一种新的二萜生物碱, 其在 C-18 位连接有 vaginatunine A 片段, brevicalcarine B (**2**)和 brevicalcarine C (**3**)是一种在 C-18 有乙酰基的 C_{19}-二萜生物碱. 此外, 还研究了这些化合物对 BV-2 细胞的抗神经炎活性, 结果表明它们均不具有显著的抗神经炎活性.

关键词 毛茛科; 短距乌头; 二萜生物碱; 抗神经炎症活性

Three New C_{19}-Diterpenoid Alkaloids from *Aconitum brevicalcaratum*

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Abstract Three new C_{19}-diterpenoid alkaloids, brevicalcarines A～C (**1**～**3**), together with seven known compounds (**4**～**10**), were isolated from the roots of *A. brevicalcaratum* (Finet et Gagnep.) Diels. Their structures were determined by HR-ESI-MS (high resolution electrospray ionization mass spectroscopy), IR (Infrared absorption spectroscopy), 1D NMR (One-dimensional nuclear magnetic resonance), 2D NMR (Two-dimensional nuclear magnetic resonance) and X-ray crystallographic analysis. Brevicalcarine A (**1**) was a new diterpenoid alkaloid with vaginatunine A fragment at C-18, while brevicalcarine B (**2**) and brevicalcarine C (**3**) were new C_{19}-diterpenoid alkaloid with an acetoxy group at C-18. In addition, the anti-neuroinflammatory activity of these compounds against BV-2 cells was investigated, which showed that none of them had significant anti-neuroinflammatory activity.

Keywords ranunculaceae; *Aconitum brevicalcaratum*; diterpenoid alkaloid; anti-neuroinflammatory activity

1 Introduction

Aconitum is a large genus of Ranunculaceae family and contains a variety of chemical constituents, including alkaloids, flavonoid glycosides, phenylpropanoids, steroids, free fatty acids, polysaccharides, *etc*.[1] Among them, diterpenoid alkaloids are the most characteristic chemical constituents in this genus, which have attracted extensive attention due to their complex structure and remarkable physiological activities.[2] *Aconitum brevicalcaratum* (Finet et Gagnep.) Diels belongs to the genus *Aconitum* of Ranunculaceae family. It is mainly distributed in northwest

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Yunnan and southwest Sichuan province, and grows on the mountain grass slope with an altitude of 2800～3800 m.[3] Phytochemical investigation showed that the main constituents in this plant were diterpenoid alkaloids.[4-6] Based on our previous findings that diterpenoid alkaloids have neuroprotective effects on neurodegenerative diseases,[7-8] we investigated the phytochemistry of *A*. *brevicalcaratum* (Finet et Gagnep.) Diels and evaluated its anti-neuroinflammatory effects. Here, we report the isolation and structural elucidation of compounds (**1**～**10**) isolated from the ethanol extract of *A*. *brevicalcaratum* (Finet et Gagnep.), as well as the neuroprotective activity of these compounds. Brevicalcarine A (**1**) was a new C_{19}-diterpenoid alkaloid with vaginatunine A fragment at C-18, while brevicalcarine B (**2**) and brevicalcarine C (**3**) were new C_{19}-diterpenoid alkaloid with an acetoxy group at C-18. The results show that none of these compounds (**1**～**10**) showed significant inhibitory activity at a concentration of 50 μmol/L.

2 Results and discussion

The ethanol extract of *A*. *brevicalcaratum* (Finet et Gagnep.) was extracted with CH_2Cl_2 to obtain the CH_2Cl_2 soluble fraction. Ten compounds were isolated from the CH_2Cl_2 soluble fraction, including three new compounds, named as brevicalcarines A～C (**1**～**3**), as well as seven known compounds (**4**～**10**) (Figure 1). The structures of the seven known compounds were identified by spectral data and compared with scaconine (**4**),[9] cammaconine (**5**),[7] apetaldine B (**6**),[10] acobretine E (**7**),[11] aconorine (**8**),[12] (6*R*,6a*S*,*P*)-(＋)-corydine (**9**),[13] and vaginatunine A (**10**)[14] reported in the literature.

Brevicalcarine A (**1**) was obtained as a white amorphous powder and showed a positive reaction with Dragendorff's reagent. Its molecular formula was determined as $C_{42}H_{55}N_3O_9$ by high resolution electrospray ionization mass spectra (HR-ESI-MS) (*m*/*z* 746.4026 $[M+H]^+$, calcd 746.4017, $C_{42}H_{56}N_3O_9$). The IR spectrum displayed absorption peaks for hydroxyl (3487 cm^{-1}) and aromatic ring (1685, 1590, 1522, 1440 cm^{-1}). The NMR spectra revealed the presence of two *ortho*-substituted phenyl groups [δ_H 8.83 (dd, *J*＝1.2, 8.4 Hz, 1H), 8.72 (dd, *J*＝1.2, 8.4 Hz, 1H), 8.02 (dd, *J*＝1.6, 8.0 Hz, 1H), 7.86 (dd, *J*＝1.6, 8.0 Hz, 1H), 7.58～7.63 (m, 1H), 7.51～7.56 (m, 1H), 7.22 (td, *J*＝1.2, 7.6, 7.6 Hz, 1H), 7.14～7.18 (m, 1H)], and protons of two monosubstituted acylamino groups [δ_H 12.05 (s, 1H), 11.93 (s, 1H)]. The above features and the comparison with the literature indicated the existence of vaginatunine A (**10**) fragment.[14] The NMR spectra revealed the presence of an *N*-ethyl group [δ_H 1.08 (t, *J*＝7.2 Hz); δ_C 13.7 q, 49.4 t], three methoxy groups [δ_H 3.38 (s, 3H), 3.32 (s, 3H), 3.28 (s, 3H); δ_C 57.9 q, 56.4 q, 56.4 q]. In addition to the signals of the above-mentioned groups, the 1D NMR and HSQC (heteronuclear single quantum coherence) spectra demonstrated the surplus of 19 carbon signals including seven methylenes (δ_C 71.4, 52.9, 41.8, 32.9, 29.6, 26.4, 25.5), nine methines (δ_C 85.4, 84.5, 82.7, 62.1, 46.5, 45.9, 45.9, 45.5, 37.0) and three quaternary carbons (δ_C 74.1, 49.1, 38.2). The aforementioned NMR features indicated that **1** was an aconitine-type C_{19}-diterpenoid with a substitution at C-18 position.[15-16] The ^{13}C NMR spectrum suggested the existence of six oxygenated carbons (δ_C 85.4 d, 84.5 d, 82.7 d, 74.1 s, 73.7 s, 71.4 t), corresponding to the molecular formula, which indicated the presence of two hydroxyl groups in addition to three methoxy groups and one anthranoyl ester group. Three methoxy groups were placed at C-1, C-14 and C-16 on the basis of heteronuclear multiple bond connectivity (HMBC) correlations from δ_H 3.28 (s) to C-1 (δ_C 85.4, d), δ_H 3.38 (s) to C-14 (δ_C 84.5, d), and δ_H 3.32 (s) to C-16 (δ_C 82.7, d), respectively (Figure 2). The long correlations from C-8 (δ_C 74.1, s) to both H-7 (δ_H 2.08～2.12, m) and H-14 (δ_H 3.67, t, *J*＝4.8 Hz) suggested the presence of a hydroxyl group at the C-8 position. According to the HMBC correlation (Figure 2) between H-18 (δ_H 4.01/4.12, each 1H, ABq, *J*＝10.8 Hz) and C-7' (168.6, s), the fragment of vaginatunine A (**10**) was localized to C-18 via an ester group. Comparing the NMR data of **1** with those of the known apetalrine B,[7] it was found that these two compounds exhibited nearly identical ^1H NMR and ^{13}C NMR resonances. The main difference between **1** and apetalrine B was the presence of a

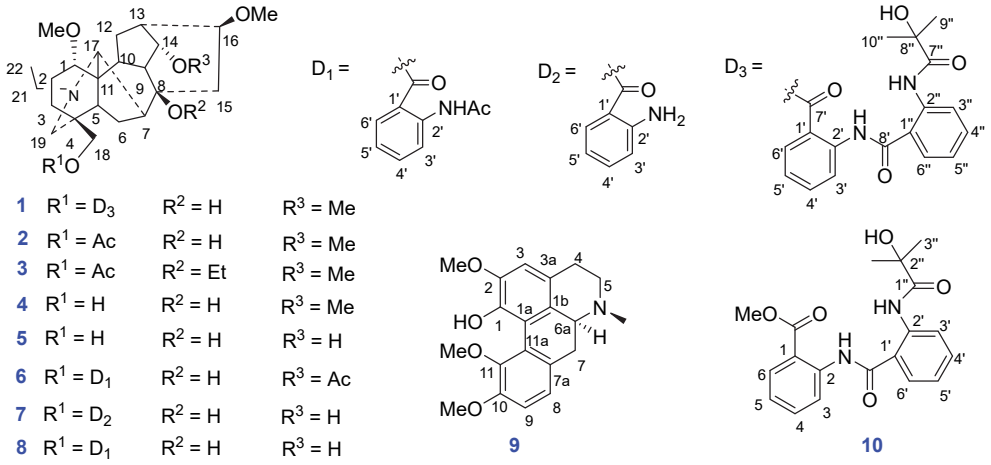

Figure 1 Chemical structures of the compounds **1**～**10**

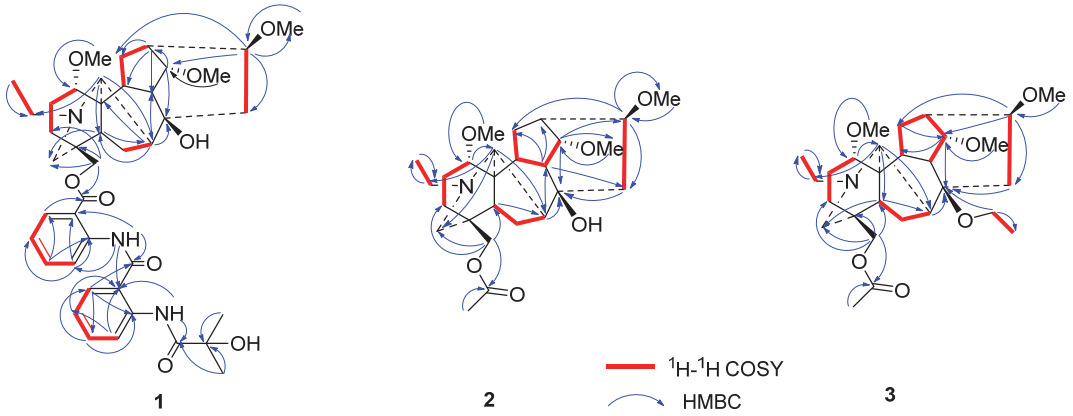

Figure 2 Key ^{1}H-^{1}H COSY and HMBC correlations of **1**～**3**

methoxy group in **1**, which was verified by the increase of 14 mass units in **1** in mass spectrometry. The proton signal of H-14 was shifted downfield from δ_H 4.05 in apetalrine B to δ_H 3.67 (t, J=4.8 Hz, 1H) in **1**. Its corresponding carbon signals were shifted upfield from δ_C 75.7 to δ_C 84.5, indicating that the hydroxyl group at H-14 in apetalrine B was substituted by methoxy group in **1**.[17] This conjecture was also supported by the HMBC (Figure 2) between H-16 (δ_H 3.17～3.20, m) and C-14 (δ_C 84.5, d), H-14 (δ_H 3.67, t, J=4.8 Hz) and C-13 (δ_C 46.5, d).

The relative configuration of compound **1** was deduced from the vicinal coupling constants (Table 1) and a nuclear overhauser effect spectroscopy (NOESY) experiment (Figure 3). The coupling constant δ_H 3.67 (t, J=4.8 Hz, 1H) of H-14 confirmed the β-configuration of H-14.[18] The cross-peak between H-14 and H-10β further confirmed the configuration of H-14. No coupling correlation between H-13 and H-16 was observed in the ^{1}H-^{1}H COSY (correlation spectroscopy) spectrum of **1**, indicating that D-ring was boat conformation and H-16 was installed in the α-configuration. This finding was supported by the cross-peak between H-16 and H-17α. The cross-peak between H-1 and H-9β indicated the α-position of OCH_3-1. Therefore, the structure of **1** was assigned as brevicalcarine A.

Brevicalcarine B (**2**) was obtained as colorless tetragonal prisms and showed a positive reaction with Dragendorff's reagent. Its molecular formula was deduced as $C_{26}H_{41}NO_6$ on the basis of a protonated molecular ion peak at m/z 464.3023 $[M+H]^+$ in the HR-ESI-MS (calcd 464.3012). The IR spectrum showed a strong and broad absorption brand at 3442 cm^{-1} for hydroxyl groups, and an absorbance peak at 1736 cm^{-1} for ester carbonyl groups. The ^{1}H NMR and ^{13}C NMR spectra of **2** exhibited characteristic features of an aconitine-type C_{19}-diterpenoid alkaloid,[15-16] bearing an acetoxy group [δ_H 2.04 (s, 3H), δ_C 171.3 s, 21.0 q], an N-ethyl group [δ_H (1.05, t, J=7.2 Hz); δ_C 13.7 q, 49.4 t], and three methoxy groups [δ_H 3.39 (s, 3H), 3.31 (s, 3H), 3.27 (s, 3H); δ_C 57.9 q, 56.4 q, 56.4 q]. In addition, it is obvious from ^{13}C NMR data and molecular formula that there is a hydroxyl group attached to the quaternary carbon (δ_C 74.1). In the HMBC spectrum (Figure 2), the acetoxy group was located at C-18, due to the long correlation between H-18 (δ_H 3.80/3.75, each 1H, ABq, J=10.8 Hz) and 18-OCO (δ_C 171.3, s). The correlations from OCH_3-1 (δ_H 3.27, s) to C-1 (δ_C 85.6, d), OCH_3-14 (δ_H 3.31, s) to C-14 (δ_C 84.5, d), and OCH_3-16 (δ_H 3.39, s) to C-16 (δ_C 82.7, d), indicated that the three methoxy groups were assigned to C-1, C-14, and C-16, respectively. The long correlations from δ_C (74.1, s) to both H-7 (δ_H 2.06～2.10, m) and H-14 (δ_H 3.67, t, J=4.8 Hz) suggested the presence of the hydroxyl group at the C-8 position. Comparing the NMR data of the known compound scaconine (**4**)[9] and **2**, it was found that the NMR data of

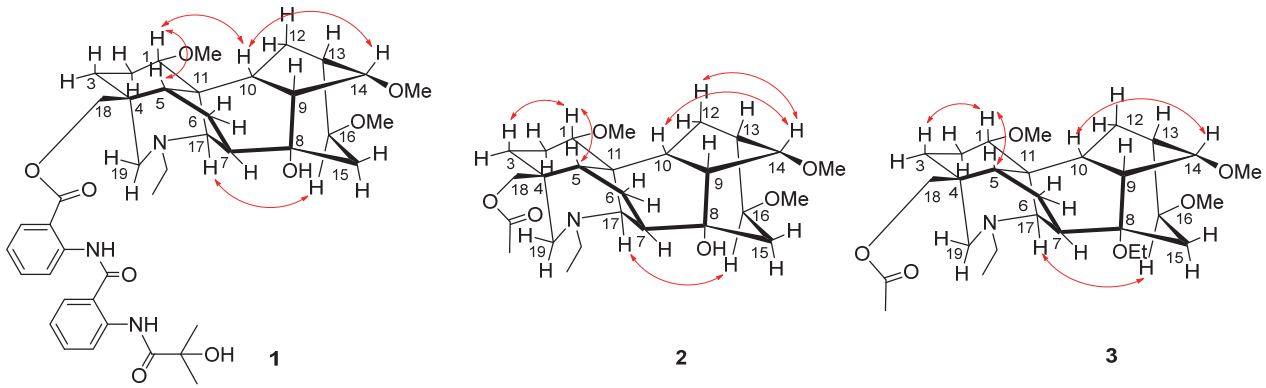

Figure 3 Key NOESY correlations of **1**～**3**

Table 1 ^{13}C NMR and ^{1}H NMR data for compounds **1**～**3** ($CDCl_3$)

No.	Compound **1**		Compound **2**		Compound **3**	
	δ_C[a]	δ_H (J in Hz)[b]	δ_C[c]	δ_H (J in Hz)[d]	δ_C[c]	δ_H (J in Hz)[d]
1	85.4 d	3.14～3.16 m	85.6 d	3.05～3.11 m	85.4 d	3.08～3.15 m
2	25.5 t	2.33～2.35 m, β	26.4 t	2.27～2.29 m, β	26.2 t	2.23～2.25 m, β
	—	2.02～2.07 m, α	—	1.94～1.98 m, α	—	1.93～1.96 m, α
3	32.9 t	1.86～1.87 m, β	32.6 t	1.69～1.77 m, β	32.0 t	1.73～1.79 m, β
	—	1.45～1.48 m, α	—	1.31～1.34 m, α	—	1.25～1.28 m, α
4	38.2 s	—	37.8 s	—	37.7 s	—
5	45.9 d	1.63～1.68 m	46.4 d	1.54～1.60 m	45.4 d	1.57～1.61 m
6	26.4 t	1.97～2.00 m, β	25.3 t	1.90～1.94 m, β	24.5 t	1.96～1.99 m, β
	—	1.83～1.85 m, α	—	2.25～2.27 m, α	—	1.33～1.38 m, α
7	45.9 d	2.08～2.12 m	45.9 d	2.06～2.10 m	41.1 d	2.33～2.37 m
8	74.1 s	—	74.1 s	—	77.5 s	—
9	45.5 d	1.82～1.84 m	45.5 d	1.80～1.86 m	42.9 d	2.26[e]
10	37.0 d	2.35～2.38 m	37.0 d	2.34～2.37 m	39.4 d	2.26[e]
11	49.1 s	—	48.9 s	—	49.4 s	—
12	29.6 t	2.31～2.33 m, β	29.6 t	2.31～2.34 m, β	29.6 t	2.20～2.23 m, β
	—	1.87～1.89 m, α	—	1.77～1.80 m, α	—	1.79～1.82 m, α
13	46.5 d	2.25～2.29 m	46.4 d	2.24～2.25 m	45.8 d	1.82～1.88 m
14	84.5 d	3.67 t (4.8)	84.5 d	3.67 t (4.8)	84.0 d	3.53 t (4.8)
15	41.8 t	2.28～2.30 m, α	41.8 t	2.29～2.31 m, α	36.2 t	2.11～2.19 m, α
	—	1.90～1.96 m, β	—	1.86～1.91 m, β	—	1.99～2.02 m, β
16	82.7 d	3.17～3.20 m	82.7 d	3.19～3.20 m	84.1 d	3.16～3.22 m
17	62.1 d	2.96 s	62.3 d	2.92 s	61.6 d	2.83 s
18	71.4 t	4.12 ABq (10.8), β	70.5 t	3.80 ABq (10.8), β	70.4 t	3.82 ABq (10.8), β
	—	4.01 ABq (10.8), α	—	3.75 ABq (10.8), α	—	3.78 ABq (10.8), α
19	52.9 t	2.63 ABq (10.8), α	52.8 t	2.50～2.55 m, α	53.2 t	2.51～2.56 m, α
	—	2.14 ABq (10.8), β	—	1.98～2.02 m, β	—	2.02～2.04 m, β
21	49.4 t	2.49～2.56 m, β	49.4 t	2.45～2.50 m, β	49.3 t	2.56～2.64 m, β
	—	2.38～2.45 m, α	—	2.36～2.42 m, α	—	2.45～2.50 m, α
22	13.7 q	1.08 t (7.2)	13.7 q	1.05 t (7.2)	13.4 q	1.09 t (7.2)
1-OCH_3	56.4 q	3.28 s	56.4 q	3.27 s	56.5 q	3.28 s
14-OCH_3	57.9 q	3.38 s	57.9 q	3.31 s	57.8 q	3.37 s
16-OCH_3	56.4 q	3.32 s	56.4 q	3.39 s	56.6 q	3.35 s
18-OCO	—	—	171.3 s	—	171.3 s	—
18-OAc	—	—	21.0 q	2.04 s	21.0 q	2.05 s
8-OEt	—	—	—	—	55.6 t	3.33～3.34 m, α
	—	—	—	—	—	3.31～3.33 m, β
	—	—	—	—	16.3 q	1.08 t (7.2)
1'	115.9 s	—	—	—	—	—
2'	141.5 s	—	—	—	—	—
3'	121.0 d	8.83 dd (1.2, 8.4)	—	—	—	—
4'	134.9 d	7.58～7.63 m	—	—	—	—
5'	123.3 d	7.14～7.18 m	—	—	—	—
6'	130.9 d	8.02 dd (1.6, 8.0)	—	—	—	—
7'	168.6 s	—	—	—	—	—
8'	167.8 s	—	—	—	—	—
1"	121.3 s	—	—	—	—	—
2"	140.0 s	—	—	—	—	—
3"	121.5 d	8.72 dd (1.2, 8.4)	—	—	—	—
4"	133.3 d	7.51～7.56 m	—	—	—	—
5"	123.7 d	7.22 td (1.2, 7.6, 7.6)	—	—	—	—
6"	127.4 d	7.86 dd (1.6, 8.0)	—	—	—	—
7"	175.9 s	—	—	—	—	—
9"	28.1 q	1.56 s	—	—	—	—

Continued

No.	Compound **1**		Compound **2**		Compound **3**	
	δ_C[a]	δ_H (J in Hz)[b]	δ_C[c]	δ_H (J in Hz)[d]	δ_C[c]	δ_H (J in Hz)[d]
10"	28.1 q	1.56 s	—	—	—	—
2'-NH	—	12.05 s	—	—	—	—
2"-NH	—	11.93 s	—	—	—	—

[a] 150 MHz; [b] 600 MHz; [c] 100 MHz; [d] 400 MHz; [e] means overlapped.

these two compounds were very similar except that **2** had an additional acetyl signal [δ_H 2.04 (s, 3H); δ_C 171.3 s, 21.0 q], which was confirmed by the increase of 42 mass units in **2** in mass spectrometry.

The relative configuration of **2** was deduced by the analyzing of ^{1}H-^{1}H coupling constants (Table 1), NOESY experiments (Figure 3) and X-ray crystallographic analysis (Figure 4). The characteristic proton signal [δ_H 3.67 (t, J=4.8 Hz, 1H)] was ascribed to H-14β.[18] The cross-peak between H-10β and H-14 further indicated the α-position of OCH_3-14. Similarly, OCH_3-1 and OCH_3-16 were installed in α-confirguration and β-confirguration, respectively, due to the presence of cross-peak between H-16 and H-17α, and between H-1 and H-5β. X-ray crystallography analysis (Figure 4) further confirmed the structure and relative configuration of **2**. Therefore, the structure of **2** was named as brevicalcarine B.

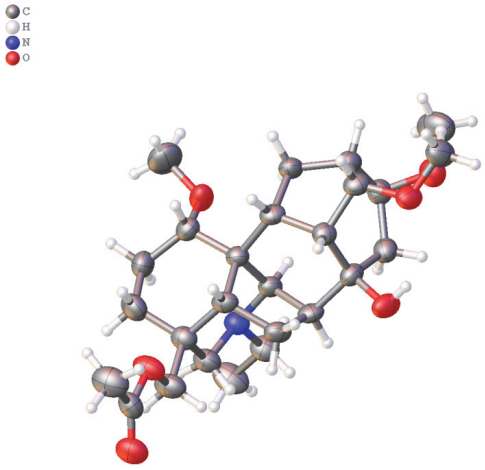

Figure 4 Crystal structure of compound **2**

Brevicalcarine C (**3**) was obtained as a white amorphous powder and gave a positive reaction with Dragendorff's reagent. Its molecular formula was determined as $C_{28}H_{45}NO_6$ based on the HR-ESI-MS ion at m/z 492.3332 (calcd for $C_{28}H_{46}NO_6$ 492.3325). The IR spectrum showed that **3** possesses hydroxyl (3453 cm^{-1}) and ester carbonyl groups (1739 cm^{-1}). The NMR data revealed the presence of an N-ethyl group [δ_H 1.09 (t, J=7.2 Hz, 3H); δ_C 13.4 q, 49.3 t], an ethoxy group [δ_H 1.08 (t, J=7.2 Hz, 3H); δ_C 16.3 q, 55.6 t], an acetoxy group [δ_H 2.05 (s, 3H); δ_C 171.3 s, 21.0 q], and three methoxy groups (δ_H 3.28, 3.35, 3.37, each 3H, s; δ_C 56.5 q, 56.6 q, 57.8 q). The ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) spectra (Table 1) showed that **3** contains nineteen carbons except for the above groups, including seven methylenes (70.4, 53.2, 36.2, 32.0, 29.6, 26.2, 24.5), nine methines (δ_C 85.4, 84.1, 84.0, 61.6, 45.8, 45.4, 42.9, 41.1, 39.4), and three quaternary carbons (77.5, 49.4, 37.7). The above-mentioned data showed that compound **3** was an aconitine-type diterpenoid alkaloid.[15-16] The NMR data for **3** were similar to those for **2** except that the hydroxyl group in **2** was replaced by the ethoxy group [δ_H 1.08 (t, J=7.2 Hz, 3H); δ_C 16.3 q, 55.6 t] in **3** at C-8, as evidenced by the 28 mass unit difference between the two compounds. The above view was also supported by the correlation of HMBC (Figure 2) among H-7 [δ_H 2.33～2.37 (m, 1H)], H-14 [δ_H 3.53 (t, J=4.8 Hz, 1H], H-15 [δ_H 2.11～2.19, 1.99～2.02 (each 1H, m)] and C-8 (δ_C 77.5 s). Furthermore, the three methoxy groups were assigned at C-1, C-14, and C-16 based on the HMBC correlation between the methoxy proton signals (δ_H 3.28, 3.35, 3.37, each 3H, s) and C-1 (δ_C 85.4, d), C-14 (δ_C 84.0, d) and C-16 (δ_C 84.1, d), respectively. Based on the HMBC correlation between H-18 (δ_H 3.82, 3.78, each 1H, ABq, J=10.8 Hz) and 18-OCO (δ_C 171.3, s), the acetoxy group was localized to C-18.

The configuration of **3** is similar to that of **2**, inferred from its NOESY experiments (Figure 3) and the coupling constants of neighboring H atoms (Table 1). The cross-peak between H-1 and H-5β, H-14 and H-10β, and H-16 and H-17α indicated that OCH_3-1and OCH_3-14 were α-oriented, while OCH_3-16 was β-oriented. In addition, the coupling constant of H-14 [δ_H 3.53 (t, J=4.8, 1H)] (Table 1) confirmed that OCH_3-14 was α-oriented.[18] Since X-ray crystallographic analysis confirmed the configuration of compound **2**, it is proposed that **3** has the same configuration. All of the above evidence illustrated the structure of **3** depicted in Figure 1.

The neuroprotective activity of each compound was tested by the Griess method as described in previous papers.[19] Quercetin was used as the positive control drug. As shown in Table 2, none of the isolated compounds showed significant inhibitory activity.

3 Experimental section

3.1 General experimental procedures

NMR spectra were obtained on a Bruker AV 400 nuclear magnetic resonance instrument (400 MHz) or a Bruker AV 600 nuclear magnetic resonance instrument (600 MHz) with tetramethylsilane (TMS) as an internal standard. HR-ESI-MS data were obtained by a Q-TOF micro mass spectrometer (Waters, Milford, America). Infrared (IR) spectra were obtained using a Thermo Fisher Nicolet 6700 spectrometer. Optical rotations were tested on a PerkinElmer 341 polari-

Table 2 Effects of compounds **1**～**10** on the production of nitric oxide (NO) in LPS-stimulated BV-2 cells and their cytotoxicity.

Compound	50 μmol/L	
	Inhibition rate/%	Survival rate/%
1	5.72±1.95	104.40±1.54
2	5.18±0.69	103.00±1.15
3	3.47±3.40	103.40±1.16
4	3.20±1.39	107.00±1.85
5	1.20±1.06	105.90±0.72
6	3.52±0.56	102.30±1.15
7	12.69±0.60	98.61±2.81
8	4.44±1.54	106.30±4.62
9	1.20±1.06	103.10±2.90
10	16.26±0.70	96.90±4.33
Quercetin (20 μmol•L^{-1})[a]	61.94±0.78	99.38±3.96
Control	0.00±0.00	100.00±0.00

[a] Quercetin was used as a positive control.

meter (PerkinElmer, Waltham, America). Column chromatography (CC) was carried out on silica gel (200～300 mesh) (Qingdao Haiyang Chmical Group Corporation, China). The TLC plates were visualized under a UV lamp at 254 nm or by spraying the Dragendorff's reagent or iodine (Chengdu Kelong Chemical Co., Ltd., Chengdu, People's Republic of China).

3.2 Plant material

The roots of *A. brevicalcaratum* (Finet et Gagnep.) Diels were collected in Basu County, Tibet Autonomous Region, People's Republic of China, in July 2019, and authenticated by associate professor Guodong Li at Yunnan University of Traditional Chinese Medicine. A voucher specimen (Swjtu-DJWT-201907) was deposited in the School of Life Science and Engineering, Southwest Jiaotong University.

3.3 Extraction and isolation

The air-dried roots of *A. brevicalcaratum* (Finet et Gagnep.) Diels (20 kg) were extracted three times with 95% (*V*/*V*) ethanol at 55 ℃. The combined filtrate was evaporated under reduced pressure to yield a viscous residue. The residue was dissolved in water (2 L) and acidified to pH 2～3 with 10% dilute hydrochloric acid, and then successively extracted with petroleum ether (5 L×5). The acidic aqueous solution was alkalized with aqueous ammonia solution to pH 9～10, then extracted with CH_2Cl_2 (5 L×8), and the solvent was removed under reduced pressure to obtain total crude alkaloids (150.0 g).

The total crude alkaloids (150.0 g) were chromatographed over silica gel with petroleum ether-ethyl acetate-Et_2NH (*V*∶*V*∶*V*=80∶1∶0.1 to 0∶10∶0.1) obtain nine fractions (Frs. A～Frs. I). Fr. C (10.7 g) was subjected to a silica gel column with petroleum ether-CH_2Cl_2-Et_2NH (*V*∶*V*∶*V*=10∶1∶0.1 to 1∶10∶0.1) to get seven subfractions (Frs. C_1～Frs. C_7). Subfraction C_1 was further purified on a silica gel column eluted with CH_2Cl_2-CH_3OH (*V*∶*V*=50∶1 to 30∶1) to give compound **3** (15 mg). Compound **2** (49 mg) was obtained by purifying subfractions C_3 with CH_2Cl_2-CH_3OH (*V*∶*V*=30∶1 to 10∶1). Subfraction C_5 was subjected to silica gel column and eluted with petroleum ether-CH_2Cl_2-CH_3OH (*V*∶*V*∶*V*=50∶80∶1 to 25∶50∶1) to get compound **6** (15 mg).

Fr. G (25.2 g) was submitted to a silica gel column with petroleum ether-CH_2Cl_2-CH_3OH (*V*∶*V*∶*V*=10∶50∶1 to 1∶10∶1) to get six subfractions (Frs. G_1～Frs. G_6). Further silica gel column purification of subfraction G_2 with CH_2Cl_2-CH_3OH (*V*∶*V*=100∶1 to 30∶1) to yield compounds **9** (20 mg) and **8** (98 mg). Compound **7** (18 mg) was isolated from subfraction G_3 with CH_2Cl_2-CH_3OH (*V*∶*V*=150∶1 to 50∶1). Subfraction G_4 was separated on a silica gel column with CH_2Cl_2-CH_3OH (*V*∶*V*=120∶1 to 50∶1) to give compounds **1** (8 mg) and **10** (23 mg). Subfraction G_6 was subjected to silica gel column with petroleum ether-CH_2Cl_2-Et_2NH (*V*∶*V*∶*V*=10∶1∶0.1 to 1∶20∶0.1) to afford compound **5** (20 mg) and a mixture which was further purified over silica gel with CH_2Cl_2-CH_3OH (*V*∶*V*=80∶1 to 50∶1) to yield compound **4** (89 mg).

Brevicalcarine A (**1**): White amorphous powder, m.p. 184.0～184.9 ℃; $[\alpha]_D^{25}$ −27.9 (*c* 0.1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data see Table 1. IR (KBr) ν_{max}: 3487, 3302, 2931, 2822, 1685, 1590, 1522, 1440, 1303, 1258, 1093, 759 cm^{-1}. HR-ESI-MS calcd for $C_{42}H_{56}N_3O_9$ $[M+H]^+$ 746.4017, found 746.4026.

Brevicalcarine B (**2**): Colorless tetragonal prisms, m.p. 92.7～93.2 ℃; $[\alpha]_D^{25}$ −25.2 (*c* 0.2, $CHCl_3$). IR (KBr) ν_{max}: 3544, 3442, 2956, 2924, 2866, 2803, 1736, 1462, 1387, 1364, 1256, 1210, 1103, 1086, 1042 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data see Table 1. HR-ESI-MS calcd for $C_{26}H_{42}NO_6$ $[M+H]^+$ 464.3012, found 446.3023.

Brevicalcarine C (**3**): White amorphous powder, m.p. 132.9～133.4 ℃; $[\alpha]_D^{25}$ −26.9 (*c* 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data see Table 1; IR (KBr) ν_{max}: 3453, 2948, 2927, 1739, 1642, 1459, 1383, 1242, 1093, 1045, 801 cm^{-1}; HR-ESI-MS calcd for $C_{28}H_{46}NO_6$ $[M+H]^+$ 492.3325, found 492.3332.

Scaconine (**4**): White amorphous powder. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data see Supporting Information; IR (KBr) ν_{max}: 3425, 2929, 2826, 1646, 1456, 1386, 1201, 1087, 796 cm^{-1}; HR-ESI-MS calcd for $C_{24}H_{40}NO_5$ $[M+H]^+$ 422.2906, found 422.2899.

Cammaconine (**5**): White amorphous powder.^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data see Supporting Information; IR (KBr) ν_{max}: 3506, 3398, 2929, 2811, 1658, 1453, 1365, 1156, 1087, 1041 cm^{-1}; HR-ESI-MS calcd for $C_{23}H_{38}NO_5$ $[M+H]^+$ 408.2750, found 408.2744.

Apetaldine B (**6**): White amorphous powder.^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data see Supporting Information; IR (KBr) ν_{max}: 3458, 3324, 2923, 2814, 1741, 1690, 1591, 1533, 1453, 1376, 1251, 1085, 755 cm^{-1}; HR-ESI-MS calcd for $C_{34}H_{47}N_2O_8$ $[M+H]^+$ 611.3332, found 611.3339.

Acobretine E (**7**): White amorphous powder.^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data

see Supporting Information; IR (KBr) ν_{max}: 3455, 3370, 2926, 2817, 1688, 1619, 1459, 1380, 1298, 1244, 1159, 1090, 754 cm^{-1}; HR-ESI-MS calcd for $C_{30}H_{43}N_2O_6$ $[M+H]^+$ 527.3121, found 527.3101.

Aconorine (**8**): White amorphous powder.^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data see Supporting Information; IR (KBr) ν_{max}: 3431, 2926, 2817, 1695, 1595, 1528, 1450, 1377, 1262, 1159, 1087, 759 cm^{-1}; HR-ESI-MS calcd for $C_{32}H_{45}N_2O_7$ $[M+H]^+$ 569.3227, found 569.3246.

(6*R*,6a*S*,*P*)-(+)-Corydine (**9**): Brown solid. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data see Supporting Information; IR (KBr) ν_{max}: 3216, 2941, 2841, 2783, 1598, 1574, 1462, 1431, 1283, 1241, 1135, 1080, 1023 cm^{-1}; HR-ESI-MS calcd for $C_{20}H_{24}NO_4$ $[M+H]^+$ 342.1705, found 342.1715.

Vaginatunine A (**10**): White amorphous powder.^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data see Supporting Information; IR (KBr) ν_{max}: 3419, 3249, 2926, 2853, 1661, 1586, 1516, 1437, 1307, 1265, 757 cm^{-1}; HR-ESI-MS calcd for $C_{19}H_{20}N_2O_5Na$ $[M+Na]^+$ 379.1270, found 379.1295.

3.4 MTT assay

Mouse microglia cell line (BV-2) was obtained from Procell Life Science & Technology Co., Ltd. (Wuhan, China). BV-2 cells were seeded in 96-well plate at a density of 5×10^3/well for 12 h and cultured in complete Minimum Essential Medium (MEM, obtained from Procell Life Science & Technology Co., Ltd., Wuhan, China, CM-0493) in a humidified atmosphere with 5% CO_2 at 37 ℃. Then, the 96-well plates were incubated with compounds **1**～**10** for 24 h, and 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL) was added to each well for 4 h. Subsequently, the medium in the wells was removed and 150 μL of dimethyl sulfoxide (DMSO) was added to each well. The absorbance was measured by a microplate reader (CMax Plus, Molecular Devices) at 492 nm. As shown in Table 2, none of the isolated compounds showed significant toxicity to BV-2 cells at 50 μmol/L.

$$\text{Cell survival rate (\%)} = (OD_{\text{drug group}} - OD_{\text{blank group}})/(OD_{\text{control group}} - OD_{\text{blank group}}) \times 100\%$$

3.5 NO production inhibitory assay

The anti-neuroinflammatory activity of the isolated compounds was performed as previously described.[20-21] Griess Reagent System Kit (Beyotime Institute of Biotechnology, China). BV-2 cells were plated in 96-well plate at a density of 5×10^4/well for 12 h. Cells were pretreated with compounds **1**～**10** (50 μmol/L) or 0.1% DMSO as control for 4 h and then co-treated for 24 h with or without 1 μg/mL of lipopolysaccharide (LPS). Subsequently, 50 μL of culture supernatant mixed with Griess reagent (reagent Iand reagent II, each 50 μL) for 10 min at room temperature in the dark. The assay was measured by microplate reader at 562 nm wavelength.

$$\text{Inhibition rate (\%)} = (OD_{\text{LPS group}} - OD_{\text{drug group}})/(OD_{\text{LPS group}} - OD_{\text{control group}}) \times 100\%$$

Supporting Information IR, HR-ESI-MS, 1D NMR and 2D NMR spectra for compounds **1**～**10** and X-ray crystallographic data of compound **2**. The Supporting Information is available free of charge via the Internet at http://sioc-journal.cn.

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