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DITERPENOID ALKALOIDS FROM *DELPHINIUM PACHYCENTRUM* HEMSL.

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Abstract – Five new C_{19}-diterpenoid alkaloids, named pachycenines A–E (**1**–**5**), along with eleven known diterpenoid alkaloids (**6**–**16**), were isolated from the whole plant of *Delphinium pachycentrum* Hemsl. Their structures were elucidated on the basis of comprehensive spectroscopic data analysis, including 1D, 2D NMR, and HR-ESI-MS. Cytotoxicity and anti-inflammatory activity of those compounds were also carried out.

Delphinium, a large genus of the Ranunculaceae family, which are distributed mainly in the temperate regions of the Northern Hemisphere.[1] The diterpenoid alkaloids are the primary characteristic constituents of *Delphinium*, which are highly oxygenated and complex natural products, exhibiting various bioactivities, such as anti-inflammatory, anti-arrhythmia, antifungal, and cytotoxic properties.[2] *Delphinium pachycentrum* Hemsl. is mainly distributed in southeast of Qinghai, Sichuan, and other places in China, and has been used for the treatment of various types of pains for a long time.[3] In previous reports, twelve diterpenoid alkaloids from this plant were isolated and identified.[4] In this study, we report the isolation and structural elucidation of five new diterpenoid alkaloids, pachycenines A–E (**1**–**5**), by 1D, 2D NMR and HR-ESI-MS experiments (Figure 1). In addition, eleven known diterpenoid alkaloids were isolated and identified as tatsiensine (**6**),[5] deacetyltatsiensine (**7**),[6] swinanine A (**8**),[5] deacetylswinanine A (**9**),[7] swinanine B (**10**),[5] pachycentine D (**11**),[4] pachycentine B (**12**),[4] dehydrodeltatsine (**13**),[8] deltatsine (**14**),[9] pachycentine A (**15**),[4] and pachycentine C (**16**).[4] Herein, we report the isolation and structural elucidation of these compounds, as well as their cytotoxicity and inhibitory effect on NO production of RAW264.7 induced by LPS cells.

Compound **1** was obtained as a white amorphous powder. Its molecular formula was determined as $C_{23}H_{37}NO_5$ by HR-ESI-MS at *m/z* 408.2737 $[M + H]^+$ (Calcd for $C_{23}H_{38}NO_5$, 408.2750). The ^1H and ^{13}C NMR spectra revealed the presence of an *N*-ethyl group [δ_H 1.11, 3H, t, J = 7.2 Hz; 2.96, 1H, m, 2.88, 1H, m; δ_C 13.7 (q), 50.3 (t)], one methyl group [δ_H 0.96, 3H, s; δ_C 27.5 (q)], and two methoxy groups [δ_H 3.34, 3.39 (each 3H, s); δ_C 51.9 (q), 57.4 (q)]. The ^{13}C NMR spectrum showed the presence of the other 19 carbon resonances, including six methines [δc 82.5, 69.6, 63.4, 54.1, 42.0, 31.5], seven methylenes [δc 59.9, 42.8, 35.0, 31.0, 29.7, 24.8, 17.6], and five quaternary carbons [δc 87.4, 83.3, 81.1, 55.3, 32.9], indicated that compound **1** was a lycoctonine-type C_{19}-diterpenoid alkaloid.[10] The locations of two methoxy groups were attributed to C-8 and C-14, based on the correlations between 8-OMe (δ_H 3.34) and C-8 (δ_C 81.1), and 14-OMe (δ_H 3.39) and C-14 (δ_C 82.5) in the HMBC spectrum. The existence of five oxygenated carbons deduced from its ^{13}C NMR spectrum suggests that **1** has three hydroxy groups, in addition to two methoxy groups. The location of the hydroxy groups at C-1, C-7 and C-10 were further confirmed by the HMBC correlations. Careful analysis of 1D and 2D NMR data, compound **1** is similar to pachycentine B,[4] except that the methoxy group in pachycentine B was replaced by a hydroxy group in compound **1** at C-1, which was supported by the HMBC correlations of H-3, H-5, and H-17 with C-1. The configuration of compound **1** was deduced from the NOESY experiment. In the NOESY spectrum, the cross-peak between H-1 and H-5β, H-9 and H-12β, H-12β and H-14 were confirmed the configuration of the α-orientation of 14-OMe and 1-OH. As of now, the absolute configuration of the skeleton of lycoctonine-type diterpenoid alkaloid has already been confirmed by X-ray crystallographic analysis.[11] Therefore, the structure and absolute configuration of compound **1** was defined as shown in Figure 1, and it was named pachycenine A.

Compound **2** was obtained as a white amorphous powder. Its molecular formula was determined as $C_{25}H_{39}NO_7$ by HR-ESI-MS at *m/z* 466.2814 $[M + H]^+$ (Calcd for $C_{25}H_{40}NO_7$, 466.2805). The ^1H and ^{13}C NMR spectra of **2** exhibited characteristic data of the lycoctonine-type C_{19}-diterpenoid alkaloid. NMR spectra revealed the presence of an *N*-ethyl group [δ_H 1.06, 3H, t, J = 7.2 Hz; δ_C 13.8 (q), 49.9 (t)], one methyl group [δ_H 0.83, 3H, s; δ_C 20.3 (q)], three methoxy groups [δ_H 3.46, 3.33, 3.30 (each 3H, s); δ_C 57.8 (q), 56.3 (q), 56.0 (q)], and a methylenedioxy group [δ_H 5.01, 4.91, (each 1H, s); δ_C 93.5 (t)]. The methoxy groups were assigned to at C-1, C-14 and C-16, due to the correlations between 1-OMe (δ_H 3.30, 3H, s) and C-1 (δ_C 76.8 d), 14-OMe (δ_H 3.46, 3H, s) and C-14 (δ_C 81.2 d), 16-OMe (δ_H 3.33, 3H, s) and C-16 (δ_C 81.6 d) in the HMBC spectrum. The location of the methylenedioxy group was established based on the HMBC between δ_H (5.01, 4.91) of the methylenedioxy group and C-7 (δ_C 88.2, s) and C-8 (δ_C 80.6, s). The remaining two OH groups were assigned to C-10 and C-13, which can be confirmed by the correlations from H-9 (δ_H 2.64, 1H, d, J = 5.2 Hz), H-12 (δ_H 1.75, 1H, d, J = 7.6 Hz) to C-13 (δ_C 84.9 s), H-9 (δ_H 2.64, 1H, d, J = 5.2 Hz), H-17 (δ_H 3.05, s) to C-10 (δ_C 83.7 s). The relative configuration of

compound **2** was deduced according to NOESY analysis. The NOESY correlations of H-1 and H-5*β*, H-12*β* and H-14 were confirmed the configuration of the *α*-orientation of 14-OMe and 1-OH. In addition, the cross-peak between H-16 with H-17*α* indicated that 16-OMe was the *β*-position. Therefore, the structure and absolute configuration of **2** was defined as shown in Figure 1, and it was named pachycenine B.

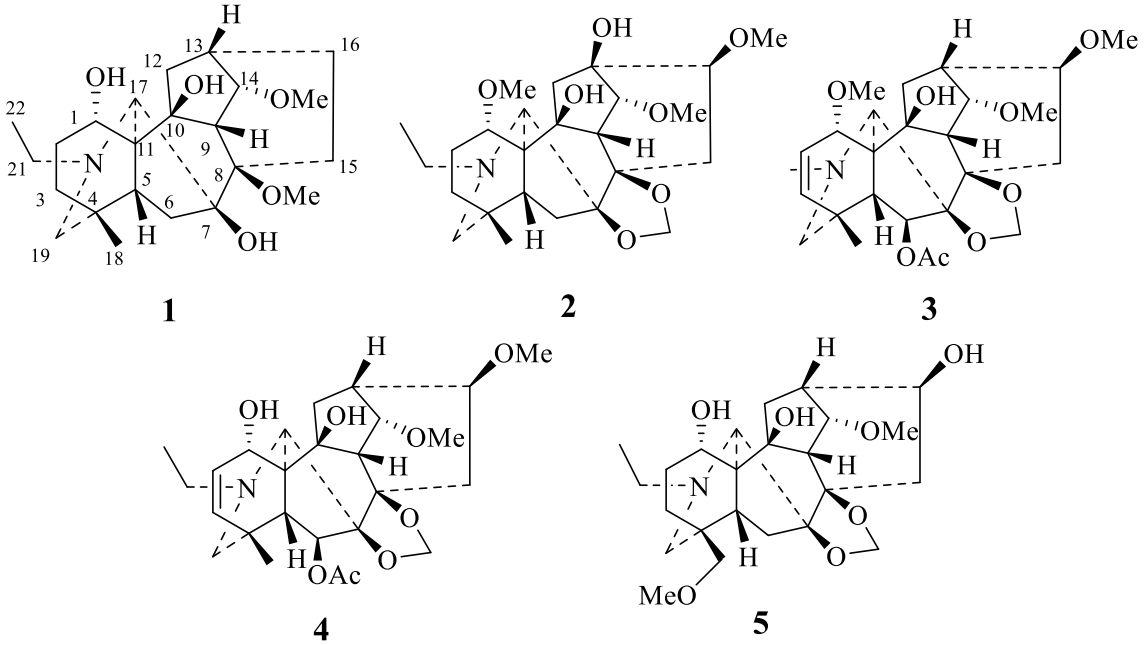

Figure 1. Structures of new compounds **1**–**5**

Compound **3** is a white amorphous powder. Its molecular formula $C_{26}H_{37}NO_8$ was established based on the HR-ESI-MS *m/z* 492.2608 $[M + H]^+$ (Calcd for $C_{26}H_{38}NO_8$ 492.2597). NMR spectra show the presence of an *N*-methyl group [δ_H 2.60, 3H, s; δ_C 43.5 (q)], one methyl [δ_H 1.03, 3H, s; δ_C 23.3 (q)], an acetyl group [δ_C 169.6 (s)], three methoxy groups [δ_H 3.39, 3.39, 3.48 (each 3H, s); δ_C 56.2 (q), 56.5 (q), 57.7 (q)] and a methylenedioxy group [δ_H 5.02, 4.98, (each 1H, s); δ_C 94.2 (t)]. ^{13}C NMR spectrum showed the presence of 19 other carbon resonances, indicating that compound **3** is a lycoctonine-type C_{19}-diterpenoid alkaloid. According to the analysis of nuclear magnetic resonance spectrum, compound **3** has very similarity with the nuclear magnetic resonance data of swininine A. The difference between compound **3** and swininine A is that the *N*-ethyl group was replaced by *N*-methyl group. The correlations between H-21 (δ_H 2.60) and C-17 (δ_C 65.3 d), C-19 (δ_C 58.6 t) in the HMBC spectrum can further supports this conclusion. The acetyl group was located at C-6 based on the HMBC correlation of H-6 (δ_H 5.48, s) with the acetyl carbon (δ_C 169.6). According to HMBC correlations between 1-OMe (δ_H 3.39, 3H, s) and C-1 (δ_C 76.3), 14-OMe (δ_H 3.48, 3H, s) and C-14 (δ_C 81.4), 16-OMe (δ_H 3.39, 3H, s) and C-16 (δ_C

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1**–**3**

(^{1}H: 400 MHz; ^{13}C: 100 MHz, $CDCl_3$)

Position	**1**		**2**		**3**	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	69.6 d	4.04 s	76.8 d	3.97dd (7.2,10.8)	76.3 d	3.85 d (3.6)
2	30.5 t	1.62 m	26.6 t	2.49 m	125.1d	6.03（4,9.6）
—	—	1.33 s	—	2.07 m	—	—
3	31.0 t	1.79 s	32.0 t	1.77 d (7.6)	136.8d	5.70 d (9.6)
—	—	1.57 s	—	1.46 m	—	—
4	32.9 s	—	38.7 s	—	34.8 s	—
5	42.0 d	1.81 s	36.3 d	2.52 d (2.8)	49.5 d	1.81 d (2.0)
6	35.0 t	2.35 d (2.8)	42.0 t	2.28 d (14.8)	78.9 d	5.48 s
—	—	1.53 s	—	2.03 d (14.8)	—	—
7	87.4 s	—	88.2 s	—	91.3 s	—
8	81.1 s	—	80.6 s	—	82.2 s	—
9	54.1 d	2.07 d (4.8)	53.5 d	2.64 d (5.2)	50.5 d	3.36 d (5.6)
10	83.3 s	—	83.7 s	—	84.0 s	—
11	55.3 s	—	56.7 s	—	56.6 s	—
12	42.8 t	2.33 s	40.1 t	2.75 d (7.2)	38.4 t	2.57 m
—	—	2.29 s	—	1.75 d (7.6)	—	1.87 m
13	31.5 d	1.29 s	84.9 s	—	38.4 d	2.78 d (15.2)
14	82.5 d	3.96 t (4.4)	81.2 d	4.23 t (4.8)	81.4 d	3.24 t (8.4)
—	—	1.97 m	—	2.49 d (6.4)	—	2.54 d (3.2)
15	17.6 t	1.89 dd (2.0, 9.6)	33.6 t	1.90 dd (5.6,10.0)	35.1 t	1.89 m
16	24.8 t	2.14 m	81.6 d	3.15 dd (6.0,9.6)	81.9 d	4.16 t (4.8)
—	—	1.37 s	—	—	—	—
17	63.4 d	2.88 s	64.6 d	3.05 s	65.3 d	2.90 s
18	27.5 q	0.96 s	20.3 q	0.83 s	23.3 q	1.03 s
19	59.9 t	2.78 d (10.8)	58.6 t	2.79 m	58.6 t	2.49 m
—	—	2.36 d (2.8)	—	2.52 d (2.8)	—	2.53 m
21	50.3 t	2.96 m	49.9 t	2.75 m	43.4 d	2.60 s
—	—	2.88 m	—	2.69 m	—	—
22	13.7 q	1.11 t (7.2)	13.8 q	1.06 t (7.2)	—	—
1-OMe	—	—	56.0 q	3.30 s	56.2 q	3.39 s
8-OMe	51.9 q	3.34 s	—	—	—	—
14-OMe	57.4 q	3.39 s	57.8 q	3.46 s	57.7 q	3.48 s
16-OMe	—	—	56.3 q	3.33 s	56.5 q	3.37 s
18-OMe	—	—	—	—	—	—
OCH_2O	—	—	93.5 t	5.01 s	94.2 t	5.02 s
—	—	—	—	4.91 s	—	4.98 s
6-OAc	—	—	—	—	169.6 s	—
	—	—	—	—	21.7 q	2.11 s

81.9), their methoxy group should be connected at C-1, C-14 and C-16 respectively. Methylenedioxy group is connected at C-7 and C-8, which can be concluded by HMBCs. The relative configuration of compound **3** was deduced by NOESY analysis. The NOESY correlations of H-5*β*/H-1, H-9*β*/H-14, and

H-17*α*/H-16 confirmed that the methoxy groups at C-1 and C-14 were *α*-oriented, and 16-OMe was *β*-oriented. Additionally, the singlet of H-6 (δ_H 5.48) implied that the dihedral angle between the H-6 and H-5*β* was approximately 90°, suggesting that H-6 was in the *α*-orientation.[12] The cross-peak between H-6 and H-19*α* further revealed that the 6-OAc was *β*-oriented. Therefore, the structure and absolute configuration of compound **3** are determined as shown in Figure 1 and named pachycenine C.

The molecular formula of compound **4** was determined as $C_{26}H_{37}NO_8$ by HR-ESI-MS. NMR spectra showed the presence of an *N*-ethyl group [δ_H 1.08, 3H, t, J = 7.2 Hz; 2.78, 2.91, (each 1H, m); δ_C 13.3 (q), 49.6 (t)], a methyl group [δ_H 1.09, 3H, s; δ_C 23.7 (q)], an acetyl group [δ_C 169.7 (s)], two methoxy groups [δ_H 3.37, 3.47 (each 3H, s); δ_C 56.3 (q), 57.7 (q)] and a methylenedioxy group [δ_H 5.02, 4.98, (each 1H, s); δ_C 94.5 (t)]. Based on the analysis of the NMR spectra, the NMR data of compound **4** showed great similarity to swinanine A, the difference was that the group at the C-1 of **4** is a hydroxyl group. It can be confirmed by H-2 (δ_H 5.88, 1H, m) and H-1 (δ_H 4.16, 1H, s) correlation in ^{1}H-^{1}H COSY spectrum. The planar structure of **4** (Figure 1) assignments were obtained from exhaustive analysis of the HMQC, HMBC and COSY data. The relative configuration of compound **3** was deduced from vicinal coupling constants (Table 1) and NOESY analysis (Figure 2). The characteristic proton signal at δ_H 4.17 (t, J = 4.8 Hz) could be assigned to H-14*β*[13,14] Similar to the spatial configuration of compound **3**, the singlet proton signal of H-6 at δ_H 5.48 demonstrated that H-6 and adjacent H-5 were in different orientations, indicating the *β*-orientation of 6-OAc. In the NOESY spectrum, the cross-peak between H-1 and H-5*β*, H-14 and H-9*β* proved that those groups at C-1 and C-14 were *α*-oriented. Furthermore, the key NOESY correlations of H-16 with H-17*α* and H-12*α* indicated the *β*-orientation of 16-OMe. Thus, the structure of compound **4** was confirmed as shown in Figure 1 and named pachycenine D.

Compound **5**, a white amorphous powder, $C_{24}H_{37}NO_7$ (HR-ESI-MS), was also a lycoctonine-type C_{19}-diterpenoid alkaloid. NMR spectra showed the presence of an *N*-ethyl group [δ_H 1.14, 3H, t, J = 7.2 Hz; 2.75, 2.88, (each 1H, m); δ_C 13.5 (q), 50.0 (t)], two methoxy groups [δ_H 3.36, 3.32 (each 3H, s); δ_C 56.4 (q), 59.4 (q)] and a methylenedioxy group oxy [δ_H 5.01, 5.12, (each 1H, s); δ_C 93.9 (t)]. Compared to compound **4**, compound **5** has no double bond between C-2 and C-3 and an acetyl group at the C-6 position. In addition, a methoxy group is attached to its C-18 position, while the C-16 position is a hydroxy group, it can be confirmed by the correlations between 18-OMe (δ_H 3.32, 3H, s) and C-18 (δ_C 78.8 t) in the HMBC spectrum, and the correlation of H-16 (δ_H 3.96, 1H, d, J = 4.8 Hz) and H-13 (δ_H 2.38, 1H, m) in the ^{1}H-^{1}H COSY spectrum. The relative configuration of compound **5** was deduced according to coupling constants and NOESY analysis. The NOESY correlations of H-1 and H-5*β*, H-9*β* and H-14, H-16 and H-17*α*, indicated the *α*-orientation of 1-OH and 14-OMe, and the *β*-orientation of 16-OH. Therefore, the structure of compound **5** were determined as shown in Figure 1 and named pachycenine E.

Table 2. ^{1}H and ^{13}C NMR spectral data for compounds **4**–**5**
(^{1}H: 400 MHz; ^{13}C: 100 MHz, $CDCl_3$)

Position	**4**		**5**	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
1	67.1 d	4.16 s	72.1 d	3.75 s
2	131.1 d	5.88 m	26.5 t	1.90 m
—	—	—	—	1.65 s
3	136.4 d	5.65 d (9.6)	29.2 t	1.82 d (8.0)
—	—	—	—	1.61 d (3.2)
4	34.6 s	—	37.4 s	—
5	50.2 d	1.86 s	38.6 d	1.80 d (8.4)
6	79.0 d	5.49 s	30.0 t	3.19 d (8.0)
—	—	—	—	1.44 d (2.8)
7	90.0 s	—	89.8 s	—
8	83.1 s	—	78.5 s	—
9	50.2 d	3.29 d (5.2)	50.2 d	1.98 d (6.8)
10	83.7 s	—	83.3 s	—
11	56.3 s	—	52.1 s	—
12	39.9 t	2.84 s	26.4 t	2.25 m
—	—	1.88 d (2.4)	—	1.26 s
13	38.1 d	2.62 d (2.8)	38.9 d	2.38 m
14	82.1 d	4.17 t (4.8)	81.7 d	3.41 s
15	35.4 t	2.50 s	36.6 t	2.69 d (8.8)
—	—	1.92 s	—	2.03 m
16	81.5 d	3.26 s	80.4 d	3.96 d (4.8)
17	64.0 d	3.03 s	63.4 d	2.93 s
18	23.7 q	1.09 s	78.8 t	3.13 d (8.8)
—	—	—	—	3.06 d (8.8)
19	56.5 t	2.50 s, 1.92 s	56.7 t	2.62 d (4.8)
—	—	—	—	2.47 d (10.8)
21	49.6 t	2.91 m	50.0 t	2.88 m
—	—	2.78 m	—	2.75 m
22	13.3 q	1.08 t (7.2)	13.5 q	1.14 t (7.2)
1-OMe	—	—	—	—
8-OMe	—	—	—	—
14-OMe	57.7 q	3.47 s	56.4 q	3.36 s
16-OMe	56.3 q	3.37 s	—	—
18-OMe	—	—	59.4 q	3.32 s
OCH_2O	94.5 t	5.02 s	93.9 t	5.01 s
—	—	4.98 s	—	5.12 s
6-OAc	169.7 s	—	—	—
	21.7 q	2.11 s	—	—

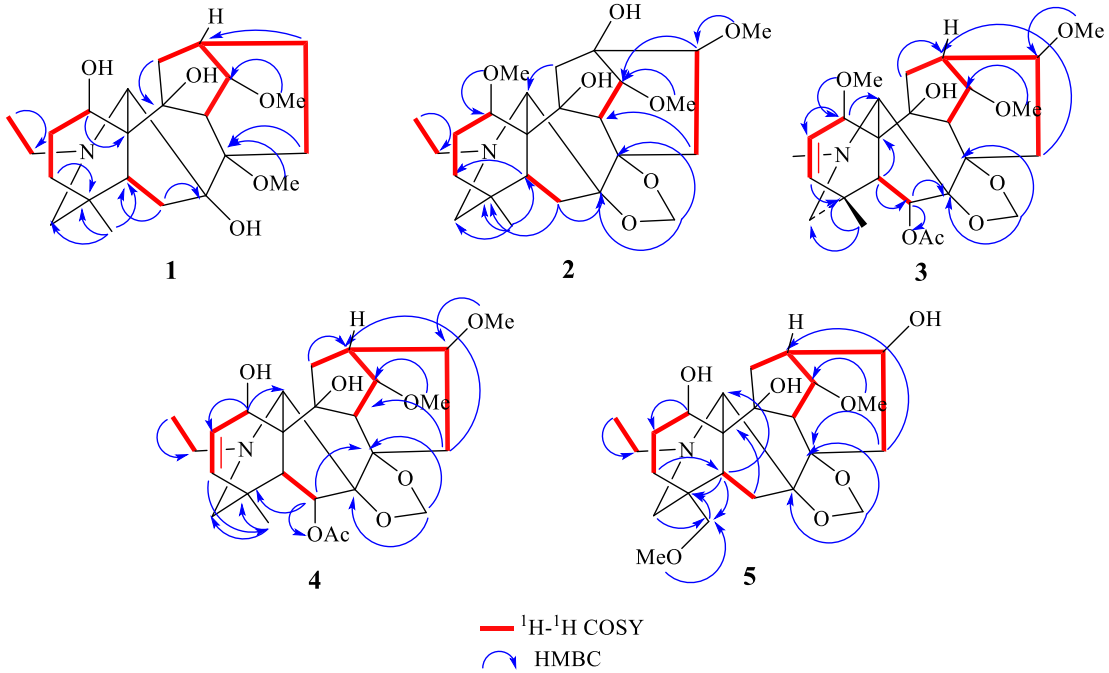

Figure 2. Key HMBC and ^{1}H-^{1}H COSY correlations for compounds **1**–**5**

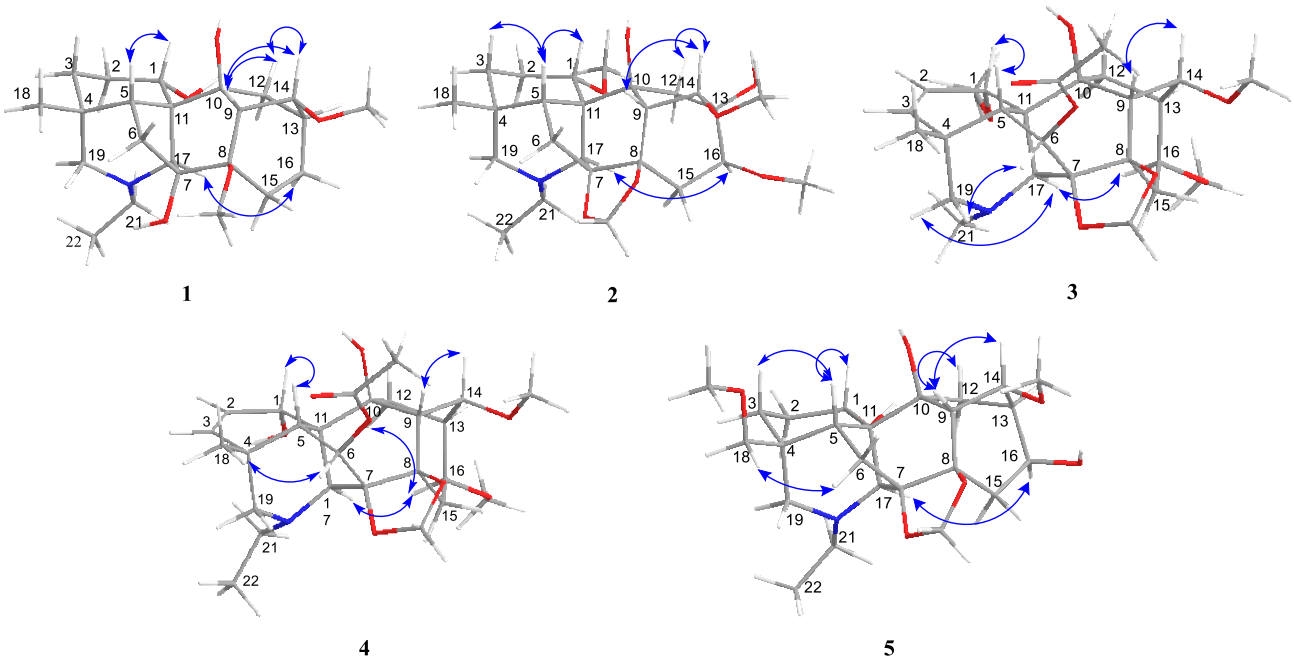

Figure 3. Key NOESY correlations of compounds **1**–**5**

Compounds **6**–**16** were evaluated for their inhibitory activities against LPS–induced NO production in RAW 264.7 cell line by Griess method,[15] celecoxib (15 μM) as positive control. However, none of them showed obviously inhibitory activity.

EXPERIMENTAL

General experimental procedures

Optical rotations were recorded on a PerkinElmer 341 polarimeter. 1D and 2D NMR spectra were measured by a Bruker AV 400 NMR spectrometer. HR-ESI-MS were carried out on a Q–TOF micro mass spectrometer (Waters, USA). Silica gel (Qingdao Haiyang Chemical Co., Ltd., China, 200–300 mesh) were used for column chromatography (CC). The TLC plates were precoated with silica gel GF_{254} (Qingdao Haiyang Chemical Co., Ltd., China).

Plant material

D. pachycentrum Hemsl. were collected in Hongyuan County, Aba Prefecture, Sichuan Province, China, in August 2021 and were identifed by Professor Guo-Dong Li of the Yunnan University of Chinese Medicine. A voucher specimen (No. 3615HS20210804) was deposited in the School of Life Science and Engineering, Southwest Jiaotong University.

Extraction and isolation

The air-dried powder (10.0 kg) of *Delphinium pachycentrum* Hemsl. was extracted three times with 95% EtOH reflux. After removing the solvent, dissolve the EtOH extract (1.1 kg) with warm water, then treated with 0.5 N hydrochloric acid (2 L) and successively extracted with petroleum ether (4 × 2 L). Then, ammonia water is added to the aqueous solution to make its pH = 10. The solutions were extracted with CH_2Cl_2 (4 × 2 L). The CH_2Cl_2 extracts were concentrated to produce the crude alkaloid extract (75.3 g). Column chromatography (CC) of the crude alkaloid extract over silica gel, using a PE/EA (2:1) mixture with increasing polarity afforded fractions A–E based on TLC analysis. Fraction A (14.6 g) was separated by silica gel CC (PE/EA/Et_2N, 15:1:0.1–1:1:0.1) to obtain six fractions A_1–A_6, compounds **6** (80 mg) and **7** (33 mg) were obtained by purifying fractions A_2 (892 mg) by silica gel CC (PE/EA/Et_2N, 60:1:0.1–1:1:0.1). Compounds **8** (411 mg), **9** (38 mg) and **10** (44 mg) were obtained by purifying fractions A_4 (10.042 g) by silica gel CC (DCM/MeOH, 120:1–10:1). Compounds **2** (39 mg) and **4** (15 mg) were obtained by purifying fractions A_5 (406 mg) by silica gel CC (PE/Acetone/Et_2N, 15:1:0.1–1:1:0.1). Fraction B (4.6 g) was loaded onto a silica gel column and eluted with DCM/MeOH (80:1–10:1) to furnish fraction B_1–B_5, Fraction B_2 (2.8 g) was separated by silica gel CC with PE/EA/Et_2N (60:1:0.5–1:1:0.5) to afford compounds **11** (433 mg) and **12** (91 mg). Fraction C (14.6 g) was separated by silica gel CC (PE/EA/Et_2N, 80:4:0.3–0:4:0.3) to obtain five fractions C_1–C_5, compounds **1** (12 mg), **13** (11 mg) and **14** (9 mg) were obtained by purifying fractions C_3 (572 mg) by silica gel CC (PE/Et_2N, 60:1–10:1), compounds **3** (13 mg), **5** (18 mg), **15** (15 mg) and **16** (22 mg) were obtained by purifying fractions C_5 (325 mg) by silica gel CC (PE/Acetone/Et_2N, 40:1:0.2–5:1:0.2).

(PE: petroleum ether, EA: ethyl acetate, Et_2N: diethylamine, DCM: dichloromethane, MeOH: methanol)

Pachycenine A (**1**)

White amorphous powder; $[\alpha]_D^{20}$ = +27.0 (*c* 0.14, $CHCl_3$); HR-ESI-MS *m/z* 408.2737 $[M + H]^+$ (calcd. for $C_{23}H_{38}NO_5$, 408.2750); ^1H and ^{13}C NMR data see Table 1.

Pachycenine B (**2**)

White amorphous powder; $[\alpha]_D^{20}$ = +18.2 (*c* 0.12, $CHCl_3$); HR-ESI-MS *m/z* 466.2814 $[M + H]^+$ (calcd. for $C_{25}H_{40}NO_7$, 466.2805); ^1H and ^{13}C NMR data see Table 1.

Pachycenine C (**3**)

White amorphous powder; $[\alpha]_D^{20}$ = +11.1 (*c* 0.38, $CHCl_3$); HR-ESI-MS *m/z* 492.2608 $[M + H]^+$ (calcd. for $C_{26}H_{38}NO_8$, 492.2597); ^1H and ^{13}C NMR data see Table 1.

Pachycenine D (**4)**

White amorphous powder; $[\alpha]_D^{20}$ = -2.0 (*c* 0.10, $CHCl_3$); HR-ESI-MS *m/z* 492.2608 $[M + H]^+$ (calcd. for $C_{26}H_{38}NO_8$, 492.2597); ^1H and ^{13}C NMR data see Table 2.

Pachycenine E (**5**)

White amorphous powder; $[\alpha]_D^{20}$ = -10.6 (*c* 0.50, $CHCl_3$); HR-ESI-MS *m/z* 452.2653 $[M + H]^+$ (calcd. for $C_{24}H_{38}NO_7$, 452.2648); ^1H and ^{13}C NMR data see Table 2.

Inhibitory activity of NO production

RAW 264.7 cells are derived from procell. RAW 264.7 cells were cultivated in the growth medium containing DMEM high sugar medium at 37 ℃ in humidified 5% CO_2/95% air. The cytotoxicity of the compounds to RAW 264.7 was evaluated by MTT test.[15] The ability of RAW 264.7 macrophages to inhibit LPS-induced NO production was measured with Griess method. RAW 264.7 cells (2 per well × 10^4 cells) were incubated in 96 well plates for 12 h, pretreated with medicated medium for 2 h, then added with LPS, and incubated in the incubator for 24 h. Absorb 50 μL of the supernatant, and then add it into Griess reagent kit in turn for mixing. The absorbance was measured at 562 nm by enzyme immunoassay. With celecoxib as the positive control, calculate the inhibitory effect of the compound on NO production.

SUPPLEMENTARY MATERIAL

NMR spectra of compounds **1**–**5** relevant to this paper are available online.

DECLARATION OF COMPETING INTEREST

The authors declare no competing financial interest.

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