ORIGINAL PAPER

# Pyrrole 2-carbaldehyde derived alkaloids from the roots of *Angelica dahurica*

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## Abstract
Six new pyrrole 2-carbaldehyde derived alkaloids, dahurines A–F (**1**–**6**), along with five known ones (**7**–**11**) and butyl 2-pyrrolidone-5-carboxylate (**12**) were isolated from the roots of *Angelica dahurica*. Their structures were determined by extensive spectroscopic and spectrometric data (1D and 2D NMR, IR, and HRESIMS) and calculated electronic circular dichroism (ECD) methods. Although compounds **7** and **8** have been chemically synthesized, they were obtained from natural materials for the first time. Compounds **2**, **3**, **4**, **10**, and **11** exhibited acetylcholinesterase inhibitory activity with $IC_{50}$ values in the range of 47.5–52.5 μM.

## Graphic abstract
Pyrrole 2-carbaldehyde derived alkaloids from the roots of *Angelica dahurica*
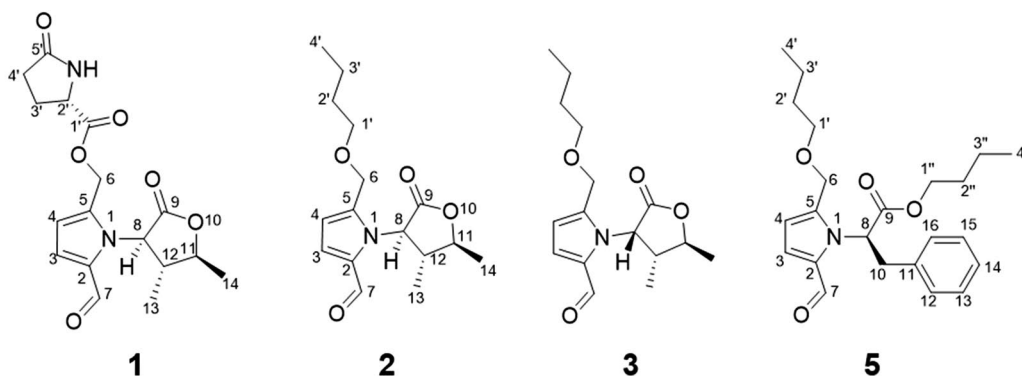
Bowen Qi and Wanqing Yang contributed equally to this work.

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# Introduction

*Angelica dahurica* is a perennial medicinal plant belonging to the family Apiaceae. The roots of *A. dahurica* are clinically used as an analgesic agent for the treatment of

Fig. 1 Structures of compounds **1**–**8** from the roots of *A. dahurica*
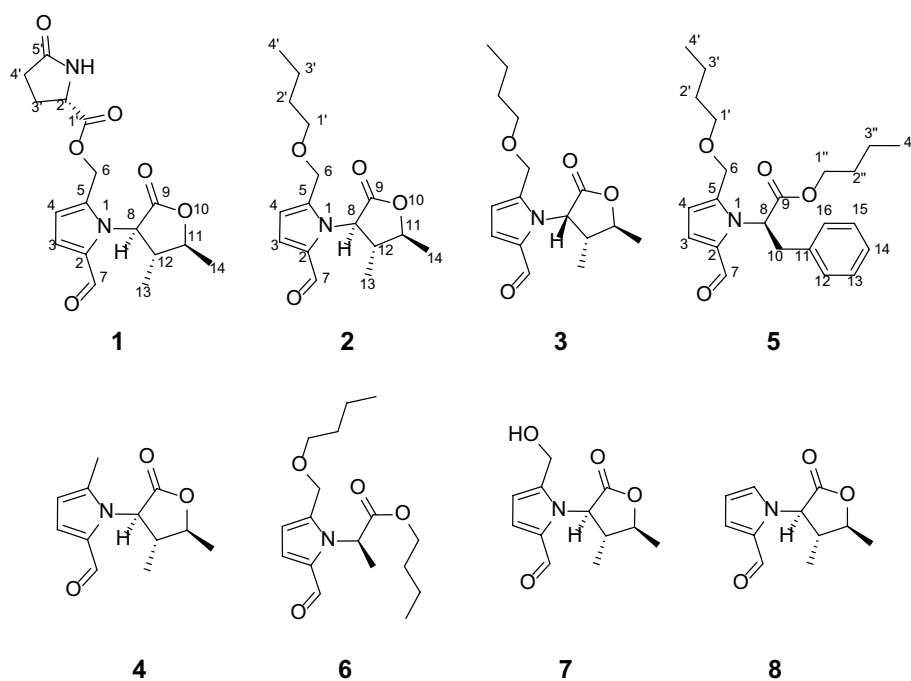


migraine headache and toothache in Chinese medicine, and the promising therapeutic effects have attracted great interests from scientific researchers, leading to publication of a mass of documents concerning the pharmacological activities and chemical constituents of *A. dahurica* [1]. It is now generally believed that the major chemical constituents of *A. dahurica* roots are coumarins, and many of these coumarins have been demonstrated as possessing significant anti-inflammatory, anti-tumor, and acetylcholinesterase (AChE) inhibitory activities [2–8]. Our previous investigation on the coumarins in the roots of *A. dahurica* by LC–MS revealed that dimeric furanocoumarins might occur in the roots of *A. dahurica* in trace amounts but very complex structures, and further LC–MS-guided separation and purification of the targeted fractions led to the determination of 10 potentially anti-inflammatory dimeric furanocoumarins [9, 10]. Notably, Lu and coworkers reported the elucidation of one pyrrole 2-carbaldehyde alkaloid named as desmodimine from the roots of *A. dahurica* [11]. Given that alkaloids are sparsely reported from *A. dahurica*, and natural pyrrole 2-carbaldehyde alkaloids exhibit interesting bioactivities such as hepatoprotective, immunostimulatory, and AChE inhibitory activities [12], further investigation on the chemical constituents of the remnant fractions of *A. dahurica* were performed, which resulted in the identification of six new pyrrole 2-carbaldehyde alkaloids, dahurines A–F (**1**–**6**), along with five known ones (**7**–**11**) and a known pyrrolidone derivative (**12**). Herein, the isolation and structural elucidation of the isolated compounds and an evaluation of their inhibitory effects on AChE are described.

## Results and discussion

The *n*-BuOH-soluble fraction of the EtOH extract of the roots of *A. dahurica* was subjected to repeated silica gel, Sephadex LH-20, and RP-C$_{18}$ gel column chromatography, followed by semipreparative RP-HPLC, to afford six new pyrrole 2-carbaldehyde derived alkaloids (**1**–**6**; Fig. 1), along with five known ones (**7**–**11**) and butyl 2-pyrrolidone-5-carboxylate (**12**).

Compound **1** was obtained as a pale yellow powder: $[\alpha]_D^{21}$ +6.0 (*c* 0.1, MeOH). Its molecular formula was assigned as $C_{17}H_{20}N_2O_6$ by the presence of a [M + H]$^+$ ion peak at *m/z* 349.1480 in the HRESIMS spectrum, which is supported by the $^{13}$C NMR data (Table 1). The IR spectrum exhibited the absorption bands of ester carbonyl (1781 cm$^{-1}$) and aldehyde (1663 cm$^{-1}$) groups. The maximum UV absorption was observed at 292 nm, which is characteristic for pyrrole-2-aldehyde [13]. The $^1$H and $^{13}$C NMR spectra of **1** showed the presence of a 5-hydroxymethyl-pyrrole 2-carbaldehyde moiety [$\delta_H$ 9.48 (1H, s, H-7), 7.05 (1H, d, *J* = 4.0 Hz, H-3), 6.47 (1H, d, *J* = 4.0 Hz, H-4), 5.36 (1H, d, *J* = 13.5 Hz, H-6a), 5.14 (1H, d, *J* = 13.5 Hz, H-6b); $\delta_C$ 179.3, 57.6, 137.1, 113.9, 126.0, 132.7], and a 5-oxopyrrolidine-2-carboxylic acid moiety [$\delta_H$ 4.22 (1H, m, H-2′), 2.43 (1H, m, H-3′a), 2.19 (1H, m, H-3′b), 2.34 (2H, m, H$_2$-4′); $\delta_C$ 177.8, 171.5, 55.4, 29.1, 24.9]. The remaining NMR signals [$\delta_H$ 4.71 (1H, d, *J* = 11.5 Hz, H-8), $\delta_H$ 4.30 (1H, m, H-11), 2.74 (1H, m, H-12), 1.63 (3H, d, *J* = 6.0 Hz, H$_3$-14), 1.16 (3H, d, *J* = 6.5 Hz, H$_3$-13); $\delta_C$ 171.2, 80.7, 63.3, 43.6, 18.6, 15.1] suggested the presence of a 4,5-dimethyldihydrofuran-2-one

**Table 1** $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR data of compounds **1–4** ($\delta$ in ppm, in CDCl$_3$)

| Position | 1[a] | | 2[a] | | 3[a] | | 4[a] | |
|---|---|---|---|---|---|---|---|---|
| | $\delta_H$, multi. ($J$ in Hz) | $\delta_C$, type | $\delta_H$, multi. ($J$ in Hz) | $\delta_C$, type | $\delta_H$, multi. ($J$ in Hz) | $\delta_C$, type | $\delta_H$, multi. ($J$ in Hz) | $\delta_C$, type |
| 2 | | 132.7, C | | 132.3, C | | 133.6, C | | 131.2, C |
| 3 | 7.05, d (4.0) | 126.0, CH | 7.01, d (4.0) | 125.9, CH | 6.97, d (4.0) | 124.8, CH | 7.00, d (4.0) | 126.9, CH |
| 4 | 6.47, d (4.0) | 113.9, CH | 6.28, d (4.0) | 112.2, CH | 6.30, d (4.0) | 112.1, CH | 6.12, d (4.0) | 110.8, CH |
| 5 | | 137.1, C | | 140.2, C | | 140.8, C | | 141.0, C |
| 6a | 5.36, d (13.5) | 57.6, CH$_2$ | 4.55, d (13.0) | 64.1, CH$_2$ | 4.56, d (13.0) | 64.5, CH$_2$ | 2.33, s | 13.0, CH$_3$ |
| 6b | 5.14, d (13.5) | | 4.50, d (13.0) | | 4.45, d (13.0) | | | |
| 7 | 9.48, s | 179.3, CH | 9.43, s | 178.8, CH | 9.42, s | 179.7, CH | 9.33, s | 177.8, CH |
| 8 | 4.71, d (11.5) | 63.3, CH | 4.98, d (11.5) | 62.6, CH | 5.34, d (12.5) | 58.2, CH | 4.54, d (11.5) | 62.7, CH |
| 9 | | 171.2, C | | 171.4, C | | 172.3, C | | 171.4, C |
| 11 | 4.30, m | 80.7, CH | 4.24, m | 80.4, CH | 4.70, m | 83.4, CH | 4.26, m | 80.5, CH |
| 12 | 2.74, m | 43.6, CH | 2.75, m | 43.5, CH | 2.52, m | 40.5, CH | 2.74, m | 43.7, CH |
| 13 | 1.16, d (6.5) | 15.1, CH$_3$ | 1.13, d (6.5) | 14.8, CH$_3$ | 0.72, d (7.5) | 12.4, CH$_3$ | 1.13, d (6.5) | 15.1, CH$_3$ |
| 14 | 1.63, d (6.0) | 18.6, CH$_3$ | 1.60, d (6.0) | 18.5, CH$_3$ | 1.49, d (6.5) | 21.1, CH$_3$ | 1.61, d (6.0) | 18.7, CH$_3$ |
| 1′ | | 171.5, C | 3.42, m | 70.1, CH$_2$ | 3.45, t (6.5) | 70.5, CH$_2$ | | |
| 2′ | 4.22, m | 55.4, CH | 1.54, m | 31.5, CH$_2$ | 1.55, m | 31.8, CH$_2$ | | |
| 3′a | 2.43, m | 24.9, CH$_2$ | 1.33, m | 19.3, CH$_2$ | 1.36, m | 19.4, CH$_2$ | | |
| 3′b | 2.19, m | | | | | | | |
| 4′ | 2.34, m | 29.1, CH$_2$ | 0.89, t (7.5) | 14.8, CH$_3$ | 0.91, t (7.5) | 14.0, CH$_3$ | | |
| 5′ | | 177.8, C | | | | | | |

[a]Assignments were carried out based on HSQC and HMBC experiments
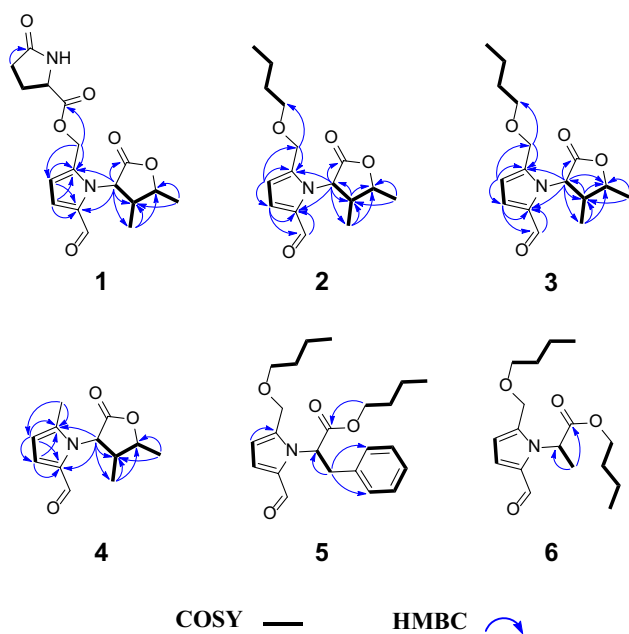


**Fig. 2** Selected COSY and HMBC correlations of compounds **1–6**

moiety in **1**, which was further confirmed by the $^1$H–$^1$H COSY correlations (Fig. 2) of H-8/H-12, H-12/H-11, H-12/H$_3$-13, and H-11/H$_3$-14, and the HMBC correlations (Fig. 2) of H-8/C-9, C-12, C-13; H$_3$-13/C-11, C-12; and H$_3$-14/C-11,

C-12. Additionally, the HMBC correlations of H$_2$-6/C-1′ and H-8/C-2, C-5 demonstrated that the moieties of 5-oxopyrrolidine-2-carboxylic acid and 4,5-dimethyldihydrofuran-2-one were located at C-6 and *N*-1, respectively. The relative configuration of **1** was determined by the NOE correlations of H$_3$-13/H-8, H-11 (Fig. 3). The absolute configuration of **1** was determined by calculated electronic circular dichroism (ECD) method. Given that the relative configuration of C-2′ could not be solved by NOESY experiment, the ECD spectra of (8*R*, 11*S*, 12*R*, 2′*S*)-**1**, (8*R*, 11*S*, 12*R*, 2′*R*)-**1**, (8*S*, 11*R*, 12*S*, 2′*R*)-**1**, and (8*S*, 11*R*, 12*S*, 2′*S*)-**1** were calculated using time-dependent density functional theory (TDDFT) at the B3LYP/6-31G level with the CPCM model. As shown in Fig. 4, the calculated spectrum of (8*R*, 11*S*, 12*R*, 2′*S*)-**1** is in good agreement with the experimental spectrum. Accordingly, the absolute configuration of **1** was defined as (8*R*, 11*S*, 12*R*, 2′*S*)-**1**, named as dahurine A.

Compound **2** was obtained as a pale yellow powder: $[\alpha]_D^{21}$ +48.0 (*c* 0.1, MeOH). Its molecular formula was identified as C$_{16}$H$_{23}$NO$_4$ by the presence of a [M+H]$^+$ ion peak at *m/z* 294.1721 in the HRESIMS spectrum, which was supported by the $^{13}$C NMR data (Table 1). Comparison of the NMR data of **2** with those of **1** revealed that the 5-oxopyrrolidine-2-carboxylic acid group in **1** was replaced by an *n*-butoxy group, which was deduced from the protons resonating at $\delta_H$ 3.42 (2H, m, H$_2$-1′), 1.54 (2H, m, H$_2$-2′), 1.33 (2H, m, H$_2$-3′), and 0.89 (3H, t, *J* = 7.5 Hz, H$_3$-4′), and the carbons
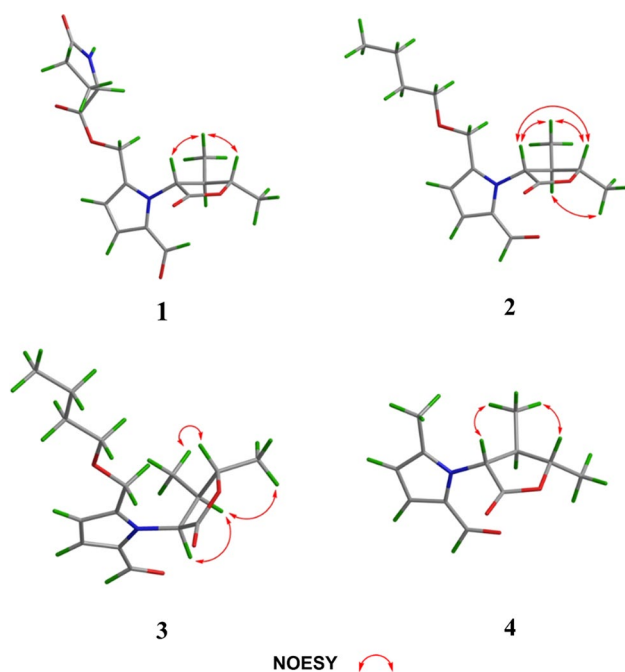
**Fig. 3** Selected NOESY correlations of compounds **1**–**4**

resonating at $\delta_C$ 70.1 (C-1′), 31.5 (C-2′), 19.3 (C-3′), and 14.8 (C-4′). The group of *n*-butoxy was determined to be linked at C-6 by the HMBC correlations between H$_2$-6 and C-1′. The planar structure of **2** was further confirmed by the HMBC correlations (Fig. 2) of H-3/C-2, C-7, C-3; H-4/C-5, C-6; H$_2$-6/C-5, C-1′; H-7/C-2; H-8/C-2, C-5, C-9, C-12, C-13; H-12/C-8, C-11; H$_3$-13/C-11, C-12; and H$_3$-14/C-11, C-12. The relative configuration of **2** was determined by the NOE correlations (Fig. 3) of H-8/H-11, H$_3$-13; H-11/H$_3$-13; and H-12/H$_3$-14. Given that the experimental CD spectrum of **2** and the calculated ECD spectra of (8*R*, 11*S*, 12*R*)-**2** are superimposed (Fig. 5), the structure of **2** was determined as shown in Fig. 1, named as dahurine B.

Compound **3** was obtained as a pale yellow powder: $[\alpha]_D^{21}$ − 74.0 (*c* 0.1, MeOH), with a molecular formula of $C_{16}H_{23}NO_4$ determined by HRESIMS and NMR spectroscopic data (Table 1). Comparison of the NMR data of **3** with those of **2** revealed that compound **3** shared a similar skeleton with that of **2**. Unambiguous assignment of the protons and carbons of **3** by 2D NMR experiments allowed establishing the planar structure of **3**, which was completely the same to that of **2**. However, the quite different retention time on the HPLC column (Fig. S47) suggested that **3** is a
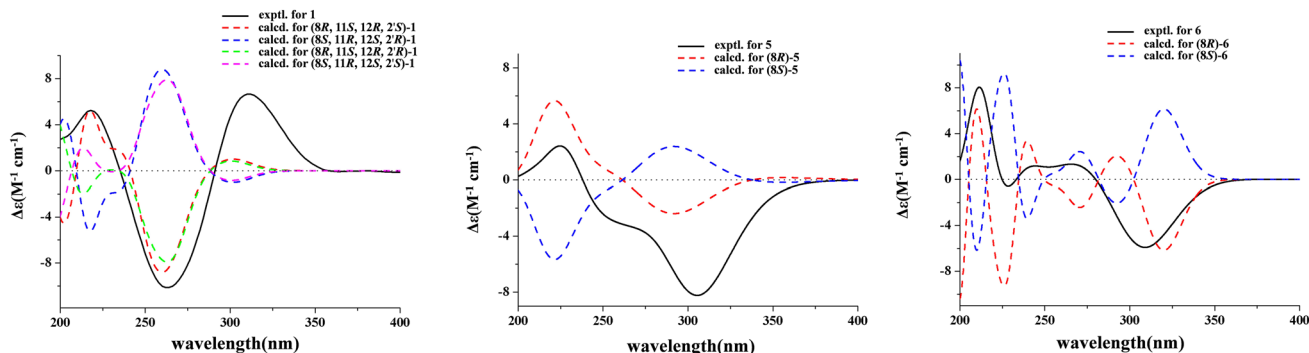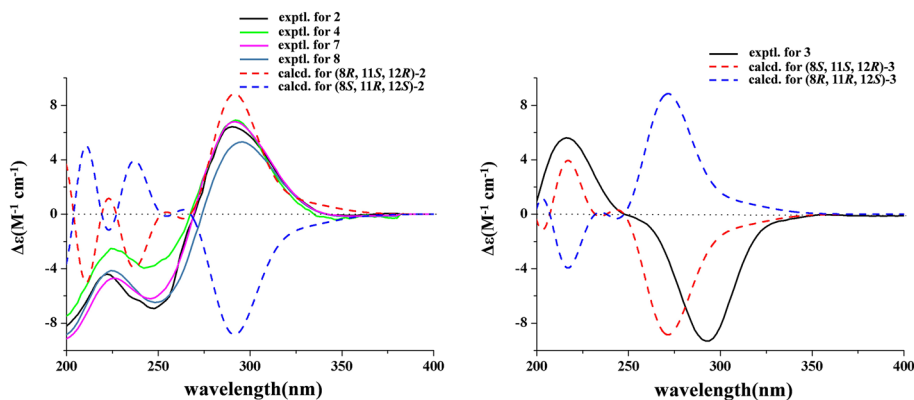


**Fig. 4** Experimental and calculated ECD spectra of compounds **1**, **5**, and **6** (in MeOH)

**Fig. 5** Experimental and calculated ECD spectra of compounds **2**, **3**, **4**, **7**, and **8** (in MeOH). The calculated ECD spectra of compounds **4**, **7** and **8** were not presented due to the high similarity of these spectra to that of **2**

stereoisomer of **2**. In the NOESY spectrum of **3**, the NOE correlations (Fig. 3) of H-8/H-12, H-12/H$_3$-14, and H-11/H$_3$-13 suggested that the orientation of H-8 in **3** is opposite to that in **2**. The absolute configuration of **3** was assigned as (8$S$,11$S$,12$R$)-**3** by comparison of its experimental and calculated ECD spectra (Fig. 5). Therefore, the structure of **3** was elucidated as shown in Fig. 1, and named as dahurine C.

Compound **4** was isolated as a pale yellow, amorphous powder: $[\alpha]_D^{21}$ + 18.0 ($c$ 0.1, MeOH). Its molecular formula was assigned as $C_{12}H_{15}NO_3$ by the presence of an [M + Na]$^+$ ion peak at $m/z$ 244.0919 in the HRESIMS spectrum. Comparison of the $^1$H and $^{13}$C NMR data of **4** with those of **2** revealed the absence of an $n$-butoxy group and the significantly up-fielded chemical shift of C-6 ($\delta_C$ 13.0, $\Delta\delta_C$ − 43.5) in **4**, suggesting that the –CH$_2$OCH$_2$CH$_2$CH$_2$CH$_3$ moiety in **2** was replaced by a methyl group [$\delta_H$ 2.33 (3H, s, H$_3$-6), $\delta_C$ 13.0]. The deduction was further confirmed by the HMBC correlations between H$_3$-6 and C-4/C-5. The relative configuration of **4** was determined by the NOE correlations of H-8/H$_3$-13 and H-11/H$_3$-13 (Fig. 3). Given that the experimental spectrum of **4** and the calculated ECD spectrum of (8$R$, 11$S$, 12$R$)-**4** are superimposed (Fig. 5), the structure of **4** was established as shown in Fig. 1, and named as dahurine D.

Compound **5** was obtained as a pale yellow powder: $[\alpha]_D^{21}$ + 2.0 ($c$ 0.1, MeOH). Its molecular formula was assigned as $C_{23}H_{31}NO_4$ by the presence of a [M + H]$^+$ ion peak at $m/z$ 386.2323 in the HRESIMS spectrum. The IR spectrum exhibited the absorption bands of ester carbonyl (1780 cm$^{-1}$), aldehyde (1659 cm$^{-1}$), and phenyl (1541 and 1487 cm$^{-1}$) groups. The $^1$H NMR spectrum of **5** indicated the characteristic signals due to a 5-hydroxymethyl-pyrrole-2-carbaldehyde moiety [$\delta_H$ 9.50 (1H, s, H-7), 6.99 (1H, d, $J$ = 4.0 Hz, H-3), and $\delta_H$ 6.05 (1H, d, $J$ = 4.0 Hz, H-4), and 3.88 (1H, br s, H-6a), $\delta_H$ 3.53 (1H, br s, H-6b)], and signals contributing to a phenylalanine moiety [$\delta_H$ 7.16 (3H, overlapped, H-13, H-14, H-15), 6.84 (2H, overlapped, H-12, H-16), 5.26 (1H, br s, H-8), 3.74 (1H, dd, $J$ = 14.0, 2.5 Hz, H-10a), and 3.32 (1H, dd, $J$ = 14.0, 2.5 Hz, H-10b)]. In addition, signals contributing to two $n$-butoxy groups were also observed in the $^1$H NMR spectrum of **5**. Comparison of the NMR data of **5** with those of methyl (2$R$)-[2-formyl-5-(methoxymethyl)-1$H$-pyrrol-1-yl]-3-(phenyl) propanoate, a pyrrole 2-carbaldehyde derived alkaloid previously isolated from *Lycium chinense* [14], revealed that the two methyl groups in the known compound were replaced by two $n$-butoxy groups. Further analysis of the 1D and 2D NMR spectra of **5** led to the establishment of the planar structure of **5**. The CD spectrum of **5** indicated negative Cotton effects at 308 nm ($\Delta\varepsilon_{max}$ − 8.3), which is similar to that of the known analogue aforementioned [14], resulting in the assignment of an 8$R$ configuration in **5**. In addition, the experimental CD spectrum of **5** is highly similar to the calculated ECD spectrum of (8$R$)-**5** (Fig. 5). Thus, the

structure of **5** was elucidated as shown in Fig. 1, and named as dahurine E.

Compound **6** was isolated as a pale yellow powder: $[\alpha]_D^{21}$ + 2.0 ($c$ 0.1, MeOH). Its molecular formula was assigned as $C_{17}H_{27}NO_4$ by the presence of a [M + Na]$^+$ ion peak at $m/z$ 332.1852 in the HRESIMS spectrum. The NMR data of **6** were similar to those of **5**, except for the replacement of the benzyl group in **5** by a methyl group. The aforementioned structure of **6** was further confirmed by the key HMBC correlations of H$_3$-10/C-8, C-9, and the $^1$H–$^1$H COSY correlations of H-3/H-4, H$_3$-10/H-8, H$_2$-1′/H$_2$-2′/H$_2$-3′/H$_3$-4′, and H$_2$-1″/H$_2$-2″/H$_2$-3″/H$_3$-4″. Akin to the CD spectrum of **5**, negative Cotton effects at 309 nm were also observed in the CD spectrum of **6**, and the experimental CD spectrum of **6** was in good agreement with the calculated spectrum of (8$R$)-**6**. Accordingly, the structure of **6** was determined as shown in Fig. 1, and named as dahurine F.

By comparison of their spectroscopic data (Table S1) with those reported, and using calculated ECD method (Fig. 5), compounds **7** and **8** were identified as (8$R$,11$S$,12$R$)-funebral and (8$R$,11$S$,12$R$)-3,4-dihydro-3-amino-4,5-dimethylfuran-2[5$H$]-one-2-formyl pyrrole, which have been chemically synthesized by Dong and coworkers [15]. Notably, this is the first time that compounds **7** and **8** were isolated from natural materials. In addition, by comparison of their NMR and specific rotation data with those reported in literature, compounds **9**–**12** were identified as 4″-butyl-2-formyl-5-(hydroxymethyl)-1$H$-pyrrole-1-butanoic acid (**9**) [16], butyl 2-formyl-5-butoxymethyl-1$H$-pyrrole-1-butanoate (**10**) [17], hemerocallisamine II (**11**) [18], and butyl 2-pyrrolidone-5-carboxylate (**12**) [19], respectively.

Compounds **1**–**12** were evaluated for their inhibitory effects on AChE using Ellman's method, using huperzine A as a positive control (IC$_{50}$ = 0.6 ± 0.1 μM). Compounds **2**, **3**, **4**, **10**, and **11** exhibited inhibition of AChE with IC$_{50}$ values of 52.0 ± 0.5, 48.2 ± 0.1, 47.5 ± 0.2, 50.4 ± 0.6, and 52.5 ± 3.4 μM, respectively. Compounds **1**, **5**–**9**, and **12** were inactive (< 50% inhibition at 100 μM).

## Experimental

### General procedures

Optical rotations were obtained on a Rudolph Autopol IV automatic polarimeter (NJ, USA). IR spectra were recorded on a Thermo Nicolet Nexus 470 FT-IR spectrophotometer (MA, USA) with KBr pellets. UV spectra were obtained using a Shimadzu UV-2450 spectrophotometer (Tokyo, Japan). NMR spectra were recorded on a Varian INOVA-500 spectrometer (CA, USA) operating at 500 MHz for $^1$H NMR and 125 MHz for $^{13}$C NMR. HRESIMS was recorded on

an LCMS-IT-TOF system, fitted with a Prominence UFLC system and an ESI interface (Shimadzu, Kyoto, Japan). Silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), LiChroprep RP-C$_{18}$ gel (40–63 μm, Merck, Germany), D101 macroporous adsorption resin (Qingdao Marine Chemical Inc., Qingdao, China), and Sephadex LH-20 (Pharmacia) were used for open-column chromatography (CC). HPLC was performed on a Shimadzu LC-20AT pump system (Shimadzu Corporation, Tokyo, Japan), equipped with a SPD-M20A photodiode array detector monitoring at 254 nm. A semi-preparative HPLC column (YMC-Pack C$_{18}$, 250 × 10 mm, 5 μm) was utilized for compound separation and purification. TLC was performed using GF$_{254}$ plates (Qingdao Marine Chemical Inc., Qingdao, China).

## Plant material

The roots of *A. dahurica* (Fisch. ex Hoffm.) Benth. et Hook. were collected in Bozhou, Anhui province, People's Republic of China, in December 2013, and plant authentication was performed by one of the authors (P.F. Tu). A voucher specimen (SPSHI-ADB-201312) is deposited in the Modern Research Center for Traditional Chinese Medicine, Beijing University of Chinese Medicine.

## Extraction and isolation

The air-dried roots of *A. dahurica* (29.6 kg) were refluxed with 80% EtOH (3 × 180 L, 2 h each). After removal of the solvent under reduced pressure, the residue (1.5 kg) was suspended in H$_2$O (4 L) and partitioned with petroleum ether (4 × 4 L), EtOAc (4 × 4 L), and *n*-BuOH (4 × 4 L), successively. The *n*-BuOH extract (805 g) was subjected to D101 macroporous adsorption resin chromatography and eluted with H$_2$O–EtOH (100:0, 80:20, 20:80, v/v) to yield three fractions (fr. A–C). Fr. C (202 g) was subjected to silica gel chromatography and eluted with a gradient of CH$_2$Cl$_2$–MeOH from 15:1 to 1:1 to give 20 subfractions (subfr. C1–C20). Subfr. C1 (10.1 g) was subjected to an RP-C$_{18}$ open column and eluted with a stepwise gradient elution using MeOH–H$_2$O to yield 12 fractions (fr. C1A–C1L). Fr. C1E (80 mg) was purified by semipreparative RP-HPLC using 20% aqueous MeCN as the mobile phase to afford **4** (7.5 mg, $t_R$ = 13.0 min), **7** (25.0 mg, $t_R$ = 18.0 min), **8** (10.0 mg, $t_R$ = 19.0 min), and **1** (1.7 mg, $t_R$ = 21.5 min). Fr. C1F (90 mg) was purified by semipreparative RP-HPLC using 50% aqueous MeOH as the mobile phase to afford **11** (12.0 mg, $t_R$ = 23.0 min), **12** (20.0 mg, $t_R$ = 25.0 min), and **10** (8.5 mg, $t_R$ = 33.5 min). Fr. C1G (70 mg) was purified by semipreparative RP-HPLC using 70% aqueous MeOH as the mobile phase to afford **2** (2.2 mg, $t_R$ = 19.0 min), **3** (5.5 mg, $t_R$ = 20.5 min), **9** (5.2 mg, $t_R$ = 25.0 min), **6** (2.2 mg, $t_R$ = 28.0 min), and **5** (1.7 mg, $t_R$ = 37.0 min).

Dahurine A (**1**): pale yellow powder; $[\alpha]_D^{21}$ +6.0 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 292 (3.79); IR (KBr) $\nu_{max}$: 2973, 2932, 2873, 1781, 1663, 1452, 1335, 1190, 1052, 781 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Table 1; positive-ion HRESIMS: *m/z* 349.1380 [M + H]$^+$ (calcd. for C$_{17}$H$_{21}$N$_2$O$_6$, 349.1394).

Dahurine B (**2**): pale yellow powder; $[\alpha]_D^{21}$ +48.0 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 291 (3.81); IR (KBr) $\nu_{max}$: 2959, 2931, 2871, 1783, 1664, 1451, 1334, 1189, 1089, 1052, 779 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Table 1; positive-ion HRESIMS: *m/z* 294.1721 [M + H]$^+$ (calcd. for C$_{16}$H$_{24}$NO$_4$, 294.1700).

Dahurine C (**3**): pale yellow powder; $[\alpha]_D^{21}$ −74.0 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 293 (3.79); IR (KBr) $\nu_{max}$: 2975, 2934, 2876, 1779, 1658, 1451, 1337, 1309, 1193, 1048, 781 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Table 1; positive-ion HRESIMS: *m/z* 294.1688 [M + H]$^+$ (calcd. for C$_{16}$H$_{24}$NO$_4$, 294.1700).

Dahurine D (**4**): pale yellow powder; $[\alpha]_D^{21}$ +18.0 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 292 (3.78); IR (KBr) $\nu_{max}$: 2974, 2933, 2876, 1779, 1659, 1451, 1337, 1310, 1194, 1049, 781 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Table 1; positive-ion HRESIMS: *m/z* 244.0919 [M + Na]$^+$ (calcd. for C$_{12}$H$_{15}$NO$_3$Na, 244.0944).

Dahurine E (**5**): pale yellow powder; $[\alpha]_D^{21}$ +2.0 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 292 (3.79); IR (KBr) $\nu_{max}$: 2974, 2933, 2876, 1780, 1659, 1541, 1451, 1310, 1241, 1087, 1049, 781 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Table 2; positive-ion HRESIMS: *m/z* 386.2323 [M + H]$^+$ (calcd. for C$_{23}$H$_{32}$NO$_4$, 386.2326).

Dahurine F (**6**): pale yellow powder; $[\alpha]_D^{21}$ +2.0 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$): 292 (3.69); IR (KBr) $\nu_{max}$: 2976, 2931, 2849, 1782, 1658, 1472, 1413, 1188, 1050, 934, 763 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Table 2; positive-ion HRESIMS: *m/z* 332.1852 [M + Na]$^+$ (calcd. for C$_{17}$H$_{27}$NO$_4$Na, 332.1832).

## Biological assays

AChE inhibitory activity was determined via the method of Mukherjee by a 96-well microplate reader based on Ellman's method [20, 21]. Briefly, 100 μL of 0.1 M PBS buffer (pH 8.0), 20 μL of AChE solution (50 mU/mL in buffer), and 20 μL of sample diluted in 0.2% DMSO buffer solution were added to a microplate and incubated at 37 °C for 20 min. Then, 40 μL of 0.6 mM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) was added. The reaction was initiated by adding 20 μL of 1.2 mM acetylthiocholine iodide (ATCh). After incubating the reaction solution at 37 °C for 30 min, the reaction was quenched by the addition of 4% sodium dodecyl sulfate (SDS) solution. The optical densities were immediately measured in a 96-well plate reader at 405 nm.

**Table 2** [1]H (500 MHz) and ^{13}C (125 MHz) NMR data of compounds **5–6** ($\delta$ in ppm, in CDCl$_3$)

| Position | 5[a] | | 6[a] | |
|---|---|---|---|---|
| | $\delta_H$, multi. ($J$ in Hz) | $\delta_C$, type | $\delta_H$, multi. ($J$ in Hz) | $\delta_C$, type |
| 2 | | 132.5, C | | 132.6, C |
| 3 | 6.99, d (4.0) | 125.6, CH | 6.94, d (4.0) | 125.1, CH |
| 4 | 6.05, d (4.0) | 110.9, CH | 6.23, d (4.0) | 111.6, CH |
| 5 | | 140.7, C | | 138.3, C |
| 6 | 3.88, br s | 63.9, CH$_2$ | 4.51, d (18.0) | 64.4, CH$_2$ |
| | 3.53, br s | | 4.46, d (18.0) | |
| 7 | 9.50, s | 178.7, CH | 9.42, s | 178.9, CH |
| 8 | 5.26, br s | 61.4, CH | 5.36, br s | 54.9, CH |
| 9 | | 169.1, C | | 170.9, C |
| 10 | 3.74, dd (14.0, 2.5) | 37.9, CH$_2$ | 1.71, d (7.0) | 17.8, CH$_3$ |
| | 3.32, dd (14.0, 11.5) | | | |
| 11 | | 138.0, C | | |
| 12 | 6.84, overlapped | 129.1, CH | | |
| 13 | 7.16, overlapped | 128.5, CH | | |
| 14 | 7.16, overlapped | 126.7, CH | | |
| 15 | 7.16, overlapped | 128.5, CH | | |
| 16 | 6.84, overlapped | 129.1, CH | | |
| 1' | 3.27, m | 69.7, CH$_2$ | 3.41, m | 70.2, CH$_2$ |
| | 3.13, m | | | |
| 2' | 1.43, m | 31.6, CH$_2$ | 1.55, m | 31.8, CH$_2$ |
| | 1.30, m | | | |
| 3' | 1.29, m | 19.2, CH$_2$ | 1.32, m | 19.5, CH$_2$ |
| 4' | 0.87, t (7.0) | 13.9, CH$_3$ | 0.89, overlapped | 14.0, CH$_3$ |
| 1'' | 4.15, t (6.5) | 65.4, CH$_2$ | 4.10, m | 65.5, CH$_2$ |
| 2'' | 1.57, m | 30.4, CH$_2$ | 1.55, m | 30.6, CH$_2$ |
| 3'' | 1.29, m | 19.0, CH$_2$ | 1.32, m | 19.2, CH$_2$ |
| 4'' | 0.90, t (7.0) | 13.6, CH$_3$ | 0.89, overlapped | 13.8, CH$_3$ |

[a]Assignments were carried out based on HSQC and HMBC experiments

The experiments were performed in triplicate. The percentage inhibition was evaluated using the equation $I$ (%) = [$1 - (A_{sample} - A_{background})/A_{blank}] \times 100\%$, where $A_{sample}$ is the absorbance of each test compound, and $A_{background}$ is the absorbance of the background without enzyme. The IC$_{50}$ values were determined using GraphPad Prism 5 software from experiments performed (GraphPad Software, Inc., San Diego, CA, USA). Huperzine A was used as a positive control (IC$_{50}$ = 0.6 ± 0.1 μM).

## ECD calculations

The relative configurations of compounds **1–4**, **7**, and **8** were initially established on the basis of their NOESY spectra and submitted to random conformational analysis with the MMFF94s force field and using the SYBYL-X 2.0 software package. The conformers were further optimized by using the time-dependent density functional theory (TDDFT) method at the B3LYP/6-31G(d) level, and the frequency was calculated at the same level of theory. The stable conformers without imaginary frequencies were subjected to ECD calculation by the TDDFT method at the B3LYP/6-31 + G(d) level with the CPCM model in methanol solution. ECD spectra of different conformers were simulated using SpecDis v1.51 with a half-bandwidth of 0.16–0.4 eV, and the final ECD spectra were obtained according to the Boltzmann-calculated contribution of each conformer after UV correction. The calculated ECD spectra were compared with the experimental data. All calculations were performed with the Gaussian 09 program package [22, 23].

## Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest.

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