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EXPERIMENTAL RESEARCH

Analgesic monoterpene indole alkaloids from *Gelsemium elegans* stems

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[Abstract] **Objective** The aim of this study is to search for potent analgesics from the stems of *Gelsemium elegans*. **Methods** Alkaloids were isolated from the samples of *G. elegans* and purified using column chromatograph and High-Performance Liquid Chromatography. The chemical structures of the isolated alkaloids were determined using extensive high resolution electrospray ionization mass spectroscopy (HRESIMS) and nuclear magnetic resonance (NMR) spectroscopic data analyses, ^{13}C NMR DP4+ analysis and electronic circular dichroism (ECD) calculations, and $Rh_2(OCOCF_3)_4$-induced ECD data analysis. The analgesic activities of all the isolates were analyzed using an acetic acid-induced writhing test in mice. **Results** Two new monoterpene indole alkaloids, elegansine A (**1**) and 14-hydroxysempervirine (**2**), and seven known monoterpene indole alkaloids were isolated from the stems of *G. elegans*. Elegansine A (**1**) represents the first example of sarpagine-type alkaloids with a $\Delta^{15(20)}$ double bond. All the alkaloids (**1**–**9**) showed potential analgesic activities. Three alkaloids, namely 14-hydroxysempervirine (**2**), 14β,20α-dihydroxydihydrorankinidine (**4**), and 14-hydroxygelsenicine (**6**), exhibited significant analgesic activities in the acetic acid-induced writhing test in mice at a dose of 5.0 mg/kg with the writhing inhibition rates of 69.5%, 69.2%, and 72.7%, respectively. **Conclusion** These results enlarged the diversity of monoterpene indole alkaloids and also provided a new basis to develop novel potent analgesics.

[Key words] *Gelsemium elegans*; Monoterpene indole alkaloids; Structure elucidation; Analgesic activity

1 Introduction

Gelsemium elegans (Garden. & Champ.) Benth. (Loganiaceae), known as "Duanchangcao" or "Humanteng" in China, is an evergreen climbing shrub widely distributed in southern China and southeast Asia[1]. *G. elegans* is generally used as a folk medicine to treat cancers, fractures, rheumatic paralysis, and neuralgia[2]. It is reportedly rich in monoterpene indole alkaloids[3-4]. To date, more than 150 monoterpene indole alkaloids have been isolated from *G. elegans*, and some of these alkaloids exhibit antitumor, analgesic, anti-inflammatory, antianxiety,

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immunoregulatory, and anti-stress activities[3,5-6]. These alkaloids from *G. elegans* have attracted much attention of organic chemists and pharmacologists owing to their complex structures and significant bioactivities.

Different studies have investigated the stems of *G. elegans* to search for novel analgesics from traditional Chinese medicines[6-9]. These studies have isolated two new monoterpene indole alkaloids, named elegansine A (**1**) and 14-hydroxysempervirine (**2**), and seven known monoterpene indole alkaloids (**3**–**9**) (Fig. 1). In the present study, we have reported the isolation, structure elucidation, and analgesic activities of these alkaloids (**1**–**9**).

2 Materials and methods

2.1 General experimental procedures

Optical rotations were obtained in MeOH by a Rudolph AUTOPOL IV Automatic Polarimeter (Waltham, USA). Ultra-violet (UV) and fourier-transform infrared spectroscopy data were recorded on a Varian Cary 50 spectrophotometer and a Bruker Vertex 70 instrument (Billerica, USA), respectively. Electronic circular dichroism (ECD) data were recorded using a JASCO J−810 spectrometer (Tokyo, Japan). Nuclear magnetic resonance (NMR) data were acquired on a Bruker AM-400 NMR spectrometer (Billerica, USA), and the residual peaks of methanol-d_4 at δ_H 3.31 and δ_C 49.15 and chloroform-*d* at δ_H 7.24 and δ_C 77.23 were used as references. High resolution electrospray ionization mass spectrometry (HRESIMS) data were measured using a Bruker micrOTOF spectrometer (Billerica, USA). High-performance liquid chromatography (HPLC) separation was conducted on an Agilent 1200 or Dionex P680 Quaternary System (Sunnyvale, USA) with a UV detector and a semi-preparative column (5 μm, 10 mm × 250 mm, Welch Ultimate XB C18 and XB-phenyl) at a flow rate of 1.5 mL/min.

2.2 Plant material

The stems of *G. elegans* were collected in Yulin, Guangxi, China, in July 2016, and were identified by Prof. Jianping Wang at Huazhong University of Science and Technology. A voucher specimen has been deposited at the School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology (No. 20160720).

2.3 Extraction and isolation

The dried stems of *G. elegans* (100 kg) were

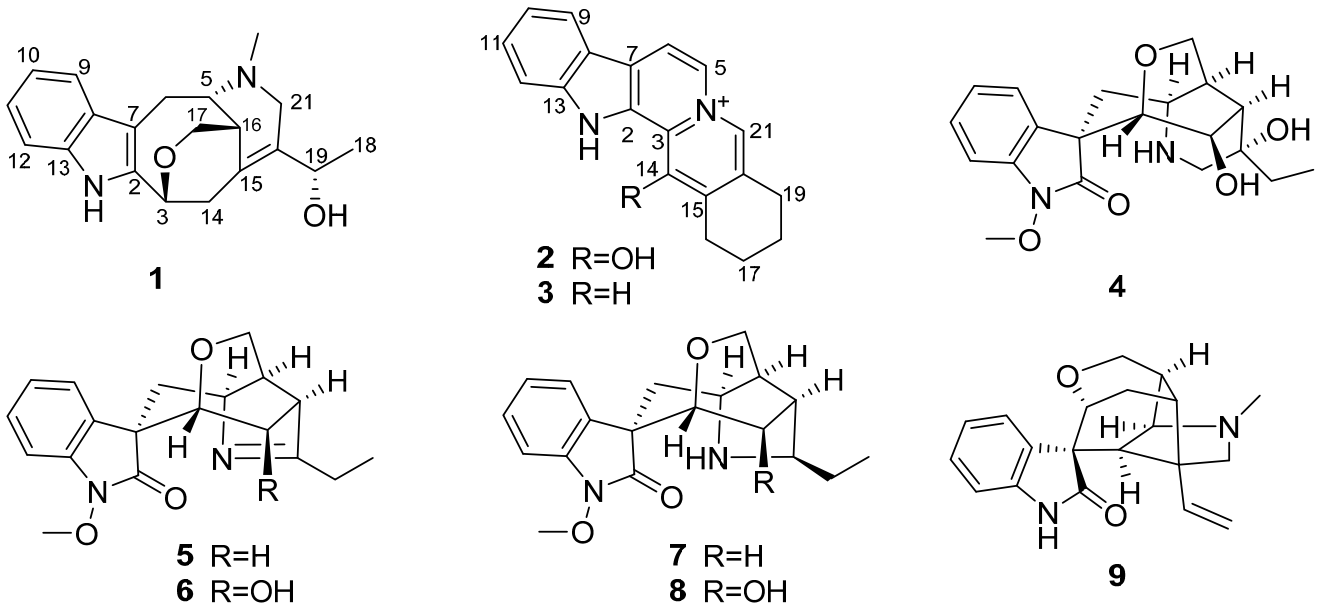

Fig. 1 Chemical structures of compounds 1–9.

powdered and then extracted with 95% EtOH (4 × 100 L, 5 days each time) at room temperature. The filtrates were combined and concentrated under reduced pressure to obtain a dark brown crude extract (4.87 kg). The extract (4.87 kg) was then suspended in H_2O (8 L), acidified with 0.3 M HCl to pH = 3, and partitioned with $CHCl_3$ to remove neutral components. The aqueous phase was basified with an ammonia solution to pH = 10, and total alkaloids (680 g) were extracted with $CHCl_3$. The total alkaloids (680 g) were fractionated by silica gel column chromatography (CC) eluting with CH_2Cl_2–MeOH (50 : 1→0 : 100) to give eight fractions (Fr. A–H). Fr. B was separated by silica gel CC to give three subfractions (Fr. B1–B3). Fr. B2 was purified by Sephadex LH-20 (MeOH) to yield compound **3** (8.5 mg), and Fr. B3 was purified by successive Sephadex LH-20 (MeOH) and RP HPLC (MeOH–H_2O–NH_3•H_2O, 80 : 20 : 0.2) to yield compound **2** (t_R = 28.4 min, 12.0 mg). Fr. E was separated by an RP C18 MPLC eluting with a gradient of MeOH–H_2O to produce seven subfractions (Fr. E1–E7). Fr. E1 was subjected to successive Sephadex LH-20 (MeOH) and silica gel CC to produce three subfractions (Fr. E1a–E1c). Fr. E1c was chromatographed by RP C18 HPLC (MeOH–H_2O–NH_3•H_2O, 55 : 45 : 0.2) to yield compound **9** (t_R = 18.5 min, 4.6 mg). Fr. E2 was separated by silica gel CC to afford eight subfractions (Fr. E2a–E2h). Fr. E2c was separated by RP C18 HPLC (MeOH–H_2O–NH_3•H_2O, 47 : 53 : 0.2) to yield compound **6** (t_R = 37.2 min, 10.2 mg). Fr. E4 was applied to silica gel CC to give eight subfractions (Fr. E4a–E4h). Fr. E4d was fractioned by RP C18 HPLC (MeOH–H_2O–NH_3•H_2O, 55 : 45 : 0.2) to yield compound **5** (t_R = 23.8 min, 35.0 mg). Fr. F was fractionated by RP C18 MPLC to yield six subfractions (Fr. F1–F6). Fr. F3 was isolated by silica gel CC to yield eight subfractions (Fr. F3a–F3h). Fr. F3c and Fr. F3e were separated by RP C18 HPLC (MeOH–H_2O–NH_3•H_2O, 80 : 20 : 0.2) to yield compounds **1** (t_R = 24.3 min, 10.6 mg) and **7** (t_R = 23.1 min, 11.4 mg), respectively. Fr. F5 was fractionated by silica gel CC to yield seven subfractions (Fr. F5a–F5g). Fr. F5d was separated by RP C18 HPLC (MeOH–H_2O–NH_3•H_2O, 60 : 40 : 0.2) to give compound **8** (t_R = 28.4 min, 7.8 mg). Fr. F5f was fractioned by RP C18 HPLC (MeOH–H_2O–NH_3•H_2O, 50 : 50 : 0.2) to gain compound **4** (t_R = 46.5 min, 15.8 mg).

2.3.1 Elegansine A (1)

Yellow oil; $[\alpha]_D^{25}$ –113 (*c* 0.1, MeOH); UV (MeOH): λ_{max} (log ε): 253 (3.925) nm; IR (KBr) ν_{max} 3 483, 2 960, 1 729, 1 066, 1 041 cm^{-1}; CD (MeOH) λ_{max} ($\Delta\varepsilon$): 232 (+69.3), 275 (−22.7) nm; ^1H NMR (400 MHz, methanol-d_4) and ^{13}C NMR (100 MHz, methanol-d_4) data, see Table 1; HRESIMS *m/z* 325.1910 $[M + H]^+$ (calcd. for $C_{20}H_{25}N_2O_2$, 325.1916).

2.3.2 14-Hydroxysempervirine (2)

Yellow powders; UV (MeOH): λ_{max} (log ε): 236 (3.512), 289 (3.201) nm; IR (KBr) ν_{max} 3378, 2926, 2854, 1690, 1598, 1501, 1025 cm^{-1}; ^1H NMR (400 MHz, chloroform-*d*) and ^{13}C NMR (100 MHz, chloroform-*d*) data, see Table 2; HRESIMS *m/z* 289.1350 $[M + H]^+$ (calcd. for $C_{19}H_{17}N_2O$, 289.1341).

2.4 Determination of absolute configuration of C-19 in elegansine A (1)

According to a previously described method[10], elegansine A (**1**) was dissolved in a dry solution of stock $Rh_2(OCOCF_3)_4$ complex in CH_2Cl_2 (molar ratio of 1 : 0.3 to 1 : 0.7). The $Rh_2(OCOCF_3)_4$-induced ECD spectrum of elegansine A (**1**) was recorded immediately every ten minutes until the ECD spectra became constant. After subtracting the inherent ECD spectrum of **1**, the induced ECD spectrum was prepared. The absolute configuration of C-19 in **1** was inferred using the bulkiness rule.

2.5 NMR and ECD calculations of 1

Table 1 ^{1}H (δ in ppm, J in Hz, 400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data for elegansine A (1) in methanol-d_4.

Position	δ_H (J in Hz)	δ_C	Position	δ_H (J in Hz)	δ_C
2		139.3	14α	2.92, dd (14.8, 2.6)	32.4
3	5.35, dd (3.8, 2.6)	73.0	14β	3.04, overlap	
5	3.13, overlap	65.8	15		130.5
6α	3.13, overlap	25.6	16	2.74, m	40.2
6β	3.04, overlap		17α	4.05, dd (11.8, 4.3)	63.2
7		110.4	17β	4.40, d (11.8)	
8		131.2	18	0.84, d (6.5)	22.1
9	7.46, dd (6.9, 1.6)	118.8	19	4.25, q (6.5)	66.0
10	6.96, td (6.9, 1.4)	119.9	20		139.0
11	7.29, td (6.9, 1.6)	122.0	21α	2.91, d (14.8)	50.6
12	7.22, dd (6.9, 1.4)	111.9	21β	3.08, d (14.8)	
13		136.5	N-CH_3	2.63, s	43.7

Table 2 ^{1}H (δ in ppm, J in Hz, 400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data for 14-hydroxysempervirine (2) in chloroform-*d*.

Position	δ_H (J in Hz)	δ_C	Position	δ_H (J in Hz)	δ_C
2		131.6	15		124.7
3		136.0	16α	3.18, t (6.0)	23.3
5	8.46, d (5.2)	138.9	16β	3.18, t (6.0)	
6	7.78, d (5.2)	112.3	17α	1.81, overlap	23.1
7		129.4	17β	1.81, overlap	
8		121.5	18α	1.81, overlap	23.0
9	8.12, d (7.9)	121.7	18β	1.81, overlap	
10	7.29, overlap	120.0	19α	2.65, t (6.0)	20.5
11	7.56, overlap	128.3	19β	2.65, t (6.0)	
12	7.56, overlap	111.7	20		123.5
13		140.2	21	7.56, s	137.0
14		147.3			

The NMR and ECD calculations of **1** were performed using the procedures described previously[6,8]. A conformational search of structure was performed by BALLOON, and the stable conformers were optimized with B3LYP/6-31G* level (gas phase), followed with the calculation of frequency.

The GIAO ^{13}C NMR was calculated at the mPW1PW91/6-311G** (IEFPCM) level of theory. The calculated isotropic magnetic shielding constants (σ) were Boltzmann averaged according to their Gibbs free energies. The calculated NMR data were analyzed using the DP4+ probability to further confirm the relative configuration of **1**.

The ECD spectra were calculated for each conformer using the TDDFT methodology at the B3LYP/6-311++G** level with MeOH as the solvent using the PCM solvation model implemented in Gaussian 09 program. The ECD spectra for each conformer were simulated using a Gaussian function with a bandwidth σ of 0.3 eV. The spectra were combined after Boltzmann weighting according to their population contributions, and UV correction was applied.

2.6 Analgesic activity evaluation of 1–9

The analgesic activities of **1**–**9** were evaluated using acetic acid-induced writhing tests in mice

(morphine was used as a positive control), according to procedures previously described in the literature[6]. The male and female Kunming mice (18−22 g each) were purchased from the Laboratory Animal Center, Tongji Medical College, Huazhong University of Science and Technology. Groups of 10 Kunming mice were used as controls and test mice. Thirty minutes after the administration of the compound (control received the vehicle, 0.9% NaCl, 5 mL/kg, i.p.), the mice were given an intraperitoneal injection of 0.8% *v/v* acetic acid solution (0.1 mL/10 g). The mice were then placed into iron net cages individually, and their writhing events were counted for 30 min. Animal experiments (approval number 2018−S748) were approved by the Laboratory Animal Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology.

3 Results and discussion

Compound **1** was isolated as a bright yellow oil. Based on the HRESIMS ion at *m/z* 325.1910 $[M + H]^+$ (calcd for $C_{20}H_{25}N_2O_2$ 325.1916) and the ^{13}C NMR data, the molecular formula of **1** was determined to be $C_{20}H_{24}N_2O_2$. The ^1H NMR data of **1** (Table 1) showed resonances for an ortho-disubstituted benzene ring system (δ_H 7.46, dd, J = 6.9, 1.6 Hz, H-9; 7.29, td, J = 6.9, 1.6 Hz, H-11; 7.22, dd, J = 6.9, 1.4 Hz, H-12; 6.96, td, J = 6.9, 1.4 Hz, H-10), two oxymethines (δ_H 5.35, dd, J = 3.8, 2.6 Hz, H-3; 4.25, q, J = 6.5 Hz, H-19), an oxymethylene (δ_H 4.40, d, J = 11.8 Hz; 4.05, dd, J = 11.8, 4.3 Hz, H_2-17), an aminomethine (δ_H 3.13, H-5), an aminomethylene (δ_H 3.08, d, J = 14.8 Hz; 2.91, d, J = 14.8 Hz, H_2-21), a methyl (δ_H 0.84, d, J = 6.5 Hz, H_3-18), and an *N*-CH_3 (δ_H 2.63, s). The ^{13}C NMR data of **1** (Table 1) exhibited 20 carbon resonances, assignable by the DEPT data to two methyls, four methylenes, eight methines, five quaternary carbons, and a carbonyl. The NMR data of **1** (Table 1) showed similarities to the known sarpagine-type alkaloid, anhydrovobasmediol, which was previously reported from the stems of *G. elegans*[11]. The main differences were the presence of a tetrasubstituted double bond (δ_C 130.5, C-15; 139.0, C-20) and an oxymethine (δ_H 4.25, q, J = 6.5 Hz, δ_C 66.0, CH-19) in **1**, replacing the trisubstituted double bond (δ_H 5.53, H-19; δ_C 119.8, C-19; 135.9, C-20) and a methine group (δ_H 2.56, m, δ_C 36.1, CH-15) in anhydrovobasmediol[11]. The same coupling constant J = 6.5 Hz of H_3-18 (δ_H 0.84, d) and H-19 (δ_H 4.25, q) and their related splitting peaks, as well was the $^1H-^1H$ COSY correlation (Fig. 2) of H_3-18 and H-19, supported the presence of the partial structure of "C(18)H_3-C(19)H(OH)−". The location of the tetrasubstituted double bond at $\Delta^{15(20)}$ was proved by the HMBC correlations from H-19 to C-15/C-16/C-20, and from H-16 (δ_H 2.74, m) to C-15 and C-20 (Fig. 2). Detailed $^1H-^1H$ COSY and HMBC data analysis

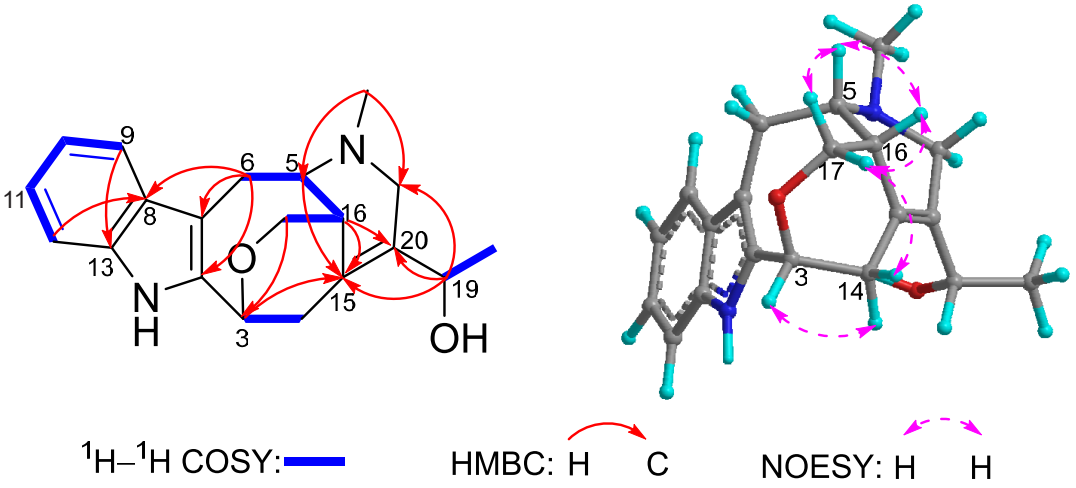

Fig. 2 $^1H-^1H$ COSY, HMBC, and NOESY correlations of elegansine A (1).

established the planer structure, as shown in Fig. 2.

Similar chemical shifts of **1** to the known sarpagine-type alkaloid, anhydrovobasmediol, and their biogenetic relationship suggested similar relative configurations except for C-19. The small coupling constant of H-3 (J = 3.8, 2.6 Hz) and NOESY correlations (Fig. 2) of H-3 to H-14a, H-14b to H-17b, H-17a to H-5, and H-5 to H-16 further indicated the relative configurations of C-3, C-5, and C-16 as R^*, S^*, and S^*, respectively. However, NOESY data analysis could not define the configuration of C-19 due to the free rotation of the side chain of "C(18)H_3-C(19)H(OH)−". To determine the relative configuration of 19-OH, the theoretical ^{13}C NMR calculations and DP4+ probability analyses of the two possible isomers 19S^* and 19R^*-**1** were performed using the GIAO method at the mPW1PW91/6-311G (d,p) level with the Gaussian 09 software[6-9]. The smaller relative errors between experimental and the calculated ^{13}C NMR chemical shift (Fig. 3) and highly DP4+ probability of approximately 100% established the relative configuration of C-19 to be S^*.

The absolute configuration of C-19 was confirmed to be S from the positive Cotton effect at 350 nm ($\Delta\varepsilon$, +2.1) (Fig. 4) in the $Rh_2(OCOCF_3)_4$-induced ECD spectrum, based on the empirical bulkiness rule of Snatzke[12-13]. The ECD spectra of (3R,5S,16S)-**1** and its enantiomer were calculated to establish the absolute configuration of **1**[14-15]. Results (Fig. 4) showed that the experimental ECD spectrum of **1** matched well with the calculated ECD spectrum of (3R,5S,16S)-**1** but was in contrast to the calculated ECD spectrum of (3S,5R,16R)-**1**. Thus, the absolute

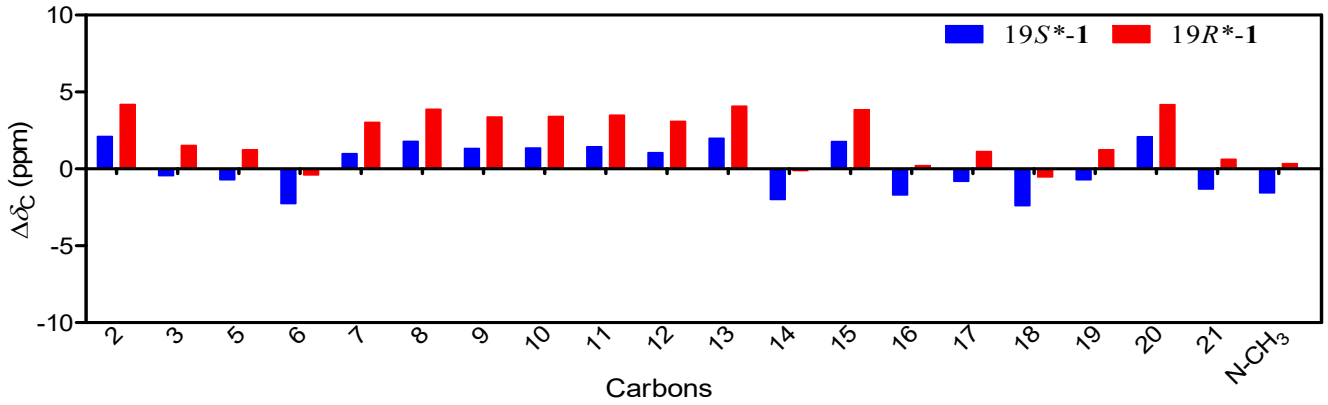

Fig. 3 Relative errors between experimental ^{13}C NMR data of 1 and the calculated ^{13}C NMR chemical shift of (19S^*)-1 and (19R^*)-1.

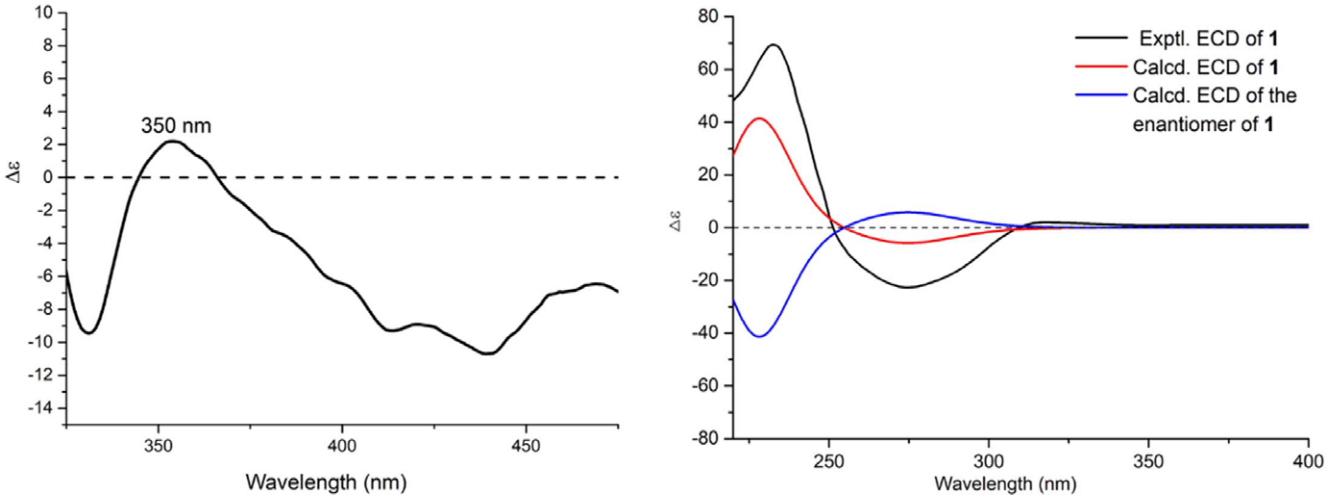

Fig. 4 $Rh_2(OCOCF_3)_4$-induced ECD spectrum of elegansine A (1) in CH_2Cl_2 and the experimental and calculated ECD spectra of elegansine A (1) and its enantiomer.

configuration of **1** was defined as 3*R*,5*S*,16*S*,19*S*, and it was named elegansine A (**1**).

Sarpagine-type indole alkaloids are mainly reported from the genera of *Rauwolfa* and *Alstonia* of the Apocynaceae family[16-17] and are relatively rare in the *Gelsemium* genus. Elegansine A (**1**) represents the first example of sarpagine-type alkaloids with a $\Delta^{15(20)}$ double bond.

Compound **2**, a yellow powder, was assigned a molecular formula of $C_{19}H_{16}N_2O$ by the HRESIMS ion at *m/z* 289.1350 $[M + H]^+$ (calcd for $C_{19}H_{17}N_2O$, 289.1341) and ^{13}C NMR data, one more oxygen atom than the co-isolated sempervirine (**3**). The NMR data of **2** (Table 2) were similar to that of sempervirine (**3**)[18], and the main difference was the deshielding of C-14 (δ_C 147.3) in **2** compared to sempervirine (**3**) (δ_C 120.7, CH-14)[18]. Thus, compound **2** might be the 14-hydroxy derivative of sempervirine (**3**). The absence of H-14 in **2** and one more oxygen atom than **3** supported the above conclusion. 2D NMR data analysis, including HSQC, $^1H–^1H$ COSY, and HMBC correlations (Fig. 5) further defined the structure of **2** as 14-hydroxysempervirine (**2**).

The structures of the six known compounds were identified to be sempervirine (**3**)[19], 14*β*,20*α*-dihydroxydihydrorankinidine (**4**)[20], gelsenicine (**5**)[21], 14-hydroxygelsenicine (**6**)[22], gelsedine (**7**)[23], 14*β*-hydroxygelsedine (**8**)[24], and gelsemine (**9**)[25], respectively, by spectroscopic data analysis and comparison with the reported data in the literature.

All isolated alkaloids **1**–**9** were evaluated for their analgesic activities in the acetic acid-induced writhing test in mice, and morphine was used as a positive control[26-29]. Results (Fig. 6) revealed that nine alkaloids showed significant analgesic activity at a dose of 5.0 mg/kg. Among them, 14-hydroxysempervirine (**2**), 14*β*,20*α*-dihydroxydihydrorankinidine (**4**), and 14-hydroxygelsenicine (**6**) showed significant analgesic activity with the writhing inhibition rates of 69.5%, 69.2%, and 72.7%, respectively.

4 Conclusion

Nine monoterpene indole alkaloids, including two new ones, were isolated from the stems of

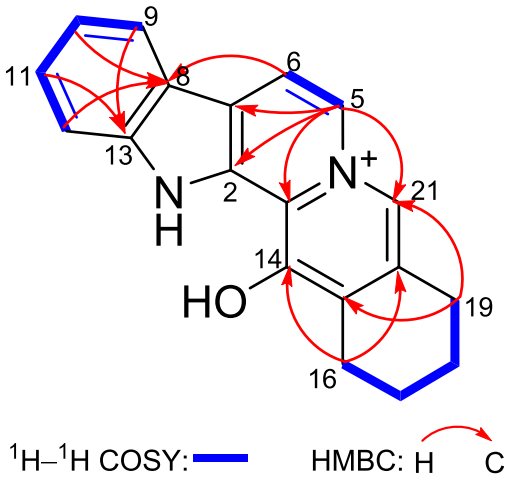

Fig. 5 $^1H–^1H$ COSY and HMBC correlations of 14-hydroxysempervirine (2).

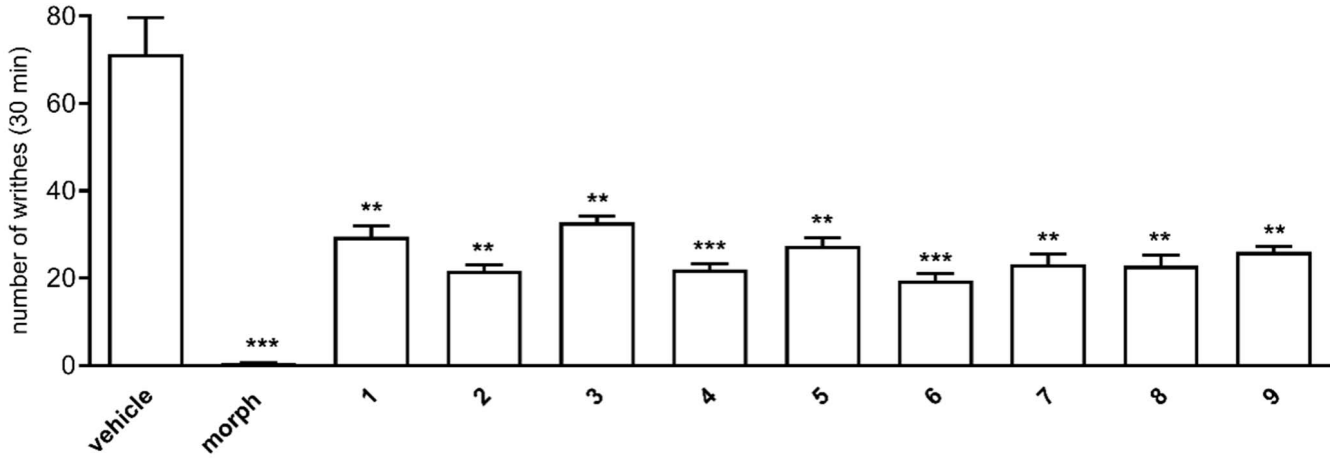

Fig. 6 Analgesic activities of alkaloids 1–9 in the writhing model at a dose of 5.0 mg/kg. *n* = 10, morphine (morph, positive control), $^{**}P < 0.01$, $^{***}P < 0.001$, *vs* vehicle.

G. elegans. Elegansine A (**1**) represents the first example of the sarpagine-type alkaloids with a $\Delta^{15(20)}$ double bond, and its absolute configuration was defined by $Rh_2(OCOCF_3)_4$-induced ECD method and ECD calculation. All the alkaloids (**1**–**9**) showed discernible analgesic activities using acetic acid-induced writhing tests in mice. Compounds **2**, **4**, and **6** exhibited significant analgesic activities in the acetic acid-induced writhing test in mice at a dose of 5.0 mg/kg. These results enriched the chemical diversity of *G. elegans* and provided a scientific basis for the development of potent analgesics.

5 Supporting information summary

UV, IR, HRESIMS, and NMR spectra for elegansine A (**1**) and 14-hydroxysempervirine (**2**); ^{13}C NMR and ECD calculation data for elegansine A (**1**).

6 Conflicts of interest

The authors have no conflict of interest to declare.

7 Acknowledgments

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