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Three novel β-carboline alkaloids from *Gelsemium elegans*

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ABSTRACT

Three novel β-carboline alkaloids characterized with a unique degraded monoterpenoid moiety, gelebolines A–C (**1**–**3**), together with 11 known alkaloids of different types, were isolated from the roots of *Gelsemium elegans*. The structures of new alkaloids were established on the basis of analysis of spectroscopic data. It is the first example for report of β-carboline from genus *Gelsemium* (Loganiaceae).

1. Introduction

Gelsemium genus (Loganiaceae) comprises three species: *G. elegans* Benth., *G. sempervirens* Ait. and *G. rankinii* Small. More than 70 alkaloids including six-type indole alkaloids such as sarpagine, koumine, humantenine, gelsedine, gelsemine, and yohimbane, have been isolated from *Gelsemium* genus [1,2]. In our continuing studies on systematic search for bioactive and structural interesting alkaloids [3,4], three new β-carboline alkaloids, named as gelebolines A–C (**1**–**3**), together with 11 known compounds including four alkaloids of humantenine-type, humantenine [5], humantenirine [5], rankinidine [5], *N*-demethoxyrankinidine [5], three alkaloids of sarpagine-type, koumidine [6], 19*E*-16-*epi*-voacarpine [7], 19*Z*-akuammidine [2b], three alkaloids of gelsedine-type, gelsedine [8], gelsenicine [9], 19-oxo-gelsenicine [2b], and koumine [10], were isolated from the roots of *G. elegans*. Herein we describe the structural elucidation of three new alkaloids. The structures of the new alkaloids were established by elucidation of their 1D and 2D NMR spectroscopic data. The structures of other 11 known alkaloids were identified by comparison of their spectral data with those of the literatures.

2. Experimental

2.1. General

General Experimental Procedure. Optical rotations were measured on a Perkin–Elmer 241 polarimeter. UV spectra were obtained on an UV-210A spectrometer. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers with TMS as the internal standard. ESIMS were carried out on a Finnigan MAT 90 mass spectrometer and VG Auto Spec-3000 instrument, respectively. Chromatographic separations were performed on silica gel (90–150 μm; Qingdao Marine Chemical Plant, Qingdao, China) columns, Sephadex LH-20 (40–70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden) columns, or Lichroprep RP-18 gel (40–63 μm; Merck, Darmstadt, Germany)

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columns. Semipreparative HPLC was performed on a Zorbax SB-C18 (10 μm; Agilent Co., Ltd. Wilmington, Delaware) column (9.4×250 mm), eluted with CH_3OH–H_2O (50:50; for 30 min at a flow rate of 3.0 ml/min; detection, UV 254 nm, 365 nm) at 30 °C. Precoated silica gel GF_{254} and HF_{254} plates (Qingdao Haiyang Chemical Plant, Qingdao, China) were used for TLC.

2.2. Plant material

The roots of *G. elegans* were collected in Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Yunnan Province, China, in April 2006. The plant was identified by Prof. De-Ding Tao and a voucher specimen (KIB 08051023) was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The air-dried root powder of *G. elegans* (11.2 kg) was extracted with MeOH for three times at room temperature and twice under reflux. The combined MeOH extracts were concentrated and the residue (135 g) was suspended in 1.5 L water, then acidified with 5% HCl to pH 3, and partitioned with EtOAc to remove the neutral components. The aqueous phase was then adjusted to pH 9 with a saturated solution of Na_2CO_3, and then extracted with $CHCl_3$. The combined organic phases ($CHCl_3$ layer) were concentrated to yield a crude alkaloid mixture (44.6 g). The crude alkaloid was subjected to silica gel column chromatography, eluted with a $CHCl_3$–MeOH gradient to yield seven fractions (Fr. A–G). Fraction D (135 mg) was subjected to silica gel column chromatography using a gradient solvent system containing petroleum ether–acetone–Et_2NH (30:1:0.1 to 5:1:0.1) to yield humantenine (18 mg), gelsenicine (23.2 mg), 19-oxo-gelsenicine (16 mg) and a subfraction fraction (Fr. D_1). Fr. D_1 was separated on a silica gel column eluted with petroleum ether–acetone–Et_2NH (5:1:0.1 to 3:1:0.1) to yield humantenirine (34 mg), rankinidine (7 mg), gelsedine (11 mg) and alkaloid mixtures, which was further separated by semipreparative HPLC (C_{18} reversed-phase silica gel column, MeOH–H_2O [70:30]) to yield geleboline B (**2**, 4.0 mg) and geleboline A (**1**, 2.5 mg). Fr. E (108 mg) was separated chromatographically on a silica gel column, eluted with petroleum ether–acetone–Et_2NH (25:1:0.1 to 2:1:0.1), to yield five compounds, *N*-demethoxyrankinidine (7 mg), 19*E*-16-epi-voacarpine (4 mg), 19*Z*-akuammidine (7 mg), koumidine (8 mg), koumine (12 mg). Geleboline C (**3**, 2.8 mg) was separated by semipreparative HPLC (C_{18} reversed-phase silica gel column, MeOH–H_2O [60:40]).

2.4. Geleboline A (**1**)

White amorphous powder, $[\alpha]_D^{20}$ +12.2 (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ε) 352 (3.62), 339 (3.60), 290.6 (4.14), 253 (4.21), 237 (4.40), 212 (4.34), 201 (4.35) nm; IR (KBr) ν_{max} 3400, 2900, 1650, 1450, 1200, 900, 750 cm^{-1}; ^1H and ^{13}C NMR see Table 1; ESI-MS *m/z*: 321.0 $[M+H]^+$; HR-ESI-MS *m/z* 321.1592 $[M+H]^+$ (calcd. for $C_{20}H_{20}N_2O_2$, 321.1525).

2.5. Geleboline B (**2**)

White amorphous powder, $[\alpha]_D^{20}$ +18.2 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 352 (3.72), 338 (3.70), 290 (4.23), 253 (4.29), 236 (4.48) nm; IR (KBr) ν_{max} 3400, 2900, 1650,

Table 1
^1H (400 MHz, *J* in Hz) and ^{13}C (125 MHz) NMR data for compounds **1**–**3**.

Pos.	**1**[a]		**2**[a]		Pos.	**3**[b]	
	δ_H	δ_C	δ_H	δ_C		δ_H	δ_C
1		142.3		142.5	1		133.3
3	8.41 (d, 5.3)	137.2	8.35 (d, 5.5)	137.1	3	8.39 (d, 5.3)	138.5
4	7.98 (d, 5.3)	114.6	7.92 (d, 5.5)	114.5	4	8.31 (d, 5.3)	119.3
4a		129.8		129.8	4a		116.7
4b		120.9		121.0	4b		121.0
5	8.18 (d, 8.0)	121.5	8.13 (d, 8.0)	121.5	5	8.26 (d, 8.0)	122.7
6	7.29 (m)	119.4	7.25 (m)	119.3	6	7.35 (t, 8.0)	121.6
7	7.60 (t, 8.0)	128.1	7.53 (t, 8.0)	128.1	7	7.63 (t, 8.0)	130.4
8	7.54 (d, 8.0)	111.6	7.49 (d, 8.0)	111.3	8	7.76 (d, 8.0)	113.5
8a		140.5		140.4	8a		143.5
9a		133.2		133.1	9a		136.6
1′	6.01 (s)	91.0	5.93 (s)	90.8	1′		202.6
3′	5.88 (s)	112.5	5.91 (s)	111.4	2′		151.9
3a′		131.3		131.5	3′		129.0
4′	2.45 (m)	22.0	2.09 (2H, m)	21.4	4′	2.52 (m)	29.9
5′	1.68 (m)	21.5	1.60 (2H, m)	22.0	5′	1.86 (m)	22.6
6′	1.54 (m)	21.3	1.67 (2H, m)	22.0	6′	1.85 (m)	22.4
7′	2.20 (m)	21.9	2.28 (2H, m)	21.2	7′	2.51 (m)	25.7
7a′		138.5		138.0	8′		168.5
OCH_3	3.81(3H, s)	56.5		15.5	COOMe	3.37 (s)	52.0
OCH_2CH_3			1.47 (3H, t, 6.5)	65.4			
OCH_2CH_3			4.13 (m), 3.85 (m)				
NH	10.84 (s)		10.91 (s)				

[a] Measured in $CDCl_3$.
[b] Measured in CD_3OD.

1450, 1200, 900, 750 cm^{-1}; ^1H NMR and ^{13}C NMR see Table 1; ESI-MS *m/z* 335.1 $[M+H]^+$; HR-ESI-MS *m/z* 335.1772 $[M+H]^+$ (calcd. for $C_{21}H_{22}N_2O_2$, 335.1681).

2.6. Geleboline C (**3**)

White amorphous powder, UV (MeOH) λ_{max} (log ε) 381 (3.42), 356 (3.58), 291 (4.14), 215 (4.50) nm; IR (KBr) ν_{max} 3500, 3306, 2929, 1764, 1710, 1627, 1493, 1429, 1249, 1141, 890, 747 cm^{-1}; ^1H and ^{13}C NMR see Table 1; ESI-MS *m/z* 335.2 $[M+H]^+$; HR-ESI-MS *m/z* 335.1396 $[M+H]^+$ (calcd. for $C_{20}H_{18}N_2O_3$, 335.1395).

3. Results and discussion

Compound **1** was obtained as a white amorphous powder. The molecular formula $C_{20}H_{20}N_2O_2$ was determined by positive HRESIMS at *m/z* 321.1592 $[M+H]^+$ (calcd. for $C_{20}H_{20}N_2O_2$, 321.1525) with 12° of unsaturation. Unlike any previous alkaloids isolated from *G. elegans*, a strong blueness fluorescence was exhibited on TLC plates under UV light ($\lambda = 365$ nm). The UV spectrum of **1** displayed absorption maxima at 352, 339, 291, 253 nm, suggesting the presence of β-carboline moieties [11]. ESI-MS analyses in the positive ion mode showed two main fragment ion peaks at *m/z* 289 ($[M–OCH_3]^+$) and 261 ($[M–OCH_3–CO]^+$), representing an acetal key ion involved potentially. The four contiguous aromatic proton signals (Table 1) at δ 8.18 (d, $J = 8.0$ Hz), 7.29 (t, $J = 8.0$ Hz), 7.60 (t, $J = 8.0$ Hz), and 7.54 (d, $J = 8.0$ Hz), respectively, were indicated in the presence of a 1, 2-disubstituted phenyl ring. The two mutually coupled doublet proton signals at δ 8.41 and 7.98, respectively, were assigned to α and β pyridine protons, according to their chemical shifts and coupling constant ($J = 5.3$ Hz). These spectroscopic characteristics suggested the presence of a β-carboline moiety in **1**. The ^{13}C NMR and DEPT spectra of **1** (Table 1) revealed the eight methine carbons and seven quaternary carbons in downfield, a methoxy carbon signal at δ_C 56.5 ppm, four high-field methylene signals at δ_C 22.0, 21.9, 21.5, and 21.2 ppm. The connection from C-4′ to C-7′ was deduced by the ^1H–^1H COSY spectrum of **1** (Fig. 1). The HMBC correlations (Fig. 1) from H-1′ to C-3a′, H-3′ to C-1′ and C-7a′, H_2-4′ to C-7a′, H_2-7′ to C-3a′, and OMe to C-3′ suggested the presence of a 3-methoxy-1,3,4,5,6,7-hexahydroisobenzofuran moiety. This moiety was connected with C-1 of the β-carboline moiety through C-1′ by the HMBC correlation of H-1′ to C-9a and C-1. The ROESY correlations of H-1′ and H-3′ with H_3-OMe indicated that H-1′ and H-3′ were *trans* form, which was further confirmed by the absence of the ROESY correlation of H-1′/H-3′. Thus, the structure of **1** was deduced as geleboline A.

Compound **2** was obtained as a white amorphous powder. The molecular formula of **2** was established as $C_{21}H_{22}N_2O_2$ with HRESIMS (*m/z* 335.1772 $[M+H]^+$) with 12° of unsaturation. The ^1H and ^{13}C NMR spectroscopic data of **2** were similar to those of **1**, except for the signals δ_C 65.4 and δ_C 15.5 for an ethoxy group instead of the methoxy signal in **1**. The HMBC correlations from H-3′ to oxygenated methylene carbon (δ_C 65.4) indicated that the ethoxy group was located at C-3′. The relative configuration of **2** was also assigned the same as **1** by their similar ROESY correlations. Therefore, the structure of **2** was assigned as geleboline B.

Compound **3** was obtained as a white amorphous powder with the molecular formula $C_{20}H_{18}N_2O_3$ according to the $[M+H]^+$ peak in the HRESIMS at *m/z* 335.1396 (calcd for 335.1395) corresponding to 13° of unsaturation. The UV spectrum of **3** also displayed the characteristic absorption maxima at 381, 356, 291 nm, suggesting the presence of β-carboline moieties [11]. The IR absorption exhibited the presence of ester (1764 cm^{-1}) and ketone (1710 cm^{-1}) carbonyl groups. Its ^1H NMR spectrum showed a set of four mutually coupled protons at δ 8.26 (d, $J = 8.0$ Hz), 7.35 (t, $J = 8.0$ Hz), 7.63 (t, $J = 8.0$ Hz), and 7.76 (d, $J = 8.0$ Hz), and two coupled proton signals at δ 8.39 (1H, d, $J = 5.3$ Hz) and 8.31 (1H, d, $J = 5.3$ Hz), 8 high-field proton signals at δ 2.52 − 1.85, and a methoxy signal at δ 3.37 (s). The ^{13}C NMR and DEPT spectra showed six methine carbons and nine quaternary carbons in down field, a methoxy carbon signal at δ 52.0, and 4 high-field methylene signals at δ 22.4, 22.6, 25.7, 29.9 (Table 1). Detailed 2D NMR studies revealed that compound **3** was composed of two moieties, one of which contained the spectroscopic characteristics of C_1-substituted-β-carboline (shown in Fig. 1). In the other moiety, 4 methylene showed a sequence of ^1H–^1H COSY correlations ($H_{4'}$–$H_{5'}$–$H_{6'}$–$H_{7'}$) (Fig. 2). The HMBC correlations of H-5′ to quaternary carbon C-3′, H-6′ to quaternary

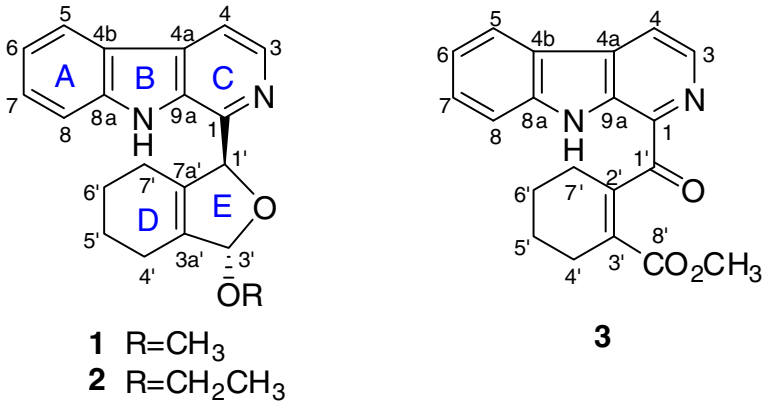


Fig. 1. Structures of alkaloids **1**−**3** isolated from *Gelsemium elegans*.

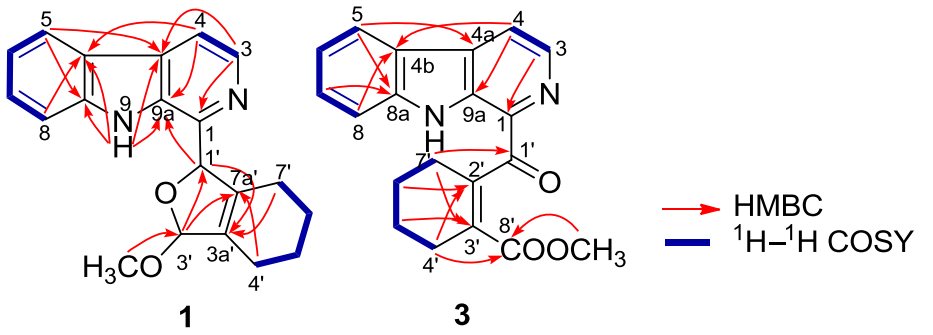
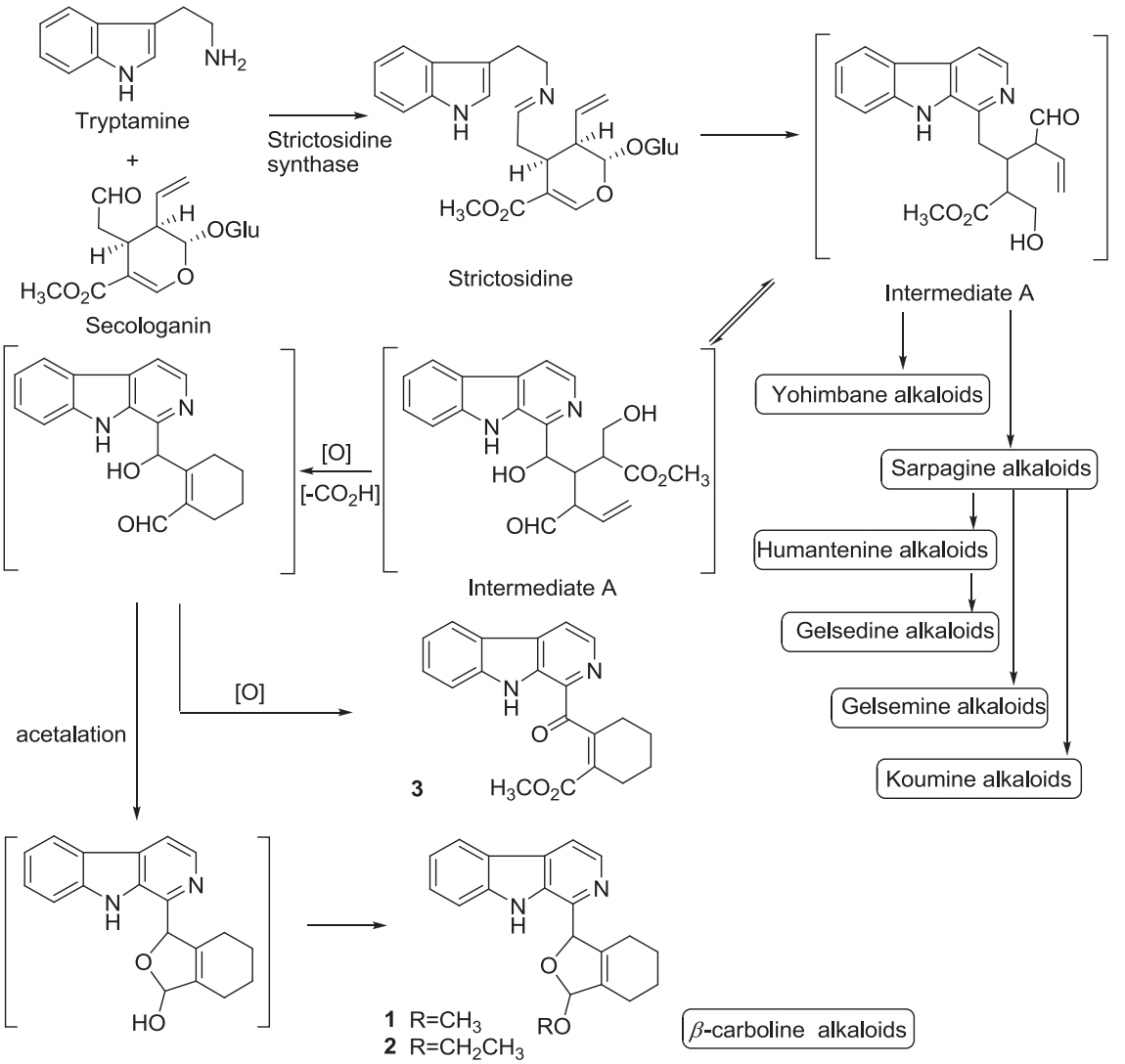


Fig. 2. ^{1}H–^{1}H COSY (bold) and key HMBC (arrow, H → C) correlations of **1** and **3**.

carbon C-2′, H-7′ to C-3′ and H-4′ to C-2′ revealed an ortho-disubstituted cyclohexene fragment. Two HMBC correlations of H-4′ (δ 2.52) to C-8′ (δ 168.5) and methoxy group signals (δ 3.37) to C-8′ suggested the ester group was located at C-3′, while HMBC correlation of H-7′ with carbonyl carbon (δ 202.6) indicated the ketone group was connected with C-1 and C-2′, respectively. Accordingly, the structure of **3** was deduced as geleboline C.



Scheme 1. Hypothetical biogenetic pathway for gelebolines A–C (**1**–**3**).

The cytotoxic activity of geleboline B (**2**) and the known alkaloids against HL-60 human leukemia and A-549 human lung cancer cell lines were evaluated by the standard protocols MTT [12] and SRB [13] methods respectively, and pseudolaric acid B [14] was used as a positive control. None showed inhibitory effects ($IC_{50} > 40\ \mu M$).

To the best of our knowledge, it is the first report of β-carboline alkaloids isolated from the genus of *Gelsemium*. Moreover, compounds **1**–**3** represented the first example of β-carboline alkaloids with a unique degraded monoterpenoid moiety. The biogenetic origin of coexistence of β-carboline alkaloids and monoterpenoid alkaloids was proposed as Scheme 1.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.fitote.2012.02.007.

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