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Gelsemium alkaloids, immunosuppressive agents from *Gelsemium elegans*

You-Kai Xu [a,*], Shang-Gao Liao [b,*], Zhi Na [c], Hua-Bin Hu [a], Yan Li [d], Huai-Rong Luo [d]

[a] CAS Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, Yunnan Province 666303, PR China
[b] School of Pharmacy, Guiyang Medical College, 9 Beijing Road, Guiyang 550004, PR China
[c] Laboratory of Tropical Plant Resource Science, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, Yunnan 666303, PR China
[d] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650204, PR China

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ABSTRACT

Bioassay-guided isolation of the stems of *Gelsemium elegans* has led to the isolation of two new *Gelsemium* alkaloids, 21-(2-oxopropyl)-koumine (**1**) and 11-methoxygelselegine (**2**), and two known alkaloids, koumine (**3**) and gelselegine (**4**). The structures of **1**–**2** were determined by spectroscopic (for both) and single-crystal X-ray diffraction (for **1**) analysis. All compounds isolated were evaluated for their potential as immunosuppressive agents and the data suggested that *Gelsemium* alkaloids of different structural types possibly have potential as immunosuppressive agents.

1. Introduction

Gelsemium elegans (Loganiaceae), a well-known toxic perennial climbing vine native to Southeast Asia, has attracted natural products chemists's great attention due to the presence of structurally complex and diversified alkaloids. More than eighty indole alkaloids of six structural skeletons (sarpagine, koumine, humantenine, gelsedine, gelsemine, and yohimbane types) have been isolated from the leaves, stems, seeds, and roots of the plant [1–11]. Despite the advances in the chemical investigations of *Gelsemium* alkaloids in recent years, only anticancer activity (and anti-epidermal cell proliferation) was reported for a limited number of compounds and only one alkaloid, koumine, was reported to show immunosuppressive activity [12,13]. *G. elegans*, for a very long time, has been used in Chinese folk medicine for the treatment of malignant tumors, skin diseases (e.g., psoriasis), rheumatism, and rheumatic arthritis. In the previous investigation of the leaves and stems of *G. elegans*, we have reported three *Gelsemium* alkaloids bearing an iridoid monoterpenoid moiety [6]. Yet, only no to moderate anticancer activity was observed for these compounds. Correlations of the traditional use of *G. elegans* with biological activities of the alkaloids produced by the plant remain unclear. Inspired by the traditional use of *G. elegans* in the treatment of rheumatic arthritis, an autoimmune disease that causes chronic joint inflammation, we carried out a bioassay-guided investigation of *G. elegans*. As a result, two new and two known *Gelsemium* alkaloids were isolated from the immunosuppressively active fractions of *G. elegans*. The structures of these new alkaloids were elucidated by means of spectroscopic (for **1** and **2**) and single-crystal X-ray diffraction (for **1**) analysis. The alkaloids isolated were evaluated for immunosuppressive and antitumor activity. We report herein the isolation and structural elucidation of these new alkaloids and their biological activity evaluation.

2. Experimental

2.1. General

Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV spectra were obtained on a UV-210A

* Corresponding authors. Tel.: +86 691 8715910/851 6908468 8712; fax: +86 691 8715070.
E-mail addresses: xyk@xtbg.ac.cn (Y-K. Xu), lshangg@163.com (S-G. Liao).

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spectrometer. CD spectra were recorded on a JASCO 810 spectrometer. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with KBr pellets. NMR spectra were recorded in CD_3OD or $CDCl_3$ on a Bruker AM-500 or AM-400 spectrometer with TMS as the internal standard. HREIMS and 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 Haiyang Chemical Plant, Qingdao, China) columns, Sephadex LH-20 (40–70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden) columns, and Lichroprep RP-18 gel (40–63 μm; Merck, Darmstadt, Germany) columns. Semi-preparative HPLC was performed on an XTerra prep RP-18 (10 μm, Waters Corp., Ireland) column (10×250 mm) eluted with MeOH/H_2O from 40:60 to 90:10 for 15 min at a flow rate of 4 mL/min; the detector used was PDA (200–400 nm) at 33 °C. Precoated silica gel GF254 and HF254 plates (Qingda Haiyang Chemical Plant, Qingdao, China) were used for TLC.

2.2. Plant material

The perennial stems (2–3 cm in diameter) of *G. elegans* were collected in Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Yunnan Province, China, in October. 2008. A voucher specimen (accession number: 2008081203) was deposited in the Herbarium of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

2.3. Extraction and bioassay-guided isolation

The air-dried perennial stems of *G. elegans* (5.0 kg) were percolated three times with 10 L of 95% EtOH at room temperature to give an ethanol extract (310 g). Immunosuppressive activity and cytotoxicity assay indicated that the extract, at 10 μg/mL, showed 30% and 40%, respectively, inhibition of T lymphocyte proliferation stimulated by Con A and B lymphocyte proliferation stimulated by LPS. The ethanolic extract (300 g) was then dissolved in water (1.0 L) to form a suspension, which was acidified with 10% H_2SO_4 to around pH 3. The acidic suspension was first partitioned with EtOAc to remove the neutral compounds, and the aqueous phase was then basified with Na_2CO_3 to around pH 10 and extracted with $CHCl_3$ to give a crude alkaloid extract (40 g). Immunosuppressive assay demonstrated that, at 1 μg/mL, the crude alkaloid showed, respectively, 10% and 37% inhibition of T lymphocyte proliferation stimulated by Con A and B lymphocyte proliferation stimulated by LPS. The crude alkaloid was therefore subjected to silica gel column chromatography ($CHCl_3$/MeOH, 100:0→1:3) to give four major fractions (1–4). Fraction 1 (2 g) was subjected to chromatography over silica gel ($CHCl_3$/MeOH, 50:1→10:1) to give 4 major fractions (Fr. 1-1 to Fr. 1-4). Fraction 1-3 (300 g) was purified by semi-preparative HPLC (MeOH/H_2O, 50/50→80/20; flow rate: 4 mL/min) to give **1** (20 mg) and **3** (50 mg). Fraction 2 (3 g) was separated by silica gel (CH_3Cl/MeOH, 30:1→5:1) to give three major fractions (Fr. 2-1 to Fr. 2-3). Fr. 2-2 was purified by semi-preparative HPLC (MeOH/H_2OH, 60:40→90:10) to give **2** (15 mg). Fraction 3 (5 g) was subjected to silica gel column chromatography ($CHCl_3$/MeOH, 10:1→3:1) to give three major fractions (Fr. 3-1 to Fr. 3-2). Fr. 3-1 was purified by semi-preparative HPLC (MeOH/H_2O, 40:60→80:20) to give **4** (100 mg).

21-(2-Oxopropyl)-koumine (**1**, Fig. 1): Colorless crystals (MeOH). M.p 256–258 °C; $[\alpha]_D$ −56 (*c* 0.1, MeOH); CD (MeOH): λ (Δε) = 265 (−3.70), 224 (2.83), 214 (−0.11); UV(MeOH), λ_{max} (log ε): 258 (1.79), 221 (1.21), 216 (1.23), 196 (1.43); IR (KBr) ν_{max} 3462, 2920, 2877, 1630, 1587, 1446, 1080, 775 cm^{-1}; ^1H and ^{13}C NMR (in CD_3OD), see Table 1; EIMS *m/z*: 362 (34), 322 (18), 308 (70), 307 (85), 306 (100), 305 (95), 291 (65), 279 (72), 277 (75), 263 (82), 251 (76), 235 (72), 223 (82), 218 (82), 206 (84), 194 (84), 180 (82), 167 (76); HREIMS *m/z* : 362.1995 $[M]^+$ (calcd for $C_{23}H_{26}N_2O_2$, 362.1994).

11-Methoxygelselegine (**2**, Fig. 1): white amorphous powder; $[\alpha]_D$ −114 (*c* 0.20, EtOH); CD (MeOH): λ_{max} (Δε): 270 (−9.08), 236 (43.70), 215 (−51.79); UV (MeOH), λ_{max} (log ε): 218 (0.46), 251 (1.13), 257 (1.12), 262 (1.13), 283 (1.17) nm; IR (KBr) ν_{max} 3482, 3408, 3186, 3049, 2951, 2836, 1686, 1626, 1502, 1433, 1222, 985, 753 cm^{-1};^1H and ^{13}C NMR (in $CDCl_3$), see Table 1; ESIMS *m/z*: 389 $[M+H]^+$; HRESIMS *m/z*: 389.2072 $[M+H]^+$ (calcd for $C_{21}H_{29}N_2O_5$, 389.2076).

X-ray single-crystal structure determination of 21-(2-oxopropyl)-koumine (1). $C_{23}H_{26}O_2N_2{\bullet}2H_2O$, *M* = 398.49; Monoclinic, space group, *P*21; a = 8.4184(1) Å, b = 15.2473 (3) Å, c = 8.9574(2) Å, α = 90°, β = 117.8410(10) °, γ = 90°, V = 1016.66(3) A^3, Z = 2, *d* = 1.302 g/cm^3, crystal dimensions 0.10 × 0.05 × 0.05 mm were used for measurement on a Bruker Smart Apex-II CCD diffractometer equipped with a graphite-monochromated Cu_{Ka} radiation (40 kV, 30 mA), Wavelength 1.54178 Å. The total number of reflections measured was 4505, of which 3191 were observed. Final indices: $R_1 = 0.0406$, $wR_2 = 0.1042$. The crystal structure of **1** was solved by direct method SHELXS-97 and expanded using difference Fourier

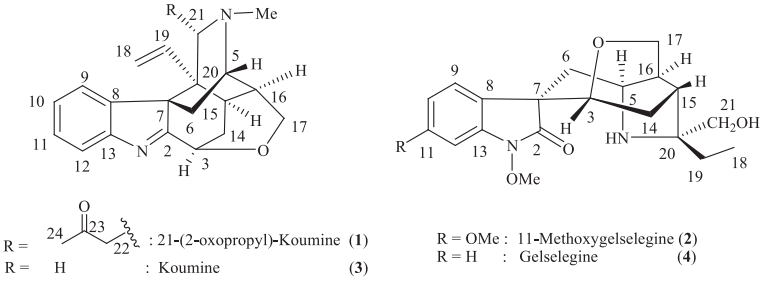

Fig. 1. Structures of compounds **1**–**4**.