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Aporphine Alkaloids from *Clematis parviloba* and Their Antifungal Activity

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A new aporphine alkaloid, β-magnoflorine (**1**), together with a known aporphine alkaloid, α-magnoflorine (**2**), were isolated from the aerial parts of *Clematis parviloba*. Their structures were elucidated on the basis of comprehensive spectroscopic techniques. In addition, both compounds showed potent antifungal activities against *Penicillium avellaneum* UC-4376.

Key words: Ranunculaceae, *Clematis parviloba*, Aporphine alkaloid, Magnoflorine, Antifungal activities

INTRODUCTION

Clematis genus, belonging to the family of Ranunculaceae, is widely distributed. Many plants of this genus are widely used as traditional Chinese medicines. *Clematis parviloba* Gardn. et Champ, a woody vine, is mainly distributed in Japan and south-west of China. Up to now, no phytochemical studies on the plant of *C. parviloba* were reported. Aiming to find bioactive secondary products from *C. parviloba*, we investigated the aerial parts of this plant and led to the isolation of a new compound, β-magnoflorine (**1**), together with a known compound, α-magnoflorine (**2**). Magnoflorine is perhaps the most widely distributed naturally occurring quaternary aporphine alkaloids (Dwuma-Badu et al., 1980). They has been found in numerous genera of flowering plant families, such as Annonaceae, Aristolochiaceae, Berberidaceae, Euphorbiaceae, Magnoliaceae, Menispermaceae, Ranunculaceae and *Rutaceae*, and so on (Dwuma-Badu et al., 1980). Moreover, magnoflorine alkaloids have ever been tested for anti-inflammatory, antnociceptive (Esra et al., 2002), antipyretic (Esra et al., 2002), antiradical (Lucia et al., 2004), antoxidant (Lucia et al., 2004), anti-hepatitis B virus (Cheng et al., 2007), and antifeedant activities (Corrado et al., 2001). In this paper, we describe the isolation, structure elucidation and antifungal activity of compounds **1** and **2**.

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MATERIALS AND METHODS

General experimental procedure

1D and 2D NMR spectra were recorded on Brucker AM-400 and DRX-500 spectrometers. Unless otherwise specified, chemical shift (δ) were expressed in ppm with reference to the solvent signals. MS were performed on a VG Autospec-3000 spectrometer under 70 eV. Optical rotations were measured with a Horiba SEPA-300 polarimeter. A Tenor27 spectrophotometer was used for scanning IR spectroscopy of compounds with KBr pellets. Column chromatography was performed with silica gel (200-300 mesh, Qing-dao Marine Chemical Inc., Qingdao, Peoples Republic of China), silica gel H (60 mm, Qing-dao Marine Chemical Inc.). Fractions were monitored by TLC and spots were visualized by spraying with potassium bismuth iodide.

Plant materials

The aerial parts of *Clematis parviloba* were collected in Xundian County of Yunnan Province, P. R. China, in December 2002. The plant material was verified by Dr. Jia-Hui Chen. A voucher specimen (No. Chen$^{\#}$200212002) was deposited at the Herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences.

Extraction and isolation

The air-dried aerial parts of *C. parviloba* (445 g) were powdered and extracted for four times with boiling 75% ethanol. After the removal of solvents, the residual syrup (79.5 g) was dissolved in water and extracted with EtOAC and *n*-butanol successively. The *n*-butanol portion (22.58 g) showed antifungal activity against *Penicillium avellaneum* UC-4376, which was subjected to column chromatography over silica gel (150 g) and eluted with water-saturated chloroform, water-saturated chloroform-MeOH (9:1, 8:2, v/v) and MeOH (9 L) to yield fractions A (15.61 g), B (1.19 g), C (0.6 g), D (2.14 g), and E (2.16 g). The antifungal assays showed fractions B, D and E had inhibitory activities against *Penicillium avellaneum* UC-4376. Fraction B was further subjected to column chromatography over silica gel (98 g) and eluted with EtOAC-MeOH (4:6, v/v) to yield compound **2** (162 mg). Fraction D was chromatographed over silica gel (80 g) and eluted with EtOAC-MeOH (4:6, v/v) to yield compound **1** (38 mg), and from fraction E, compound **1** (87 mg) was also obtained through the same isolation procedure.

β-Magnoflorine (1)

Yellow powder; m.p. 197-198°C; $[\alpha]_D^{26}$ +240.0 (*c* 0.1, MeOH); UV $_{max}$ (MeOH): 205, 227, 273; IR (KBr); ν_{max} 3435, 2932, 2365, 1634, 1440, 1384, 1310, 1249, 1066 cm^{-1}; FABMS *m/z* [%]: 342 [M^+, 100]; negative-ion HR-ESIMS: *m/z* 342.1694 (calcd. For $C_{20}H_{24}NO_4$ 342.1705); For ^{1}H- and ^{13}C-NMR see Table I.

α -Magnoflorine (2)

Yellow powder; m.p. 243-244°C; $[\alpha]_D^{26}$ +150.0 (*c* 0.1, MeOH); UV_{max} (MeOH): 205, 227, 273; IR (KBr) ν_{max} 3436, 2930, 2366, 1634, 1457, 1385, 1311, 1249, 1212 cm^{-1}; FABMS *m/z* [%]: 342 [M^+, 100]. For ^{1}H- and ^{13}C-NMR see Table I.

Antifungal assay

Inhibitory activities of compounds **1** and **2** against *Penicillium avellaneum* UC-4376, *Candida albicans*, *C. glabrata*, *Saccharomyces cerevisiae*, *Cryptococcus neoformans*, *Trichosporon beigelii* and *Pyricularia oryzae* were determined by disk diffusion assay on agar plates as described previously (Espinel-Ingroff et al., 1999).

RESULTS AND DISCUSSION

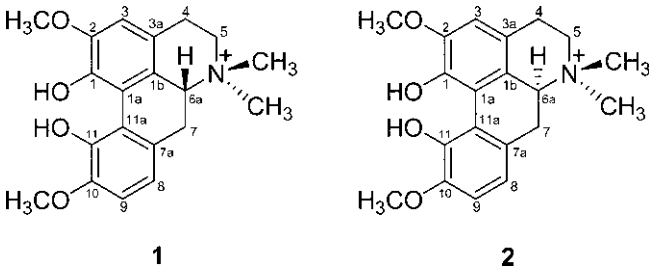
Compounds **1** and **2** can react with potassium bismuth iodide, suggesting that they were alkaloids. The ^{13}C-NMR data of **1** were very similar to those of **2** (Table I). Both compounds possessed 20 carbon signals, including two methyls, three methylenes, four methines, nine quaternary carbons and 2 methoxyl groups. Compound **1** was assigned the molecular formular of $C_{20}H_{24}NO_4$ based on the HR-ESIMS and NMR data. The NMR assignments for **1** and **2** were carried out ambiguously with HMQC, HMBC and ^{1}H-^{1}H COSY experiments. The ^{1}H- and ^{13}C-NMR data of **1** and **2** were consistent with those of magnoflorine reported in literature (BarbosaFilho et al., 1997; Marsaioli et al., 1979), which showed that **1** and **2** were magnoflorines. However, the antifungal activities of **1** and **2** have obvious difference, which indicated that compounds **1** and **2** must have difference in their stereochemistry. This deduction was supported by the optical rotations of **1** $[\alpha]_D^{26}$ +240.0 (*c* 0.1, MeOH) and **2** $[\alpha]_D^{26}$ +150.0 (*c* 0.1, MeOH). The ROESY experiments indicated that the protons at C-6a in **1** and **2** were either α- or β-form. Considered that the ^{1}H- and ^{13}C-NMR data of **2** is more close to the reported data of α-magnoflorine (BarbosaFilho et al., 1997; Marsaioli et al., 1979), compound **2** was assigned to be *S*-form aporphine. Accordingly, compound **1** was assigned to be *R*-form aporphine. Therefore, com-

Table I. The NMR Data of **1** and **2** (CD_3OD)

Position	1[a]		2[a]	
	δ_H [mult., $J(H_Z)$] [b]	δ_C	δ_H [mult., $J(H_Z)$] [b]	δ_C
1	/	149.0 (s)	/	150.4 (s)
1a	/	123.1 (s)	/	123.3 (s)
1b	/	121.1 (s)	/	121.1 (s)
2	/	152.7 (s)	/	152.9 (s)
CH_3O-2	3.72 (s)	56.0 (s)	3.76 (s)	56.4 (s)
3	6.40 (s)	109.7 (d)	6.48 (s)	109.5 (d)
3 a	/	116.5 (s)	/	116.1 (s)
4 α	3.09 (m)	24.6 (t)	2.63 (m)	24.7 (t)
4 β	2.49 (m)		3.17 (m)	
5 α	3.06 (m)	62.1 (t)	3.45 (m)	62.4 (t)
5 β	3.38 (m)		3.11 (m)	
N-CH_3-α	2.68 (s)	43.6 (s)	3.23 (s)	54.0 (s)
N-CH_3-β	3.17 (s)	53.9 (s)	2.79 (s)	43.6 (s)
6 a	3.67 (m)	70.7 (d)	3.48 (m)	71.0 (d)
7 α	2.26 (t, 12.0)	31.5 (s)	2.96 (m)	31.6 (s)
7 β	2.86 (m)		2.45 (t, 11.6)	
7 a	/	126.1 (s)	/	126.1 (s)
8	6.43 (d, 7.9)	117.5 (d)	6.49 (d, 7.1)	117.5 (d)
9	6.64 (d, 8.0)	110.7 (d)	6.67 (d, 7.5)	110.5 (d)
10	/	151.4 (s)	/	151.6 (s)
CH_3O-10	3.80 (s)	56.3 (s)	3.81 (s)	56.1 (s)
11	/	149.8 (s)	/	149.6 (s)
11a	/	123.0 (s)	/	123.4 (s)

[a] ^{1}H and ^{13}C NMR spectra were obtained at 400 and 125 MHz respectively.

[b] Coupling constants were presented in Hertz, δ in ppm. Unless otherwise indicated, all proton signals integrated to 1H.



1 **2**

Fig. 1. The structures of compounds **1** and **2**

pounds **1** and **2** were determined to be β-magnoflorine and α-magnoflorine (Fig. 1), respectively.

The minimal inhibition amounts of compound **1** against *P. avellaneum* UC-4376 is 10 μg/disc, and against *P. oryzae* at 100 μg/disc. The minimal inhibition amount of compound **2** against *P. avellaneum* UC-4376 is 5 μg/disc, and no inhibitory activities against *P. oryzae* were observed at 100 μg/disc. Amphotericin B (purchased from SIGMA) was used as positive control, 0.04 μg/disc (inhibitory zone diameter 8 mm).

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