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Plagranline A, a Novel *β*-carboline and the First Alkaloid Isolated from *Platycodon grandiflorus*

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Abstract: A novel *β*-carboline, namely Plagranline A (**1**), and a known alkaloid (**2**) were isolated from the roots of *Platycodon grandiflorus*. Their structure was elucidated using a combination of spectroscopic analyses and computational chemistry, including electronic circular dichroism (ECD) and optical rotatory dispersion (ORD). Alkaloids **1** and **2** exhibited moderate inhibitory effects against NO production. Interestingly, **1** and **2** are the first alkaloid constituents reported from the plant *P. grandifloras.*

Keywords: *Platycodon grandiflorus*; *β*-carboline; computational chemistry; alkaloid; anti-inflammatory activity.

1. Plant Source

P. grandiflorus roots were collected from Taihe, Anhui Province of China, in July 2021. The plant was identified by Qing-shan Yang of the Anhui University of Chinese Medicine. A voucher specimen (No. 202101) was deposited at Anhui University of Chinese Medicine.

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2. Previous Studies

Platycodon grandiflorus (Jacq.) A. DC. is a renascent herb belonging to the family Campanulaceae, which is widely distributed in Asian countries. As a well-known food material, it is frequently used as an ingredient in healthy foods and vegetable dishes. Meanwhile, it is used in folk medicine to treat cough, lung abscess, chest pain, sore throat, and diabetes [1]. Chemical investigations on *Platycodon grandiflorus* have mainly focused on and characterized triterpenoid saponins [2-4], flavonoids [5], phenolic acids (lignans) [6], polyacetylene [7], as well as sterols [1] and polysaccharides [8]. Moreover, there were a handful of records of the alkaloid content of the plant with UPLCQTOF-MS/MS techniques in the literature [9]. Among the above constituents, triterpenoid saponins, especially Platycodin D have been studied further, while polysaccharides have also been investigated. In summary, there are only limited chemical studies on *Platycodon grandiflora*, and their structural variation has not been further investigated in recent years.

3. Present Study

Two *β*-carboline-type alkaloids (**1-2**) (Figure 1) were isolated from the roots of *Platycodon grandifloras*, providing better insights into its constituents.

The dried roots of *P. grandiflorus* (20 kg) were reflux extracted with 80% ethanol (2 h×3). The solution was evaporated under reduced pressure to obtain an extract (1.2 kg). The crude extract was suspended in distilled water (1.5 L), and then partitioned with EtOAc and *n*-BuOH (each 1.5 L × 3 times). The EtOAc-soluble extract (276 g) was separated by silica gel CC elution with a step gradient of CH_2Cl_2/MeOH (100:0 to 0:100, v/v) to obtain six fractions (Fraction A–Fraction F). Fraction E (39 g) was subjected to C_{18} MPLC using MeOH–H_2O (20–80%, v/v) to yield five subfractions (E-1 to E-5). Subfraction E-2 (5 g) was chromatographed on Sephadex LH-20 (MeOH) twice and was further purified on the HPLC preparative column with CH_3CN-H_2O (20–35%, v/v, 40 min) to afford **2** (5.2 mg, 28 min). Subfraction E-5 (1 g) was chromatographed on Sephadex LH-20 (MeOH) and was further purified on the HPLC preparative column with CH_3CN-H_2O (35–50%, v/v, 40 min) to afford **1** (0.8 mg, 35 min).

*Plagranline A (**1**)*: Yellow amorphous powder; $C_{20}H_{20}N_2O_4$, $[\alpha]_D^{24}$ = -21.3 (*c* 0.6, CH_3OH); UV (CH_3OH) λ_{max} (logε): 202 (4.39), 278 (3.95), 362 (3.68) and 379 (3.67) nm, ^1H (800 MHz) and ^{13}C (200 MHz) NMR data $(CD_3)_2CO$, acetone-*d6*), Table 1; HRESIMS *m/z* 375.1314 $[M+Na]^+$ (calcd. for $C_{20}H_{20}N_2O_4Na$ 375.1315).

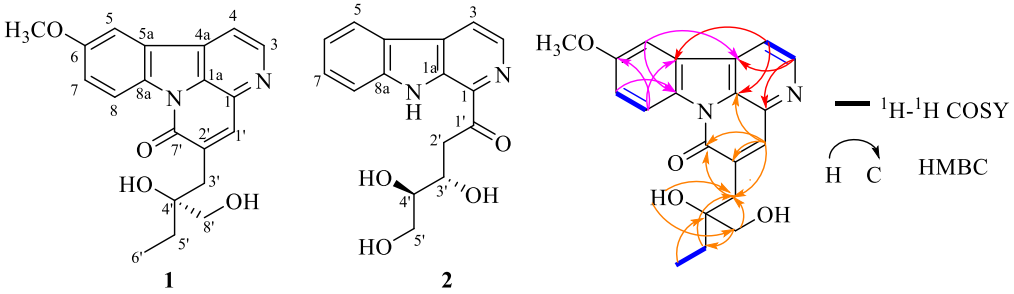

Figure 1. ^1H-^1H COSY correlations and the selected HMBC correlations of alkaloid **1** and the structures of alkaloids **1-2**

Alkaloid **1** was obtained as a yellow amorphous powder with maximum UV absorbance at 202, 278, 362, and 379 nm, which revealed the presence of a conjugated group. Its molecular formula was $C_{20}H_{20}N_2O_4$ based on HRESIMS with twelve degrees of unsaturation. The ^1H NMR data of 1 (Table 1)

and the HSQC spectrum showed the characteristic signals of an aromatic ring, including a group of ABX system spin coupling [7.94 (1H, d, *J* = 2.5 Hz, H-5), 7.36 (1H, dd, *J* = 8.8, 2.5 Hz, H-7) and 8.54 (1H, d, *J* = 8.8 Hz, H-8)], and a pair of olefinic protons at δ_H 8.83 (1H, d, *J* = 4.9 Hz, H-3) and 8.20 (1H, d, *J* = 4.9 Hz, H-4)]. The ^{13}C NMR and DEPT data (Table 1) revealed several conspicuous quaternary carbon signals at δ_C 125-140 ppm. Meanwhile, by combining the above hydrogen signals, in particular, an unusual pair of coupling constants (4.9 Hz) suggested that **1** was a *β*-carboline type. Furthermore, the key HMBC correlations from H-3 (δ_H 8.83) to C-1 (δ_C 137.4)/C-4a (δ_C 130.5), from H-4 (δ_H 8.20) to C-1a (δ_C 132.4)/C-5a (δ_C 127.3) and from H-5 (δ_H 7.94) to C-4a (δ_C 130.5)/C-8a (δ_C 134.5), as well as, the correlations of H-3 (δ_H 8.83) with H-4 (δ_H 8.20) and of H-7 (δ_H 7.36) with H-8 (δ_H 8.54) in the ^{1}H-^{1}H COSY spectrum, supported the above deduction. Moreover, the remaining olefinic proton (δ_H 8.06) indicated that the correlations with C-1a (δ_C 132.4)/C-2′ (δ_C 138.7)/C-7′ (δ_C 162.3) in the HMBC spectrum, pointed to the presence of *α*, *β*-unsaturated lactam ring between N_9-C_1 and $C_{1'}$- $C_{7'}$. Finally, five high field signals in the 2D spectra were distinctly observed: the ^{1}H-^{1}H COSY correlation with H-5′ and H-6′, together with the HMBC correlation from H-8′ (δ_H 3.37/3.41) to C-3′ (δ_C 38.4)/C-5′ (δ_C 30.7), from H-5′ (δ_H 1.64/1.69) to C-3′ (δ_C 38.4) and from H-6′ (δ_H 1.00) to C-4′ (δ_C 75.5), suggested the presence of 2-methylbutane-1,2-diol group. The group was located at C-2′ that confirmed by the crucial correlations from H-1′ (δ_H 8.06) to C-3′ (δ_C 38.4) and from H-3′ (δ_H 2.97/3.13) to C-7′ (δ_C 162.4). Therefore, the planar structure of **1** was assigned.

Because one chiral center is present in the structure of **1**, the ECD spectrum was applied to unambiguously confirm its absolute configuration, which was further supported with the aid of specific rotation. The calculated ECD spectra were determined using time-dependent density functional theory (TD-DFT) calculations at the M06–2X-D3/def2-TZVP level with MeOH as the solvent [10] using the polarizable continuum model (PCM), as well as, the calculated specific rotation was established by TDDFT at the APFD/6-311++g(2d,2p) (589 nm). As a result, the calculated ECD or ORD showed a good match with their experimental ones. Consequently, the absolute configuration of **1** was determined as 4′*R* (Figure 2 and S13-Figure 1).

Alkaloid **2** was identified as (3*S*,4*R*)-1-(*β*-carbolin-1-yl)-3,4,5-trihydroxy-1-pentanone by comparing their NMR spectroscopic data with those reported in the literature [11]. Interestingly, alkaloids **1** and **2** are the first alkaloids to be identified from *P. grandiflorus*, which have expanded the chemical diversity of this plant.

Table 1. ^{1}H (800 MHz) and ^{13}C (200 MHz) NMR spectroscopic data for **1** in $(CD_3)_2CO$ (δ in ppm and *J* in Hz)

No.	δ_H	δ_C	NO.	δ_H	δ_C
1		137.4, C	1′	8.06 (s)	139.8, C
1a		132.4, C	2′		138.7, C
3	8.83 (d, 4.9)	146.9, CH	3′	2.97 (d, 13.7) 3.13 (d, 13.7)	38.4, CH_2
4	8.20 (d, 4.9)	117.1, CH	4′		75.5, C
4a		130.5, C	4′-OH	3.91 (s)	
5	7.94 (d, 2.5)	107.9, CH	5′	1.64 (q, 7.5) 1.69 (q, 7.5)	30.7, CH_2
5a		127.3, C	6′	1.00 (t, 7.5)	7.8, CH_3
6		159.1, C	7′		162.3, C
7	7.36 (dd, 8.8, 2.5)	118.6, CH	8′	3.37 (dd, 11.3, 5.6) 3.41 (dd, 11.3, 6.9)	66.6, CH_2
8	8.54 (d, 8.8)	118.5, CH			
8a		134.5, C			
6-OCH_3	3.97 (s)	56.2, CH_3			

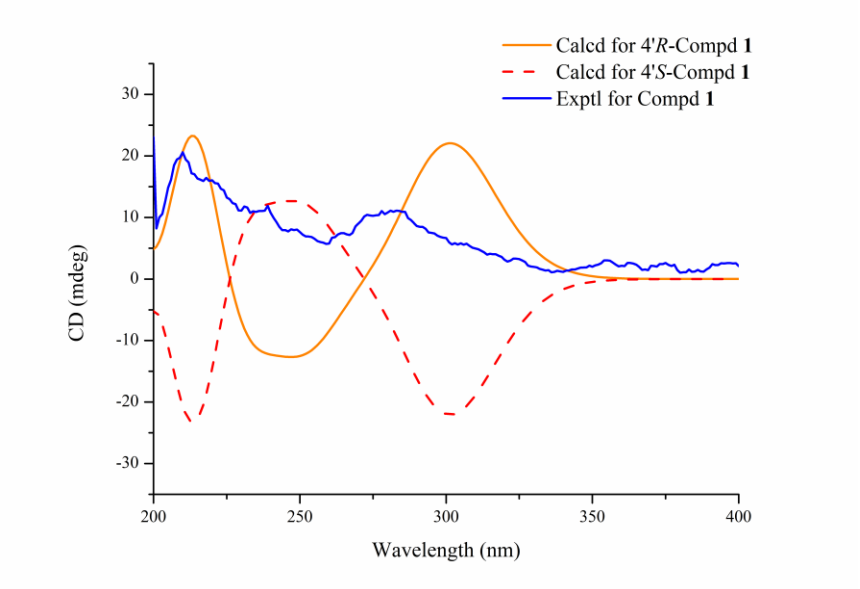

Figure 2. Calculated and experimental and ECD of **1**

Table 2. IC_{50} values of alkaloids **1–2** inhibiting NO production in RAW 246.7 cells

Compound	IC_{50} (μM)[a]
1	36.18±1.02
2	37.45±1.75
L-NMMA[b]	32.95±0.44

[a] IC_{50} values were expressed as mean ± SD (n = 3).
[b] L-NMMA = L-NG-Monomethylarginine hydrochloride was used as the positive control.

Alkaloids **1** and **2** were assessed for their abilities to inhibit NO production in LPS-stimulated RAW 264.7 cells [12, 13]. The results (Table 2) indicated that compounds **1** and **2** displayed moderate inhibitory effects against NO production with IC_{50} values of 36.18 and 37.45 μM, respectively.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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