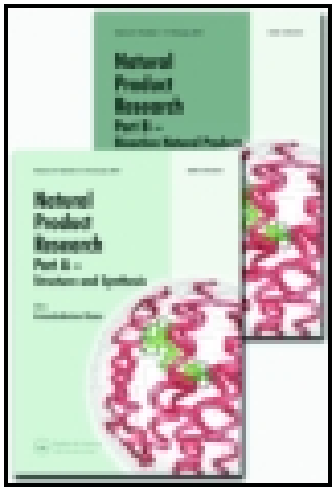

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Two new β-carboline alkaloids from the roots of Gypsophila oldhamiana

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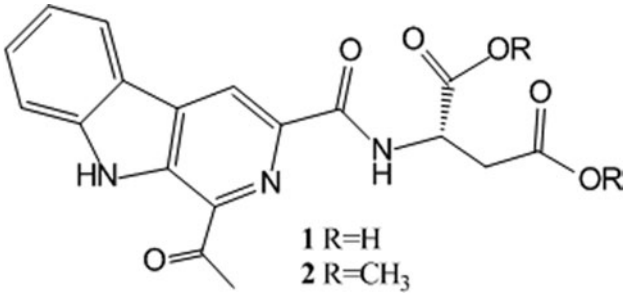

Phytochemical investigation of the roots of *Gypsophila oldhamiana* afforded two new β-carboline alkaloids, oldhamiaines A and B (**1** and **2**), along with a known analogue (**3**). Their structures were elucidated by using spectroscopic and chemical methods. This is the first report of β-carboline alkaloids in the genus *Gypsophila.*

Keywords: Caryophyllaceae; *Gypsophila oldhamiana*; β-carboline alkaloids

1. Introduction

The genus *Gypsophila* (Caryophyllaceae) comprises approximately 150 species that are widely distributed in Asia and Europe. Of these, more than 18 species grow in mainland China, especially in Xinjiang province (Lu 1994), and some of which have been used as pharmaceutical and ornamental plants for a long time (Nie et al. 2010). *Gypsophila oldhamiana* Miq., a perennial herbaceous plant, is mainly distributed in the north of China, and its roots have been used as a traditional folk medicine Shan-Yin-Chai-Hu for the treatment of fever, consumptive disease and infantile malnutrition syndrome (Jiansu College of New Medicine 1977). Previous phytochemical investigations on the roots of *G. oldhamiana* have led to the isolation of a series of bioactive natural products, including triterpenoid saponins (Luo & Kong 2006), sterols (Liu et al. 1995) and cyclic peptides (Wang et al. 2013). Therefore, as a continuation of our studies, two new β-carboline alkaloids (**1**–**2**), along with one known analogue (**3**), were obtained from the roots of this plant. Herein, we report the isolation and elucidation of the new β-carboline alkaloids.

2. Results and discussion

Compound **1** was obtained as a yellow powder, presumably being endowed with an *N* function on the basis of TLC examinations by using the Dragendorff's reagent. The molecular formula of

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1 was determined as $C_{18}H_{15}N_3O_6$ (13 unsaturation degrees) based on its HR-ESI-MS which showed an $[M - H]^-$ ion peak at *m/z* 368.0894 (calcd for 368.0888). The IR spectrum showed absorption bands at 3420, 1679 and 1669 cm^{-1} assignable to amino, carbonyl and amide functionalities. Absorption maxima in the UV spectrum of **1** were observed at 372, 285 and 219 nm, suggesting the presence of β-carboline chromophore (Kuo et al. 2003). In the ^1H NMR (DMSO-d_6) spectrum of **1**, resonances for four mutually coupled, aromatic protons at [δ_H 8.45 (1H, d, $J = 8.0$ Hz), 7.35 (1H, t, $J = 7.5$ Hz), 7.63 (1H, t, $J = 7.5$ Hz) and 7.84 (1H, d, $J = 8.0$ Hz)] were indicative of the occurrence of a disubstituted aromatic ring. In addition, a broad singlet NH signal at δ_H 12.19 (1H, br. s, D_2O exchangeable) and an aromatic singlet at δ_H 9.09 (1H, s) were also displayed. These signals were indicative of a β-carboline skeleton (Gözler & Shamma 1990; Sun et al. 2004).

The ^{13}C NMR (DMSO-d_6) spectrum of **1**, along with the information obtained from the HSQC experiment, showed 18 C-atom signals, of which 11 carbons were assigned to the β-carboline alkaloid skeleton. Furthermore, the following four carbon resonances (δ_C 49.1 and 39.9; 200.6 and 25.5), as well as the presence of proton signals [δ_H 4.47 (1H, br. m), 2.71 (1H, dd, $J = 8.0$, 16.0 Hz) and 2.80 (1H, dd, $J = 2.0$, 16.0 Hz); 2.88 (3H, s)] suggested the occurrence of a —$CHCH_2$— moiety and an acetyl group. In the HMBC spectrum, the correlations from Me-15 (δ_H 2.88) to C-1 (δ_C 133.9) and C-14 (δ_C 200.6) were observed, supporting the location of the acetyl group at C-1. The correlations of H-19 (δ_H 2.68–2.82) with C-18 (δ_C 49.1), C-21 (δ_C 172.7) and C-20 (δ_C 173.4), as well as H-17 (δ_H 9.12) with C-18 (δ_C 49.1) and C-16 (δ_C 163.1) led to the construction of the fragment CONHCH(COOH) CH_2COOH. This side chain was located at C-3 as deduced from the ^3J correlations of H-4 (δ_H 9.09) and C-16 (δ_C 163.1) (Figure S7). Acid hydrolysis of **1** liberated L-aspartic acid, which was identified by comparing the retention time and optical rotation with the standard sample. Thus, the absolute configuration at C-18 was determined as *S* (Matsumura et al. 1984) and compound **1** was formulated as 1-acetyl-16-aspartyl-β-carboline-3-carboxylate named as oldhamiaine A (Figure 1).

Compound **2**, purified as a yellow amorphous solid, was also characterised as a β-carboline derivative, and the molecular formula was established as $C_{20}H_{19}N_3O_6$ from HR-ESI-MS. The ^1H and ^{13}C NMR spectra displayed signals very similar to those of **1**, with the major differences being on the presence of two additional methoxyl signals at δ_H 3.69, 3.64 (3H, s, each). The HMBC ^3J-correlations of H-22 (δ_H 3.69) and C-20 (δ_C 171.2), as well as H-23 (δ_H 3.64) and C-21 (δ_C 171.1) indicated these two methoxyls were esterified with the C-20, C-21, respectively. Similar to **1**, compound **2** displayed a positive optical rotation, indicating that the absolute configuration of C-18 was also *S*. On the basis of the above-mentioned results, compound **2** was identified as 1-acetyl-20,21-dimethoxy-16-aspartyl-β-carboline-3-carboxylate, named as oldhamiaine B. To verify that **2** is an authentic natural product, compound **1** was treated with MeOH by simulating isolation situation, and compound **2** was not detected in the supernatant using HPLC analysis (Supporting Information Figures S13–S15). This result indicated that **2** is indeed a naturally occurring metabolite.

Compound **3** was identified as 1-acetyl-3-methoxycarbonyl-β-carboline by comparing its spectroscopic data with the literature (Faini et al. 1978).

This is the first report of β-carboline-type alkaloids isolated from the genus *Gypsophila*, which adds to the number of reports regarding the absence of alkaloids in some species of the Caryophyllaceae family (Chen et al. 2010; Tian et al. 2012).

The cytotoxicity of compounds **1**–**3** against three human cancer cell lines MCF-7 (human breast cancer), HepG-2 (human hepatoma cancer) and U2OS (human osteosarcoma cancer) were tested using the methyl thiazol tetrazolium method (Lu et al. 2009). However, the result showed that these compounds exhibited no cytotoxic activities ($IC_{50} > 100$ μM).

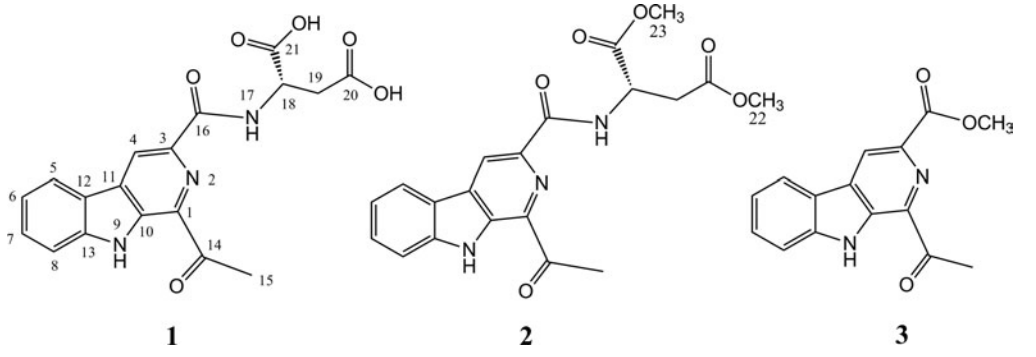

Figure 1. The structures of compounds **1**–**3**.

3. Experimental

3.1. *General experimental procedures*

Optical rotations were measured with a JASCO P-1020 polarimeter (Jasco, Tokyo, Japan). UV spectra were recorded using a UV-2450 UV–visible spectrophotometer (Shimadzu, Tokyo, Japan). IR spectra (KBr disks) were recorded on a Bruker Tensor 27 spectrometer (Bruker, Karlsruhe, Germany). NMR spectra were measured on a Bruker AV-500 NMR instrument (^{1}H: 500 MHz, ^{13}C: 125 MHz, Bruker), with tetramethylsilane as internal standard. HR-ESI-MS were measured with an Agilent 6520B Q-TOF mass instrument (Agilent Technologies, Santa Clara, CA, USA). Extracts were chromatographed on silica gel (Qingdao Marine Chemical Co., Ltd, Qingdao, China), octadecyl silane (40–63 μM, Fuji, Tokyo, Japan), Sephadex LH-20 (Pharmacia, Uppsala, Sweden) and purified on an Agilent 1100 Series preparative HPLC system (Agilent Technologies, Santa Clara, CA, USA).

3.2. *Plant material*

The roots of *G. oldhamiana* were collected in Lianyungang City, Jiangsu Province, China, and identified by Prof. Minjian Qin, the Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing, China. A voucher specimen (No. 20110616) has been deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

3.3. *Extraction and isolation*

The air-dried roots of *G. oldhamiana* (2.0 kg) were refluxed with 80% EtOH three times (3 × 10 L). The solution was concentrated, and the obtained crude extract (202.0 g) was suspended in H_2O and successively partitioned with petroleum ether, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH). The EtOAc portion (total 10.0 g) was fractioned on a silica gel column with a gradient of $CHCl_3$–MeOH (10:1, 5:1, 5:3, 1:1, v/v) to afford four fractions (A–D). Subsequently, fraction B (2.6 g) was further separated by Sephadex LH-20 column (MeOH) to give three subfractions (B1–B3). Subfraction B2 (620.0 mg) was subjected to an RP-C_{18} CC with MeOH–H_2O (60:40 to 80:20, v/v) to yield three major subfractions B2a–B2c. Finally, fraction B2c was further purified by preparative HPLC (MeOH–H_2O–trifluoroacetic acid (TFA), 60:40:0.1, 10 mL/min), to yield **1** (5.0 mg). Subfraction B3 (580.0 mg) was performed on RP-C_{18} column eluted with a step gradient of MeOH–H_2O (50:50 to 90:10), and then purified by preparative HPLC (MeOH–H_2O–TFA, 60:40:0.1, 10 mL/min) to afford **2** (3.0 mg) and **3** (2.0 mg).

3.4. *Oldhamiaine A (1)*

Yellow powder; $[\alpha]_D^{25}$ + 14.0 (c = 0.02, MeOH); UV (MeOH) λ_{max} (log ε): 376 (3.45), 286 (4.29), 220 (4.20) nm; IR (KBr) ν_{max}: 3420, 2919, 2851, 1679, 1669, 1593, 1385, 1205, 1140, 1049, 841, 803, 724 cm^{-1}; ^1H NMR (500 MHz, DMSO- d_6): δ 9.09 (1H, s, H-4), 8.45 (1H, d, J = 8.0 Hz, H-5), 7.35 (1H, t, J = 7.5 Hz, H-6), 7.63 (1H, t, J = 7.5 Hz, H-7), 7.84 (1H, d, J = 8.0 Hz, H-8), 12.19 (1H, br. s, H-9), 2.88 (3H, s, H-15), 9.12 (1H, d, J = 7.0 Hz, H-17), 4.47 (1H, br. m, H-18), 2.71 (1H, dd, J = 8.0, 16.0 Hz, Ha-19), 2.80 (1H, dd, J = 2.0, 16.0 Hz, Hb-19). ^{13}C NMR (125 MHz, DMSO- d_6): δ 133.9 (C-1), 138.3 (C-3), 117.7 (C-4), 122.2 (C-5), 120.8 (C-6), 129.3 (C-7), 113.3 (C-8), 134.9 (C-10), 132.0 (C-11), 120.3 (C-12), 142.4 (C-13), 200.6 (C-14), 25.5 (C-15), 163.1 (C-16), 49.1 (C-18), 39.9 (C-19), 172.7 (C-20), 173.4 (C-21); ESI-MS: m/z 368 $[M - H]^-$, 251 $[M - H - C_4H_5O_4]^-$, 165 $[M - H - C_7H_9O_6N]^-$; HR-ESI-MS: m/z 368.0894 $[M - H]^-$ (calcd for $C_{18}H_{14}N_3O_6$, 368.0888).

3.4.1 *Acid hydrolysis of **1***

A mixture of compound **1** (2.0 mg), 6 M HCl (1 mL) and dimethyl sulfoxide (DMSO, 1 mL) was heated at 80°C for 4 h, and then poured into ice water. The reaction mixture was extracted with $CHCl_3$ and the acid aqueous layer was neutralised with $AgNO_3$ to obtain an AgCl precipitate. After filtering, the aqueous mixture was concentrated under reduced pressure to dryness. The residue was purified by preparative HPLC (MeOH–H_2O, 40:60) to yield a white powder, which was identified to be L-aspartic acid by comparing the retention time on HPLC (MeOH–H_2O, 40:60, t_R = 6.9 min) and optical rotation dispersion ($[\alpha]_D^{25}$ + 13.1 (c = 1.0, MeOH)) with an authentic sample.

3.5. *Oldhamiaine A (2)*

Yellow amorphous solid; $[\alpha]_D^{25}$ + 8.2 (c = 0.02, MeOH); UV (MeOH) λ_{max} (log ε): 375 (3.25), 286 (4.10), 205 (4.38) nm; IR (KBr) ν_{max}: 3483, 2952, 1735, 1702, 1681, 1533, 1450, 1201, 964, 794 cm^{-1}; ^1H NMR (500 MHz, DMSO- d_6): δ 9.11 (1H, s, H-4), 8.46 (1H, d, J = 8.0 Hz, H-5), 7.35 (1H, t, J = 7.8 Hz, H-6), 7.64 (1H, t, J = 7.8 Hz, H-7), 7.85 (1H, d, J = 8.0 Hz, H-8), 12.20 (1H, br. s, H-9), 2.91 (3H, s, H-15), 9.17 (1H, d, J = 8.0 Hz, H-17), 5.07 (1H, dt, J = 6.0, 8.0 Hz, H-18), 2.99 (1H, dd, J = 6.0, 16.5 Hz, Ha-19), 3.09 (1H, dd, J = 6.0, 16.5 Hz, Hb-19), 3.69 (3H, s, H-22), 3.64 (3H, s, H-23). ^{13}C NMR (125 MHz, DMSO- d_6): δ 133.9 (C-1), 137.6 (C-3), 118.2 (C-4), 122.3 (C-5), 120.9 (C-6), 129.4 (C-7), 113.3 (C-8), 135.0 (C-10), 131.9 (C-11), 120.3 (C-12), 142.3 (C-13), 200.7 (C-14), 25.7 (C-15), 163.9 (C-16), 48.6 (C-18), 35.8 (C-19), 171.2 (C-20), 171.1 (C-21), 52.4 (C-22), 51.7 (C-23); ESI-MS: m/z 396 $[M - H]^-$, 353 $[M - H - C_2H_3O]^-$; HR-ESI-MS: m/z 420.1169 $[M + Na]^+$ (calcd for $C_{20}H_{19}N_3O_6Na$, 420.1196).

3.5.1 *Simulating reaction between **1** and methanol in the isolation situation*

A portion of compound **1** (0.5 mg) in MeOH (2 mL) was mixed with 1 g of silica gel in water bath at 50°C for 12 h. Then, the supernatant was collected and subjected to HPLC analysis, and compound **2** was not detected.

4. Conclusions

In this article, two new β-carboline alkaloids, named oldhamiaines A and B (**1** and **2**), together with a known analogue, were isolated from the roots of *G. oldhamiana.* The structure of oldhamiaines A and B were identified as 1-acetyl-16-aspartyl-β-carboline-3-carboxylate, 1-

acetyl-20,21-dimethoxy-16-aspartyl-β-carboline-3-carboxylate, respectively, by 1D, 2D NMR and MS spectra. This is the first report on the isolation of β-carboline-type alkaloids from the genus *Gypsophila*.

Supplementary material

Supplementary material relating to this article is available online, alongside Figures S1–S15.

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