A new isocoumarin from the aerial parts of *Aconitum gymnandrum*

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ABSTRACT

A new isocoumarin, along with 10 known compounds, was isolated from the aerial parts of *Aconitum gymnandrum*. Their structures were elucidated by spectroscopic methods including extensive 1D and 2D NMR techniques. Among the known compounds, compound 11 was obtained as a natural product for the first time, which was previously reported as a synthetic product. In addition, compounds 1–5, 7 and 9 were tested for their cytotoxicity against four human cancer cell lines. The results showed that compounds 3, 4 and 7 displayed cytotoxicity against lung cancer A549 and gastric cancer MGC80, respectively, whereas 5 and 9 showed selective cytotoxicity against hepatocellular carcinoma HepG2.

1. Introduction

*Aconitum* (Ranunculaceae) is a large resource of medicinal plants. About 36 kinds of *Aconitum* species have been used for treating rheumatism and analgesia in China (Fu 2004). *Aconitum gymnandrum* is the sole representative of the monotypic subgenus *Gymnacoitum* (Stapf.) Rapaics (Wang et al. 2009). This species is widely distributed in alpine meadows and hillsides.
along the edges of the Qinghai-Tibetan Plateau as well as on the Qinghai-Tibetan platform itself. It differs from other congeneric species by being pollinated by both insects and wind to adapt to the arid habitats (Duan et al. 2010). The aerial parts of *A. gymnandrum* are used as a traditional Tibetan medicine to treat rheumatic numbness and joint pain. The water extracts of stems and leaves of *A. gymnandrum* exhibited anti-inflammatory activities (Fu et al. 2005). *A. gymnandrum* is also a kind of poisonous plant in natural grassland, and it’s reported that the methanol extracts of *A. gymnandrum* had strong anti-feeding effects against larva of *Pieris rapae* (Hu et al. 2011). Phytochemical investigation of this species was rare and previous research revealed that diterpenoid alkaloids were the major components of *Aconitum* plants (Wu & Zhu 1984; Jiang et al. 1986; Ding et al. 1993; Atta-ur-Rahman & Choudhary 1999; Guo et al. 2014; Yin et al. 2014). As a part of our research for new bioactive secondary metabolites from the Tibetan medicine, our group conducted the phytochemical research of this species. This led to the isolation of a new isocoumarin, 6,7-dimethoxy-8-hydroxy-3-[β-(p-hydroxyphenyl)ethyl]-3,4-dihydroiso-coumarin (1), together with 10 known compounds (2–11) (Figure 1). In addition, the cytotoxicity of the compounds 1–5, 7 and 9 against four human cancer cell lines was evaluated. Herein, we report the isolation, structural elucidation and cytotoxicity of these compounds.

### 2. Results and discussion

Repeated column chromatography (CC) over silica gel and semi-preparative HPLC of the ethanol extracts from the aerial parts of *A. gymnandrum*, led to the isolation of a new isocoumarin, named 6,7-dimethoxy-8-hydroxy-3-[β-(p-hydroxyphenyl)ethyl]-3,4-di-hydroisocoumarin (1), together with 10 known compounds (2–11) (Figure 1).

![Chemical structures of compounds 1–11.](image)
Compound 1 was obtained as a white amorphous powder. The molecular formula of compound 1 was established as C_{19}H_{20}O_{6} on the basis of the HRESIMS [M + H]^+ ion at m/z 345.1336 (Calcd for C_{19}H_{21}O_{6} 345.1333) and NMR data (Table S1). The IR spectrum of 1 showed absorptions due to the hydroxyl (3419 cm\(^{-1}\)) group, alkyl (2985 and 2931 cm\(^{-1}\)) group and benzene ring (1658, 1513, and 1457 cm\(^{-1}\)) functionalities. All 19 carbon resonances were well resolved in the \(^{13}\)C NMR (Table S1) and HSQC spectra and assigned as one carbonyl (δ\(_C\) 171.8), 12 aromatic carbons (δ\(_C\) 102–160), one oxymethyne (δ\(_C\) 80.3), two methoxyl moieties (δ\(_C\) 56.7 and 56.0) and three methylenes (δ\(_C\) 33.6, 37.8 and 31.2). In \(^{1}\)H NMR spectrum, one singlet aromatic proton at δ\(_H\) 6.28 (1H, s) and a set of AB coupled signals [δ\(_H\) 7.14 (2H, d, J = 8.7 Hz, H-2′ and H-6′), 6.83 (2H, d, J = 8.7 Hz, H-3′ and H-5′)] arising from an 1,4-disubstituted benzene ring were observed. In addition, characteristic proton signals due to an oxymethyne [δ\(_H\) 4.47, (dt, 13.0, 8.3)], and two methoxy groups [δ\(_H\) 3.75, 3.82 (each 3H, s)] were displayed in \(^{1}\)H NMR spectrum. The aforementioned data of 1 were closely related to those of the aglycone of a known isocoumarin isolated from Agrimonia pilosa, agrimono-lide-6-β-O-D-glycoside (Pei et al. 1989), suggesting that 1 could be an isocoumarin. The major difference was the presence of an additional methoxy in 1, related to agrimonolide (Yamato & Hashigaki 1976). The structure of 1 was further accomplished on the basis of HSQC, HMBC and \(^{1}\)H–\(^{1}\)H COSY experiments. \(^{1}\)H–\(^{1}\)H COSY spectrum of 1 (Figure S1) revealed the presence of a 2-butanol moiety (C4-C3-Cα-Cβ). In the HMBC spectrum of 1 (Figure S1), correlations of anomeric protons at δ\(_H\) 7.14 (H-2′ and H-6′) with C-β (δ\(_C\) 31.2) and C-4′ (δ\(_C\) 159.7) confirmed the linkage β-(p-hydroxyphenyl) ethyl moiety at C-3 of the isocoumarin. Subsequently, HMBC correlation of the proton at δ\(_H\) 2.82 (H-4) with C-5 (δ\(_C\) 107.9), anomeric proton at δ\(_H\) 6.28 (H-5) with C-7 (δ\(_C\) 135.2), and methoxys at δ\(_H\) 3.75 and 3.82 with C-6 (δ\(_C\) 158.4) and C-7 (δ\(_C\) 135.2), respectively, indicated the location of the methoxys at C-6 and C-7. On the basis of these spectroscopic data, a planar structure of 1 was proposed and named 6,7-dimethoxy-8-hydroxy-3-[β-(p-hydroxyphenyl)ethyl]-3,4-dihydroisocoumarin. And the absolute configuration of 1 was determined through the comparison of optical rotation with the known compound. The \(^{3}\)S-isocoumarin exhibit negative optical rotation (Kato et al. 2010) and compound 1 was levorotatory ([α]\(^{25}\)D -22.5°) suggesting that 1 has the absolute configuration \(^{3}\)S.

The known compounds were identified as benzoylmesaconine (2) (Jiang et al. 2012), mesaconitine (3) (Ye et al. 2013), benzoyllypcaconine (4) (Ye et al. 2013), (+)-1-hydroxy-pinoresinol (5) (Liu et al. 2013), (6R,7E,9R)-9-hydroxy-4,7megastigma-dien-3-one (6) (D’Abrosca et al. 2004), ferulic acid (7) (Kim et al. 2015), dihydroconiferyl alcohol (8) (Zhang et al. 2006), 4-hydroxybenzoic acid (9) (Kim et al. 2015), vanillic acid (10) (Kim et al. 2015), 2,3-dimethoxybenzoic acid (11) (Kuo et al. 1996) (Figure 1), by comparison of their NMR spectroscopic data with those reported in the literature.

Compounds 1–5, 7 and 9 were evaluated for their cytotoxicity against four human cancer cell lines (gastric cancer MGC80, liver cancer HepG2, lung cancer A549 cells and colon cancer Hct116). The results showed that compounds 3 displayed selective cytotoxicity against lung cancer A549 with IC\(_{50}\) values of 35.31 μg mL\(^{-1}\), and compounds 4 and 7 displayed selective cytotoxicity against gastric cancer MGC80 with IC\(_{50}\) values of 24.7 and 38.1 μg mL\(^{-1}\), respectively, while 5 and 9 showed selective cytotoxicity against liver cancer HepG2 with IC\(_{50}\) values of 48.70 and 27.40 μg mL\(^{-1}\), respectively. Compounds 1 and 2 showed no cytotoxicity against any of the four human cancer cell lines at a concentration of 50 μg mL\(^{-1}\).
3. Experimental

3.1. General

Optical rotations were measured with a HORIBA SEPA300 high sensitive polarimeter. IR spectra were recorded on a Bio Rad FTS 135 series spectrometer in dry film. UV spectra were recorded on a Shimadzu UV2401A UV–Vis spectrophotometer. ESI MS and HRESI-MS were run on an API QSTAR Pular-1 spectrometer. NMR spectra measured in CDCl₃ or CD₃OD solution and recorded on a Bruker Avance III-600 spectrometer at 25 °C, using TMS as an internal standard. Chemical shifts were reported in units of δ (ppm) and coupling constants (J) were expressed in Hz. CC were carried out over silica gel (200–300 mesh, Qingdao Haiyang Chemical Co.) and Rp-18 (40–63 μm, Merck). Pre-coated silica gel plates (Qingdao Haiyang Chemical Co.) were used for TLC. Detection was done under UV light (254 nm and 365 nm) and by spraying the plates with 10% sulphuric acid followed by heating. An Agilent series 1200 (Agilent Technologies) were used for HPLC. A Hanbon preparative HPLC (Hanbon Sci & Tech, Jiangsu) and C18-HC column 10 μm 100 Å (250 mm × 20 mm, Acchrom, Beijing, China) was used for semi-preparative HPLC separations.

3.2. Plant material

The aerial parts of A. gymnamdru were collected in Qi-lian, Qing-hai Province, People’s Republic of China, in August 2013. The identification of the plant material was verified by professor Li-juan Mei (Northwest Institute of Plateau Biology, Chinese Academy of Science). A voucher specimen (No. 0295343) has been deposited in the Key Laboratory of Tibetan Medicine Research.

3.3. Extraction and isolation

The air-dried and powdered aerial parts of A. gymnamdru (5 kg) were extracted four times with 95% ethanol aqueous (3t, 3 h each time) under reflux at 60 °C. Evaporation of the solvent (under vacuum, rt) gave a residue (440 g), which was suspended in water. 2% hydrochloric acid was added to adjust the pH of the solution to 3.0, and then filtered the insolubles. The pH of the filtrate was adjusted to 10.0, and then the filtrate was extracted with chloroform to give the chloroform fraction (56 g). The chloroform fraction was submitted to silica gel (200–300 mesh) CC, eluting with a CHCl₃–Me₂CO gradient system (20:1, 9:1, 8:2, 7:3, 6:4, 5:5), to give six fractions A-F. Fraction B (9:1, 22 g) was subjected to silica gel CC, eluted with petroleum ether-EtOAc (9:1, 8:2, 7:3, 6:4, 1:1), yielded mixtures B1-B5. Fraction B2 (8:2, 2.1 g) was subjected to semi-preparative HPLC (50% MeOH, flow rate 15 mL/min) to give 4 (10.1 mg), 5 (6.2 mg) and 6 (12.5 mg). Fraction B3 (7:3, 3.6 g) was further chromatographed over Rp-18 column to yield mixtures B31-B33. Fraction B31 was subjected to semi-preparative HPLC (25% MeCN, flow rate 15 mL/min) to give 1 (2.8 mg), 2 (3.2 mg) and 3 (2.9 mg). Fraction B4 (6:4, 2.8 g) was further chromatographed over semi-preparative HPLC (10–60% MeCN, flow rate 15 mL/min) to yield B41-B43. Fraction B42 was subjected to semi-preparative HPLC (20–30% MeCN, flow rate 15 mL/min) to give 7 (2.1 mg), 8 (5.2 mg) and 9 (7.9 mg). 10 (1.7 mg) and 11 (4.3 mg) were isolated from fraction B43 with the same method.
3.4. **36,7-dimethoxy-8-hydroxy-3-[β-(p-hydroxyphenyl)ethyl]-3,4-dihydroisocoumarin (1)**

White powder; [α]_D^{25} = -22.5° (c 0.07, CH₃OH); IR (KBr) v_max (cm⁻¹): 3419, 2931, 1658, 1513, 1457, 1376, 1287, 1232, 1174 and 1097; UV (MeOH) λ_max nm (log ε): 201 (4.39), 222 (4.41), 274 (4.09), 304 (3.74); \(^1\)H NMR (600 MHz, CD₃OD) δ, ppm: 4.47 (1H, dt, J = 13.0, 8.3 Hz, H-3), 2.82 (2H, m, H-4), 6.28 (1H, s, H-6), 2.08 (1H, m, H-α), 1.97 (1H, m, H-α), 2.81 (1H, m, H-β), 2.73 (1H, ddd, J = 13.9, 9.3, 7.1 Hz, H-β), 7.14 (2H, d, J = 8.7 Hz, H-2', 6'), 6.83 (2H, d, J = 8.7 Hz, H-3', 5'), 3.75 (3H, s, OCH₃-6), 3.82 (3H, s, OCH₃-7); \(^{13}\)C NMR (150 MHz, CD₃OD) δ, ppm: 171.8 s (C-1), 80.3 d (C-3), 33.6 t (C-4), 107.9 d (C-5), 158.4 s (C-6), 135.2 s (C-7), 157.6 s (C-8), 102.3 s (C-9), 137.5 s (C-10), 37.8 t (C-α), 31.2 t (C-β), 134.4 s (C-1'), 130.5 d (C-2', 6'), 115.1 d (C-3', 5'), 159.7 s (C-4'), 55.8 q (OCH₃-6), 61.0 q (OCH₃-7); HR-ESI-MS: m/z 345.1336 [M + H]^+ (Calcd 345.1333).

3.5. **In vitro cytotoxic activity**

Four human cancer cell lines, gastric cancer MGC80, liver cancer HepG2, lung cancer A549 and colon cancer Hct116 were used in the cytotoxic assay. All the cells were cultured in RPMI-1640 or DMEM medium (HyClone, USA), supplemented with 10% fetal bovine serum (HyClone, USA). The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method in 96-well microplates. (Mosmann 1983) Briefly, adherent cells (100 μL) were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with an initial density of 0.5 × 10⁵–1 × 10⁵ cells/mL. Each tumour cell line was exposed to the test compound dissolved in DMSO in triplicates for 48 h at 37 °C with doxorubicin as positive control. Then, MTT (50 μL) was added to each well, and the tumour cells were incubated for another 4 h at 37 °C. After the supernatant liquor was removed, SDS (200 μL) was added to each well. The optical density was measured at 595 nm on a microplate reader. Cell viability was detected and a cell growth curve was graphed. IC₅₀ values were calculated by Reed and Muench's method. (Reed & Muench 1938).

4. **Conclusions**

In this study, a new isocoumarin together with 10 known compounds was isolated from the aerial parts of *A. gymnandrum*. Among the known compounds, compound 11 was firstly reported as a natural product, which was previously reported as a synthetic product. Herein, the structure elucidation of the new compound was reported. Furthermore, the cytotoxic activity of compounds 1–5, 7 and 9 was tested for their cytotoxicity against four human cancer cell lines (MGC80, HCT116, A549 and HepG2) by the MTT assay. The results showed that compounds 3, 4 and 7 displayed cytotoxicity against lung cancer A549 and gastric cancer MGC80, respectively, whereas 5 and 9 showed cytotoxicity against hepatocellular carcinoma HepG2.

**Supplementary material**

Experimental details relating to this article are available online, alongside Table S1 and Figures S1-S7.
Disclosure statement
No potential conflict of interest was reported by the authors.

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