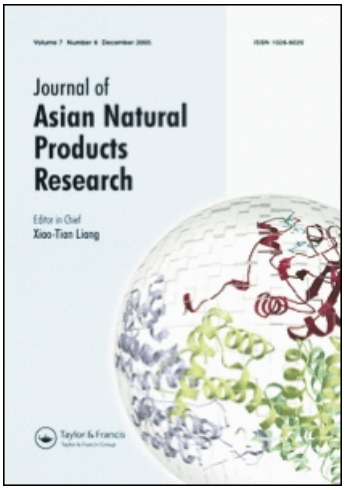

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Two new homoisoflavonoids from the fibrous roots of *Ophiopogon japonicus* (Thunb.) Ker-Gawl

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Two new homoisoflavonoids ophiopogonone D (**1**) and ophiopogonanone G (**2**) were isolated from the fibrous roots of *Ophiopogon japonicus*. The structures of these two compounds were determined on the basis of spectroscopic means including HR-ESI-MS, 1D, and 2D NMR experiments. The cytotoxic activities of **1** and **2** against Hela and Hep2 cells are described.

Keywords: *Ophiopogon japonicus* (Thunb.) Ker-Gawl; homoisoflavonoids; cytotoxicity; Liliaceae

1. Introduction

Ophiopogon japonicus (Thunb.) Ker-Gawl (known as Maidong in China) is an evergreen perennial, widely distributed in mainland China, especially in Sichuan and Zhejiang Provinces. It has often been used in traditional Chinese medicine and folk medicine of Vietnam as an expectorant, antitussive, and tonic agent. Its tubers were used for the treatments of cardiovascular and cerebral vascular diseases in combination with *Panax ginseng* and *Schisandra chinensis* clinically [1,2]. Previous phytochemical studies of *Ophiopogonis* tubers resulted in the isolation and structural elucidation of steroids [3–6], homoisoflavonoids [7,8], amides [9], as well as monoterpenoids [10,11]. Our phytochemical investigation on the fibrous roots of *O. japonicus* led to the isolation of two new homoisoflavonoids ophiopogonone D (**1**) and ophiopogonanone G (**2**). In this paper, we report the isolation and structural elucidation of the new compounds. The cytotoxic activities of them against Hela and Hep2 cells are also described.

2. Results and discussion

Compound **1** was obtained as a brown powder, which exhibited a blue fluorescence under UV light at 365 nm. The molecular formula was deduced to be $C_{17}H_{14}O_6$ based on a molecular ion peak at *m/z* 315.0864 $[M+H]^+$ in its HR-ESI-MS spectrum. The IR spectrum showed absorption bands at 3352, 1640 (C=O), and 1380 (CH_3) cm^{-1}, and the UV spectrum exhibited absorption maxima at 267, 301, and 332 nm, which were similar to those of ophiopogonone derivatives [7,12].

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The ^{1}H NMR spectrum revealed the presence of an aromatic methyl at δ 1.96 (3H, s), a benzylmethylene at δ_H 3.74 (2H, s), aromatic ABX-type protons at δ_H 6.84 (1H, d, $J = 8.0$ Hz), 6.13 (1H, dd, $J = 8.0$, 2.0 Hz), and 6.28 (1H, d, $J = 2.0$ Hz), an aromatic proton at δ_H 6.37 (1H, s), an olefinic proton at δ_H 7.82 (1H, s), and a hydroxyl proton at δ_H 13.04 (1H, s).

The proton signal at δ_H 13.04 (1H, s) indicated the presence of a chelated hydroxyl group assignable to C-5. The location of the methyl at δ_H 1.96 (3H, s) was concluded to be at C-6 according to HMBC correlations as shown in Figure 1. The aromatic proton at δ_H 6.37 (1H, s) was assigned to H-8 because it showed HMBC correlations with C-10 and C-11. The carbon signal at δ_C 164.8 was assigned to C-7 by its HMBC correlation with H-8. From the chemical shift of C-7, it was deduced to be substituted with a hydroxyl group at this position. The substitution style of B ring was determined to be 2′,4′-dihydroxy by HMBC correlations as shown in Figure 1. The proton signal at δ_H 3.74 (2H, s) was assigned to H-9 for its HMBC correlations with C-2, 3, 4, and C-1′, 2′. Thus, **1** was established to be 5,7-dihydroxy-6-methyl-3-(2,4-dihydroxybenzyl)-chromone, a new compound named ophiopogonone D.

Compound **2** was obtained as a brown powder, $[\alpha]_D^{20}$ -15.3 ($c = 0.1$, MeOH). The IR spectrum of **2** exhibited absorption bands at 3405, 1638 (C=O), and 1620 cm^{-1}. The UV spectrum of **2** showed absorption maxima at 296 and 350 nm. The molecular formula of **2** was deduced as $C_{17}H_{16}O_6$ based on a molecular ion peak at *m/z* 317.1021 $[M+H]^+$ in its HR-ESI-MS spectrum. The ^{1}H NMR spectrum revealed the presence of an aromatic methyl group at δ_H 1.85 (3H, s), aromatic ABX-type protons at δ_H 6.80 (1H, d, $J = 8.0$ Hz), 6.15 (1H, dd, $J = 8.0$, 2.0 Hz), and 6.30 (1H, d, $J = 2.0$ Hz), an aromatic proton at δ_H 5.86 (1H, s), and a hydroxyl proton at δ_H 12.50 (1H, s). These signals were very similar to those of compound **1** except for the presence of several signals at δ_H 2.99 (1H, dd, $J = 13.5$, 5.0 Hz), 2.85 (1H, m), 2.45 (1H, dd, $J = 13.5$, 10.0 Hz), 4.16 (1H, dd, $J = 11.2$, 2.6 Hz), and 4.00 (1H, dd, $J = 11.2$, 7.3 Hz) and the lack of an olefinic proton. The signal at δ_C 7.1 (C-12) further indicated that compound **2** was an ophiopogonanone derivative [7,8,12]. The absolute configuration (*R*) of **2** at C-3 was determined by comparing its CD spectral data with those in Refs [13,14]. There were positive Cotton effects at 257 and 320 nm and a negative Cotton effect at 297 nm. The final structure of **2** was determined to be 5,7-dihydroxy-6-methyl-3(*R*)-(2,4-dihydroxybenzyl)chroman-4-one by HMQC and HMBC analyses (Figure 2), a new compound named ophiopogonanone G.

These two compounds were tested for *in vitro* cytotoxicity against Hela and Hep2

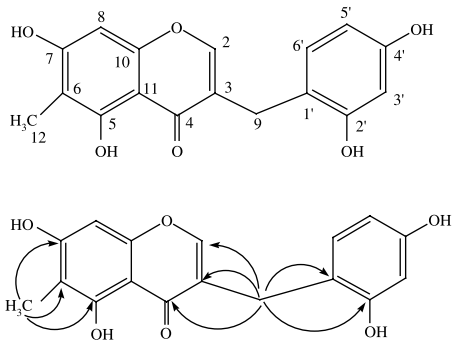

Figure 1. Structure and key HMBC correlations of compound **1**.

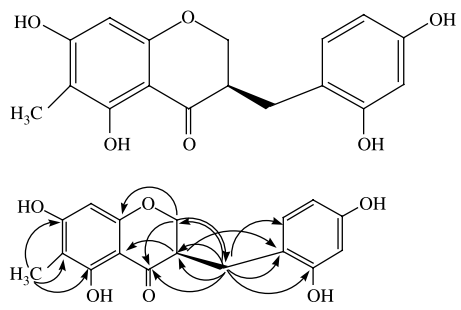

Figure 2. Structure and key HMBC correlations of compound **2**.

Table 1. Cytotoxicities of **1** and **2** against Hela and Hep2 cells (IC_{50}, μg/ml).

Compound	Hela	Hep2
1	20.87	21.29
2	108.9	294.6

cells. The IC_{50} values of **1** and **2** are listed in Table 1. All of them showed weak cytotoxicity ($IC_{50} > 3$ μg/ml) against Hela and Hep2 cells.

3. Experimental

3.1 General experimental procedures

UV spectra were obtained from an Agilent 8453 UV–vis spectrometer. NMR spectra were recorded on a JNM-ECA 400 MHz spectrometer in DMSO-d_6, δ in ppm and J in Hz. IR spectra were obtained from a Hitachi EPI-2 spectrometer. The HR-EI-MS spectra were recorded on a Bruker APEX IV FT-MS (7.0 T) mass spectrometer. The CD spectra were recorded on a Jasco J-815 spectrometer. HPLC was carried out on Agilent 1100 with a DAD detector using Zorbax XDB-C_{18} column (9.4 × 250 mm). Silica gel (200–300 mesh; Qingdao Marine Chemical Group, Qingdao, China) and Sephadex LH-20 (Amersham Biosciences AB, Uppsala, Sweden) were used for column chromatography. Fractions were monitored by TLC (pre-coated silica gel GF254 plates made by Qingdao Marine Chemical Group).

3.2 Plant material

The fibrous roots of *O. japonicus* (Thunb.) Ker-Gawl were purchased from Anguo Market, Hebei Province, China, in September 2005 and identified by one of the authors (Prof. P.-F. Tu). A voucher specimen (MD20050906) has been deposited in the Herbarium of Peking University Modern Research Center for Traditional Chinese Medicine.

3.3 Extraction and isolation

The fibrous roots of *O. japonicus* (15 kg) were extracted with EtOH (75%) under reflux and then filtered by gauze. The EtOH extract was concentrated by reduced pressure rotary evaporation and the residue was suspended in H_2O and extracted with petroleum ether, EtOAc, and *n*-BuOH successively. The EtOAc-soluble fraction was evaporated and the residue (80.0 g) was separated into eight fractions by column chromatography on silica gel, eluted gradiently with petroleum ether–acetone (1:0, 100:1, 50:1, 30:1, 15:1, 10:1, 5:1, 2:1, 0:1). Fraction 4 (14.5 g) was subjected to column chromatography on polyamide eluted with MeOH–H_2O (0:10, 1:9, 2:8, 3:7, 5:5, 10:0) to afford six fractions. Fraction 5 (1.8 g) was purified on Sephadex LH-20 with MeOH as the eluent and repeated HPLC (ODS column, 5 μM, 9.4 × 250 mm, flow rate 2.0 ml/min, UV 254 nm) eluted with MeOH–H_2O (7:3) to afford **1** (3 mg, 15.4 min) and **2** (5 mg, 19.1 min).

3.3.1 Ophiopogonone D (*1*)

A brown powder; a blue fluorescence under UV light at 365 nm. UV λ_{max} (MeOH): 267, 301, 332 nm. IR (KBr) ν_{max}: 3352, 1640, 1380 cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz): δ_H 7.82 (1H, s, H-2), 6.37 (1H, s, H-8), 3.47 (2H, s, H-9), 1.96 (3H, s, H-12), 6.28 (1H, d, $J = 2.0$ Hz, H-3′), 6.13 (1H, dd, $J = 8.0$, 2.0 Hz, H-5′), 6.84 (1H, d, $J = 8.0$ Hz, H-6′), 13.04 (1H, s, 5-OH). ^{13}C NMR (100 MHz) spectral data, see Table 2. HR-ESI-MS m/z: 315.0864 $[M+H]^+$ (calcd for $C_{17}H_{15}O_6$, 315.0869).

3.3.2 Ophiopogonanone G (*2*)

A brown powder, $[\alpha]_D^{20}$ −15.3 ($c = 0.1$, MeOH). IR (KBr) ν_{max}: 3405, 1638 (C═O), 1620 cm^{-1}. UV λ_{max} (MeOH): 296, 350 nm. ^1H NMR (DMSO-d_6, 400 MHz): δ_H 4.14 (1H, dd, $J = 11.2$, 2.6 Hz, H-2a), 4.00 (1H, dd, $J = 11.2$,

Table 2. ^{13}C NMR spectral data of **1** and **2** in DMSO-d_6 (100 MHz).

Position	1	2	Position	1	2
2	153.8	68.8	1′	115.0	114.6
3	121.0	44.3	2′	156.8	156.9
4	180.8	197.2	3′	102.4	102.4
5	158.4	160.9	4′	155.5	156.1
6	106.8	103.4	5′	106.0	106.0
7	164.8	164.0	6′	130.4	131.1
8	92.7	94.4			
9	23.9	26.8			
10	155.8	160.2			
11	103.5	100.1			
12	7.4	7.1			

7.3 Hz, H-2b), 2.85 (1H, m, H-3), 5.86 (1H, s, H-8), 2.99 (1H, dd, $J = 13.5$, 5.0 Hz, H-9a), 2.45 (1H, dd, $J = 13.5$, 10.0 Hz, H-9b), 1.85 (3H, s, H-12), 6.30 (1H, d, $J = 2.0$ Hz, H-3′), 6.15 (1H, dd, $J = 8.0$, 2.0 Hz, H-5′), 6.80 (1H, d, $J = 8.0$ Hz, H-5′), 12.54 (1H, s, 5-OH). ^{13}C NMR (DMSO-d_6, 100 MHz) spectral data, see Table 2. HR-ESI-MS *m/z*: 317.1021 $[M+H]^+$ (calcd for $C_{17}H_{17}O_6$, 317.1025).

3.4 Cytotoxicity against Hela and Hep2 cell lines

The cytotoxicities against Hela and Hep2 cells were determined using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide colorimetric assay on 96-well microplates as described in a previous paper [15]. The OD value was read on a SYNERY™ 4 multi-mode microplate reader (BioTek Instrument, Inc., Winooski, VT, USA) at 490 nm. The cytotoxicity was expressed as IC_{50} value (μg/ml), which was the mean of three determinations and reduced the viable cell number by 50%. Computer program (GraphPad Prism) was used to calculate the IC_{50} (50% inhibition of cell proliferation) values.

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