Three New Homoisoflavanones from the *Ophiopogon japonicus* KER-GAWLER (Liliaceae)

by **Yongyi Wang**, **Jinzhong Xu**, **Lei Zhang**, and **Haibin Qu***

Institute of Pharmaceutical Informatics, School of Pharmaceutical Science of Zhejiang University, Hangzhou 310058, P. R. China (phone/fax: +86-571-88208428; e-mail: quhb@zju.edu.cn)

Three new homoisoflavanones, **1**–**3**, together with a known one, **4**, were obtained from the AcOEt extract of the tuberous roots of *Ophiopogon japonicus* (Liliaceae). They were identified as (3*R*)-2,3-dihydro-7-hydroxy-5-methoxy-3-(4-methoxybenzyl)-6,8-dimethyl-4*H*-chromen-4-one (**1**), (3*R*)-3-(1,3-benzodioxol-5-ylmethyl)-2,3-dihydro-7-hydroxy-5-methoxy-6,8-dimethyl-4*H*-chromen-4-one (**2**), (3*R*)-3-(1,3-benzodioxol-5-ylmethyl)-2,3-dihydro-7-hydroxy-5-methoxy-6-methyl-4*H*-chromen-4-one (**3**), and ophiopogonanone A (**4**). Their structures were determined on the basis of extensive NMR-spectroscopic and mass-spectrometric analyses. The three new compounds are rare homoisoflavanones which contain a MeO group at C(5). Compounds **1** and **2** showed weak cytotoxicity against the HepG2 (human hepatoma G2), KB (human oral epidermoid carcinoma), and MCF-7 (human breast adenocarcinoma) cell lines in an MTT assay. Compound **3** exhibited weak cytotoxicity against HepG2 and MCF-7, and moderate cytotoxicity against KB cell lines. Compound **4** showed moderate cytotoxicity against HepG2, KB, and MCF-7 cell lines.

Introduction. – Homoisoflavanones are an important class of natural products whose numbers have grown from 20 in 1981 to 157 at the present time. They are found to occur in seven plant families including Liliaceae, Hyacinthaceae, Alliaceae, Fabaceae, Polygonaceae, Orchidaceae, and Gentianaceae [1]. Pharmacological studies have revealed antibacterial [2], antitumor [3], antiallergic, antihistaminic, and angioprotective activities [4]. Additionally, some homoisoflavanones have been found to be potent angiogenesis inhibitors [5]. *Ophiopogon japonicus* KER-GAWLER (Liliaceae) is a widely used traditional Chinese medicine. It is mainly distributed in Sichuan and Zhejiang Province in China. Up to now, several homoisoflavanones have been isolated from this plant [6–10]. To find new and biologically active homoisoflavanones, the chemical constituents of *O. japonicus* were further investigated. In this work, three new homoisoflavanones were obtained from the AcOEt extract of the tuberous roots of *O. japonicus*.

Results and Discussion. – Compound **1** was isolated as a yellow amorphous powder, and displayed a *pseudo*-molecular-ion peak at *m*/*z* 343 ($[M+H]^+$) in the ESI-MS. The molecular formula was established as $C_{20}H_{22}O_5$ from the $[M+Na]^+$ peak at *m*/*z* 365.1308 in the HR-ESI-TOF-MS. The ^{1}H-NMR spectrum (*Table 1*) revealed a set of characteristic signals at δ(H) 4.27 (*dd*, *J* = 11.5, 4.0) and 4.09 (*dd*, *J* = 11.4, 7.0) for the CH_2(2) group[1]), at δ(H) 2.68–2.72 (*m*) for a the H–C(3) group, and at δ(H) 2.63 (*dd*,

[1]) Arbitrary atom numbering. For systematic names, see *Exper Part.*

Table 1. *^{1}H-NMR Spectral Data* (500 MHz) *of* **1** *and* **2** *in $CDCl_3$ and* **3** *in (D_6)DMSO.* δ in ppm, J in Hz.

	1[1])	**2**[1])	**3**[1])
H_α–C(2)	4.27 (*dd*, *J* = 11.5, 4.0)	4.29 (*dd*, *J* = 11.0, 4.0)	4.20 (*dd*, *J* = 11.5, 4.5)
H_β–C(2)	4.09 (*dd*, *J* = 11.4, 7.0)	4.11 (*dd*, *J* = 11.0, 7.5)	4.01 (*dd*, *J* = 11.5, 8.5)
H–C(3)	2.68–2.72 (*m*)	2.69–2.73 (*m*)	2.69–2.73 (*m*)
H–C(8)			6.19 (*s*)
H_α–C(9)	2.63 (*dd*, *J* = 14.0, 11.0)	2.61 (*dd*, *J* = 14.0, 10.0)	2.55 (*dd*, *J* = 14.0, 9.5)
H_β–C(9)	3.11 (*dd*, *J* = 13.5, 4.0)	3.15 (*dd*, *J* = 14.0, 4.5)	2.99 (*dd*, *J* = 14.0, 5.5)
H–C(2′)	7.12 (*d*, *J* = 8.5)	6.72 (*d*, *J* = 1.5)	6.81 (*d*, *J* = 1.0)
H–C(3′)	6.82 (*d*, *J* = 8.5)		
H–C(5′)	6.82 (*d*, *J* = 8.5)	6.73 (*d*, *J* = 8.0)	6.81 (*d*, *J* = 8.0)
H–C(6′)	7.12 (*d*, *J* = 8.5)	6.67 (*dd*, *J* = 8.0, 1.5)	6.66 (*dd*, *J* = 8.0, 1.0)
H–C(7′)		5.92 (*s*)	5.96 (*s*)
MeO–C(5)	3.76 (*s*)	3.81 (*s*)	3.80 (*s*)
MeO–C(4′)	3.76 (*s*)		
Me–C(6)	2.10 (*s*)	2.10 (*s*)	1.92 (*s*)
Me–C(8)	2.06 (*s*)	2.07 (*s*)	

J = 14.0, 11.0) and 3.11 (*dd*, *J* = 13.5, 4.0) for the benzylic CH_2(9) group, indicating that **1** possessed a homoisoflavanoid skeleton [11]. Furthermore, it exhibited two Me groups at δ(H) 2.10 (*s*) and 2.06 (*s*), two MeO groups at δ(H) 3.76 (*s*, 6 H), one set of an *AA′BB′* system at δ(H) 7.12 (*d*, *J* = 8.5), 6.82 (*d*, *J* = 8.5) due to the aromatic H-atoms of ring *B*. The ^{13}C-NMR spectrum (*Table 2*) indicated characteristic signals for C(4), C(2), C(3) and C(9) C-atoms at δ(C) 192.2, 68.9, 48.6, and 32.2, respectively. In the HMBC experiment (*Fig. 1*), the correlation between δ(H) 4.27 (H_α–C(2)) and δ(C) 159.8 enabled to assign the signal at δ(C) 159.8 (C(10)). The correlation of δ(H) 2.06 with δ(C) 159.8 (C(10)) and δ(C) 159.2 suggested that the Me group was attached at C(8), and that the signal at δ(C) 159.2 corresponded to C(7). Furthermore, a signal at δ(H) 2.10 showed correlations with δ(C) 159.2 (C(7)) and δ(C) 158.0, implying that another Me group was connected to C(6), and the signal at δ(C) 158.0 was assigned to C(5). In addition, the correlation between δ(H) 3.76 and δ(C) 158.0 (C(5)) suggested that one MeO group was linked to C(5). The location of the other MeO group at C(4′)

Table 2. *^{13}C-NMR Spectral Data* (125 MHz) *of* **1** *and* **2** *in $CDCl_3$ and* **3** *in (D_6)DMSO.* δ in ppm.

	1[1])	**2**[1])	**3**[1])		**1**[1])	**2**[1])	**3**[1])
C(2)	68.9	68.9	69.3	C(2′)	130.3	109.6	109.8
C(3)	48.6	48.6	47.9	C(3′)	114.2	146.4	146.2
C(4)	192.2	191.9	190.4	C(4′)	158.4	148.0	147.9
C(5)	158.0	158.1	160.4	C(5′)	114.2	108.5	108.6
C(6)	111.6	111.5	112.7	C(6′)	130.3	122.3	122.5
C(7)	159.2	159.0	163.1	C(7′)		101.1	101.3
C(8)	106.9	106.9	98.7	*Me*O–C(5)	61.4	61.4	61.0
C(9)	32.2	32.8	32.5	*Me*O–C(4′)	55.4		
C(10)	159.8	159.7	162.0	*Me*–C(6)	8.1	8.1	8.5
C(11)	108.6	108.7	109.8	*Me*–C(8)	8.1	8.1	
C(1′)	130.7	132.6	133.1				

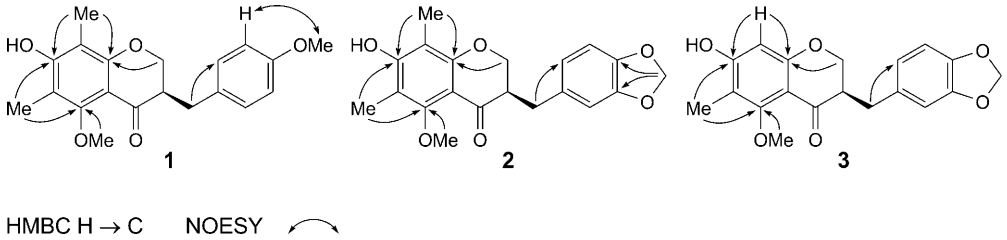

Fig. 1. *Key NOESY and HMBC correlations for* **1**–**3**

was deduced from the NOESY cross-peak of δ(H) 3.76 with δ(H) 6.82 (H–C(3′) and H–C(5′)).

Taken together, the structure of the new compound **1** was established as (3*R*)-2,3-dihydro-7-hydroxy-5-methoxy-3-(4-methoxybenzyl)-6,8-dimethyl-4*H*-chromen-4-one, corresponding to 5-*O*,8-dimethylophiopogonanone B (*Fig. 2*).

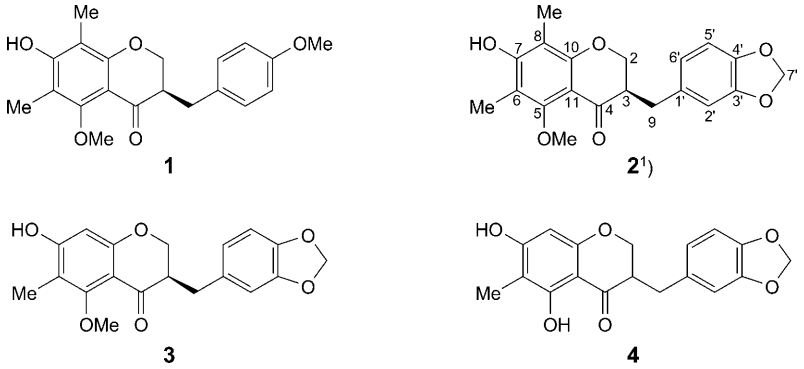

Fig. 2. *Structures of compounds* **1**–**4** *isolated from* O. japonicus

Compound **2** was obtained as a pale amorphous powder and displayed a *pseudo*-molecular-ion peak at *m*/*z* 357 ($[M+H]^+$) in the ESI-MS. The molecular formula was established as $C_{20}H_{20}O_6$ from the ($[M+Na]^+$) peak at *m*/*z* 379.1158 in the HR-ESI-TOF-MS. Comparison of the ^{1}H- and ^{13}C-NMR spectra for compounds **2** and **1** indicated that both compounds possessed the same *A*-ring substitution, but differed in the *B*-ring fragment. The ^{1}H-NMR spectrum of **2** indicated a set of an *ABX* system at δ(H) 6.73 (*d*, *J* = 8.0), 6.67 (*dd*, *J* = 8.0, 1.5), 6.72 (*d*, *J* = 1.5) and one signal at δ(H) 5.92 (*s*) for an OCH_2O group. The OCH_2O group was connected to C(3′) and C(4′)[1]) based on the analysis of HMBCs (*Fig. 1*). Consequently, the structure of the new compound **2** was determined as (3*R*)-3-(1,3-benzodioxol-5-ylmethyl)-2,3-dihydro-7-hydroxy-5-methoxy-6,8-dimethyl-4*H*-chromen-4-one, corresponding to 5-*O*,8-dimethylophiopogonanone A (*Fig. 2*).

Compound **3** was obtained as a pale amorphous powder, which displayed a *pseudo*-molecular-ion peak at *m*/*z* 343 ($[M+H]^+$) in the ESI-MS. The molecular formula was established as $C_{19}H_{18}O_6$ from the ($[M+Na]^+$) peak at *m*/*z* 365.1014 in the HR-ESI-TOF-MS. Compound **3** had one signal for an aromatic H-atom (δ(H) 6.19 (*s*)) more

and one Me group signal less than compound **2**, as observed in the ^{1}H-NMR spectra. In the HMBC experiment (*Fig. 1*), the H-atom signal at δ(H) 6.19 (*s*) showed correlations with δ(C) 162.0 (C(10)[1]) and 163.1 (C(7)), suggesting that the H-atom was connected to C(8). Hence, the structure of the new compound **3** was confirmed to be (3*R*)-3-(1,3-benzodioxol-5-ylmethyl)-2,3-dihydro-7-hydroxy-5-methoxy-6-methyl-4*H*-chromen-4-one, corresponding to 5-*O*-methylophiopogonanone A (*Fig. 2*).

Circular dichroism experiments of compounds **1**–**3** showed a negative peak at around 290 nm and positive peaks at around 250 and 315 nm, indicating the (*R*)-configuration at C(3) [12].

Compounds **1**–**4** were tested for cytotoxic activity against the HepG2, KB, and MCF-7 human tumor cell lines using the MTT (3-(4,5-dimethyl-1,3-thiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay [13]. Compounds **1** and **2** exhibited weak cytotoxicity against HepG2, KB, and MCF-7 human tumor cell lines with IC_{50} values of 96.89, 87.60, and 86.75 μmol/l, as well as 79.49, 52.89, and 54.38 μmol/l, respectively. Compound **3** exhibited weak cytotoxicity against HepG2 and MCF-7 cell lines with IC_{50} values of 72.25 and 91.20 μmol/l, respectively, and moderate cytotoxicity against KB cell lines with IC_{50} values of 43.09 μmol/l. Compound **4** showed moderate cytoxicity against HepG2, KB, and MCF-7 cell lines with IC_{50} values of 38.4, 18.55, and 19.02 μmol/l, respectively.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh; *Puke Chemical Factory*, Qingdao, P. R. China). Optical rotations: *JASCO 1030* polarimeter. UV Spectra: *PGENERAL UV-T6* spectrophotometer, λ_{max} in nm. CD Spectra: *JASCO J-815* spectrophotometer. IR Spectra: *JASCO FT-4100* spectrophotometer, $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker Avance-500* (500 MHz); δ in ppm, *J* in Hz. MS: *Agilent Finnigan LCQ-Advantage* ion-trap mass spectrometer (for ESI) and *Q-Tof 6210* micro mass spectrometer (for HR-ESI); in *m*/*z*.

Plant Material. The roots of *O. japonicus* were collected at Cixi area in Zhejiang Province, P. R. China, in July 2008, and identified by Dr. *B. Wu*, Pharmaceutical Informatics Institute, Zhejiang University. A voucher specimen (OJ080703) was deposited with the Pharmaceutical Informatics Institute, Zhejiang University, P. R. China.

Extraction and Isolation. The air-dried roots of *O. japonicus* (30 kg) were refluxed with 90% EtOH (2 × 90 l, 2 h each). The extracts were concentrated under reduced pressure to a syrup, which was suspended in H_2O (6 l), followed by successive partitioning with petroleum ether (PE; 3 × 6 l), AcOEt (3 × 6 l), and BuOH (3 × 6 l), resp. The AcOEt extract (120.5 g) was subjected to CC (SiO_2; $CHCl_2$/MeOH 100:0 to 1:1) to afford eight fractions (*Frs. 1–8*). *Fr. 5* (32.3 g) was further chromatographed by CC (SiO_2; PE/acetone 8:1 to 1:1) to afford nine subfractions (*Frs. 5.1–5.9*). *Fr. 5.6* (2.8 g) was further purified on prep. HPLC (*Zorbax SB-C18* column (21.2 mm × 250 mm, 7 μm); MeCN/H_2O 45:55; flow rate 6 ml/min) to give compounds **1** (21.6 mg, t_R 59.0 min), **2** (7.2 mg, t_R 62.1 min), **3** (9.4 mg, t_R 69.0 min), and **4** (8 mg, t_R 43.2 min). The elution of these compounds was monitored at UV 280 nm.

*(3*R*)-2,3-Dihydro-7-hydroxy-5-methoxy-3-(4-methoxybenzyl)-6,8-dimethyl-4*H*-chromen-4-one* (**1**). Yellow amorphous powder. $[\alpha]_D^{27} = +60.5$ ($c = 0.08$, EtOH). UV: 286 (4.33), 250 (3.56), 225 (4.57). CD ($c = 2.92 \cdot 10^{-4}$ mol/l, EtOH): +2099 (249), −4585 (288), +1767 (320), −1449 (353). IR (KBr): 3526, 2926, 1655, 1598, 1514, 1467, 1374, 1301, 1249, 1186, 1118. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. ESI-MS (pos.): 343 ($[M+H]^+$). HR-ESI-TOF-MS: 365.1308 ($[M+Na]^+$, $C_{20}H_{22}NaO_5^+$; calc. 365.1365).

*(3*R*)-3-(1,3-Benzodioxol-5-ylmethyl)-2,3-dihydro-7-hydroxy-5-methoxy-6,8-dimethyl-4*H*-chromen-4-one* (**2**). Pale amorphous powder. $[\alpha]_D^{27} = +67.1$ ($c = 0.076$, EtOH). UV: 287 (4.32), 251 (3.57), 206 (4.55). CD ($c = 2.64 \cdot 10^{-4}$ mol/l, EtOH): +1323 (249), −5799 (292), +1867 (323), −1281 (352). IR (KBr): 3411, 2928, 1658, 1590, 1486, 1243, 1188, 1118, 1037. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. ESI-MS (pos.): 357 ($[M+H]^+$). HR-ESI-TOF-MS: 379.1158 ($[M+Na]^+$, $C_{20}H_{20}NaO_6^+$; calc. 379.1157).

*(3*R*)-3-(1,3-Benzodioxol-5-ylmethyl)-2,3-dihydro-7-hydroxy-5-methoxy-6-methyl-4*H*-chromen-4-one* (**3**). Pale amorphous powder. $[\alpha]_D^{27} = +42.3$ ($c = 0.078$, EtOH). UV: 285 (4.15), 251 (3.39), 206 (4.42). CD ($c = 2.78 \cdot 10^{-4}$ mol/l, EtOH): +489 (248), −2334 (291), +985 (318), −694 (348). IR (KBr): 3230, 2932, 1662, 1593, 1492, 1276, 1165, 1095, 1095, 1043. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. ESI-MS (pos.): 343 ($[M+H]^+$). HR-ESI-TOF-MS: 365.1014 ($[M+Na]^+$, $C_{19}H_{18}NaO_6^+$; calc. 365.1001).

REFERENCES

[1] B. M. Abegaz, J. Mutanyatta-Comar, M. Nindi, *Nat. Prod. Commun.* **2007**, *2*, 475.
[2] K. Du Toit, E. E. Elgorashi, S. F. Malan, D. A. Mulholland, S. E. Drewes, J. Van. Staden, *S. Afr. J. Bot.* **2007**, *73*, 236.
[3] J. Mutanyatta, B. G. Matapa, D. D. Shushu, B. M. Abegaz, *Phytochemistry* **2003**, *62*, 797.
[4] G. Amschler, A. W. Frahm, A. Hatzelmann, U. Kilian, D. Müller-Doblies, U. Müller-Doblies, *Planta Med.* **1996**, *62*, 534.
[5] J. H. Kim, J. H. Kim, Y. Y. Suk, H.-O. Jun, H. J. Kwon, K. H. Park, K.-W. Kim, *Mol. Vision* **2008**, *14*, 556.
[6] A. Tada, R. Kasai, T. Saitoh, J. Shoji, *Chem. Pharm. Bull.* **1980**, *28*, 1477.
[7] Y. Watanabe, S. Sanada, Y. Ida, J. Shoji, *Chem. Pharm. Bull.* **1985**, *33*, 5358.
[8] T. Asano, T. Murayama, Y. Hirai, J. Shoji, *Chem. Pharm. Bull.* **1993**, *41*, 391.
[9] N. T. H. Anh, T. V. Sung, A. Porzel, K. Franke, L. A. Wessjohann, *Phytochemistry* **2003**, *62*, 1153.
[10] N. Kaneda, H. Nakanishi, T. Kuraishi, T. Katori, *Yakugaku Zasshi* **1983**, *103*, 1133.
[11] S. C. Jain, S. K. Sharma, R. Kumar, V. K. Rajwanshi, B. R. Babu, *Phytochemistry* **1997**, *44*, 765.
[12] M. Namikoshi, T. Saitoh, *Chem. Pharm. Bull.* **1987**, *35*, 3597.
[13] S. P. C. Cole, *Cancer Chemother. Pharmacol.* **1986**, *17*, 259.

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