Homoisoflavonoids from *Ophiopogon japonicus*

by **Chang-Xin Zhou**[a]), **Li Zou**[a]), **Jian-Xia Mo**[a]), **Xue-Yao Wang**[a]), **Bo Yang**[b]), **Qiao-Jun He**[b]), and **Li-She Gan***[a])

[a]) Institute of Modern Chinese Medicine, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, P. R. China (phone: +86-571-88208457; fax: +86-571-88208445; e-mail: lsgan@zju.edu.cn)

[b]) Institute of Pharmacology and Toxicology, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, P. R. China

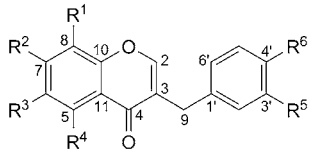
Phytochemical study on the root tubers of *Ophiopogon japonicus* resulted in the isolation and identification of 13 homoisoflavonoids, including three new compounds, 8-formyl-7-hydroxy-5,4′-dimethoxy-6-methylhomoisoflavone (**1**), 6-formylisoophiopogonone B (**2**), and 8-formylophiopogonanone B (**4**), and the ten known homoisoflavonoids **3**, and **5**–**13**. The absolute configurations of 8-formylophiopogonanone B (**4**) and 8-formyl-7-hydroxy-5,4′-dimethoxy-6-methylhomoisoflavanone (**5**) were confirmed by time-dependent density-functional-theory (TD DFT) calculations of their theoretical electronic circular dichroism (ECD) spectra. The structure of the formerly reported '6-aldehydoisoophiopogonone B' was revised to 8-formylophiopogonone B (**3**). All compounds were evaluated for their cytotoxic activities against the human-lung-tumor A549 cell line, and compounds **3**, **9**, **10**, and **13** exhibited promising antiproliferative activities with IC_{50} values of 10.01, 6.40, 0.84, and 1.66 μM, respectively.

Introduction. – The root tubers of *Ophiopogon japonicus* KER-GAWLER (Liliaceae) is a famous traditional chinese medicine (*Maidong*) and popular soup ingredient for nourishing 'Yin' and treating pulmonary and cardio-cerebrovascular diseases [1]. Previous phytochemical studies have demonstrated that steroidal saponins [2], homoisoflavonoids [3], and polysaccharides [4] are the main constituents of *O. japonicus*. Among them, the steroidal saponins and polysaccharides have been regarded as the active components for the antimyocardial ischemia effects of *O. japonicus* [4][5], while the homoisoflavonoids are still far from being thoroughly studied. Since the early isolation of homoisoflavonoids from *O. japonicus* in the 1980s [6], over 30 ones have been identified. Pharmacological studies in recent years have shown promising anti-inflammation [7], cytotoxic [8], and antioxidant [9] activities of these homoisoflavonoids.

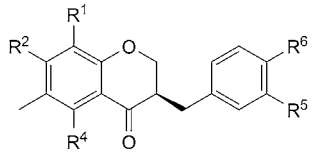
In the present study, 13 homoisoflavonoids were isolated from the 95% EtOH extract of *O. japonicus*, including the three new ones **1**, **2**, and **4**[1]), and the ten known ones **3**, and **5**–**13** (*Fig. 1*). The structures of these homoisoflavonoids were identified by spectroscopic methods, including HR-MS and 2D-NMR techniques, and the absolute configurations of compounds **4** and **5** were further confirmed by time-dependent density-functional-theory (TD DFT) calculations of their theoretical electronic

[1]) Trivial atom numbering; for systematic names, see *Exper. Part.*

circular dichroism (ECD) spectra. As to the known compounds, the structure of formerly reported '6-aldehydoisoophiopogonone B' [10] was corrected to 8-formyl-ophiopogonone B (**3**). The NMR, UV, IR, specific optical rotation, and ECD data of 8-formyl-7-hydroxy-5,4′-dimethoxy-6-methylhomoisoflavanone (**5**) were reported for the first time. All compounds were evaluated by a cytotoxic sulforhodamine B assay against the A549 cell line.



	R^1	R^2	R^3	R^4	R^5	R^6
1[1])	CHO	OH	Me	MeO	H	MeO
2[1])	Me	OH	CHO	OH	H	MeO
3[1])	CHO	OH	Me	OH	H	MeO
6[1])	CHO	OH	Me	OH	–OCH$_2$O–	
7[1])	Me	MeO	Me	OH	H	MeO



	R^1	R^2	R^4	R^5	R^6
4	CHO	OH	OH	H	MeO
5	CHO	OH	MeO	H	MeO
8	H	OH	OH	H	MeO
9	Me	OH	OH	MeO	OH
10	Me	OH	OH	H	MeO
11	CHO	OH	OH	–OCH$_2$O–	
12	Me	MeO	OH	H	MeO
13	Me	OH	OH	–OCH$_2$O–	

Fig. 1. *Compounds* **1**–**13**, *isolated from* Ophiopogon japonicus

Results and Discussion. – Compound **1** was isolated as a yellow powder and showed a pink spot on TLC when treated with $FeCl_3$ (1% $FeCl_3$ in 50% aq. EtOH). The negative-ion-mode HR-ESI-MS of **1** exhibited a pseudomolecular-ion peak at *m*/*z* 353.1034 ($[M-H]^-$), which indicated a molecular formula $C_{20}H_{18}O_6$. The IR spectrum revealed the presence of an OH group (3422 cm^{-1}), aliphatic H-atoms (2923 cm^{-1}), and a conjugated ketone moiety (1645 and 1622 cm^{-1}). The UV spectrum of **1** exhibited three absorption maxima at 232, 258, and 298 nm, similar to that of methylophiopogonone A (= 3-(1,3-benzodioxol-5-ylmethyl)-5,7-dihydroxy-6,8-dimethyl-4*H*-1-benzopyran-4-one) [11]. In the ^{1}H-NMR spectrum (*Table 1*), two aromatic *d* at δ(H) 7.19 and 6.85 (each *d*, J = 8.4 Hz, 2 H) suggested the presence of a 1,4-disubstituted benzene ring. An aliphatic H-atom *s* at δ(H) 3.72 (2 H), a Me *s* at δ(H) 2.18, and an aldehyde H-atom at δ(H) 10.35 (*s*) revealed that compound **1** might be a 2,3-unsaturated 3-benzylchroman-4-one-type homoisoflavonoid (homoisoflavone = 3-(phenylmethyl)-4*H*-1-benzopyran-4-one) [12]. The ^{13}C-NMR (including DEPT-135) spectrum confirmed the above hypothesis with 18 signals (two overlapped, *Table 2*). The typical aldehyde and α,β-unsaturated ketone signals appeared at δ(C) 191.7 and 174.6, respectively. The 4-substituted benzyl group was confirmed by a CH_2 signal at δ(C) 30.6 and two signals at δ(C) 130.1 and 114.1 (each two C-atoms). The substitution positions of the Me, CHO, and MeO groups were determined by 2D-NMR techniques. The HSQC experiment firstly allowed the assignments of all the H-atoms to their bonding C-atoms. Key HMBC cross peaks (*Fig. 2*) from the typical signals of H–C(2) at δ(H) 7.40 (*s*), and of the aldehyde H-atom at δ(H) 10.35 (*s*) to the same C-atom at δ(C) 158.9 (C(10)) located the CHO group at C(8). The Me group was then assigned to C(6) by its

Table 1. *^{1}H-NMR Data* (400 MHz, $CDCl_3$) *of Compounds* **1**–**5**[1]). δ in ppm, J in Hz.

H-Atom	**1**	**2**	**3**	**4**	**5**
H–C(2) or CH_2(2)	7.40 (*s*)	7.53(*s*)	7.54 (*s*)	4.26 (*dd*, J = 7.6, 11.6, H_a), 4.44 (*dd*, J = 4.8, 11.6, H_b)	4.22 (*dd*, J = 8.0, 11.2, H_a), 4.41 (*dd*, J = 4.0, 11.2, H_b)
C(3) or H–C(3)	–	–	–	2.88–2.93 (*m*)	2.79–2.82 (*m*)
CH_2(9)	3.72 (*s*)	3.70(*s*)	3.74 (*s*)	2.73 (*dd*, J = 10.0, 14.0, H_a), 3.21 (*dd*, J = 4.4, 14.0, H_b)	2.66 (*dd*, J = 10.4, 13.6, H_a), 3.19 (*dd*, J = 4.4, 14.0, H_b)
H–C(2′,6′)	7.19 (*d*, J = 8.4)	7.19(*d*, J = 8.4)	7.19 (*d*, J = 8.4)	7.14 (*d*, J = 8.4)	7.12 (*d*, J = 8.4)
H–C(3′,5′)	6.85 (*d*, J = 8.4)	6.87(*d*, J = 8.4)	6.87 (*d*, J = 8.4)	6.88 (*d*, J = 8.4)	6.83 (*d*, J = 8.4)
OH–C(5)	–	14.04 (*s*)	13.78 (*s*)	12.95 (*s*)	–
MeO–C(5)	3.91 (*s*)	–	–	–	3.86 (*s*)
Me–C(6)	2.18 (*s*)	–	2.09 (*s*)	2.00 (*s*)	2.03 (*s*)
CHO–C(6)	–	10.35 (*s*)	–	–	–
OH–C(7)	12.92 (*s*)	12.62 (*s*)	12.98 (*s*)	12.98 (*s*)	12.92 (*s*)
Me–C(8)	–	2.10 (*s*)	–	–	–
CHO–C(8)	10.35 (*s*)	–	10.22 (*s*)	10.06 (*s*)	10.14 (*s*)
MeO–C(4′)	3.79 (*s*)	3.80 (*s*)	3.80 (*s*)	3.81 (*s*)	3.77 (*s*)

Table 2. *^{13}C-NMR Data* (100 MHz, $CDCl_3$) *of Compounds* **1**–**5**[1]). δ in ppm.

C-Atom	**1**	**2**	**3**	**4**	**5**
CH(2) or CH_2(2)	150.1	153.7	152.5	70.0	69.6
C(3) or CH(3)	127.1	123.5	125.1	46.3	47.9
C(4)	174.6	182.2	180.8	197.4	189.4
C(5)	164.7	165.8	165.7	167.2	166.4
C(6)	118.1	106.1	108.4	105.4	113.7
C(7)	166.6	165.1	167.3	168.3	167.6
C(8)	105.7	102.9	104.4	103.8	106.3
CH_2(9)	30.6	29.8	29.9	31.8	31.8
C(10)	158.9	159.7	158.2	164.8	165.0
C(11)	111.3	103.9	102.3	100.7	107.0
C(1′)	130.0	129.3	129.1	129.1	129.8
CH(2′,6′)	130.1	130.0	130.0	130.0	129.9
CH(3′,5′)	114.1	114.2	114.2	114.2	114.0
C(4′)	158.4	158.5	158.6	158.6	158.3
*Me*O–C(5)	61.8	–	–	–	61.7
Me–C(6)	7.5	–	6.4	6.0	7.0
*C*HO–C(6)	–	192.7	–	–	–
Me–C(8)	–	6.4	–	–	–
*C*HO–C(8)	191.7	–	189.7	191.1	192.5
*Me*O–C(4′)	55.3	55.3	55.3	55.3	55.1

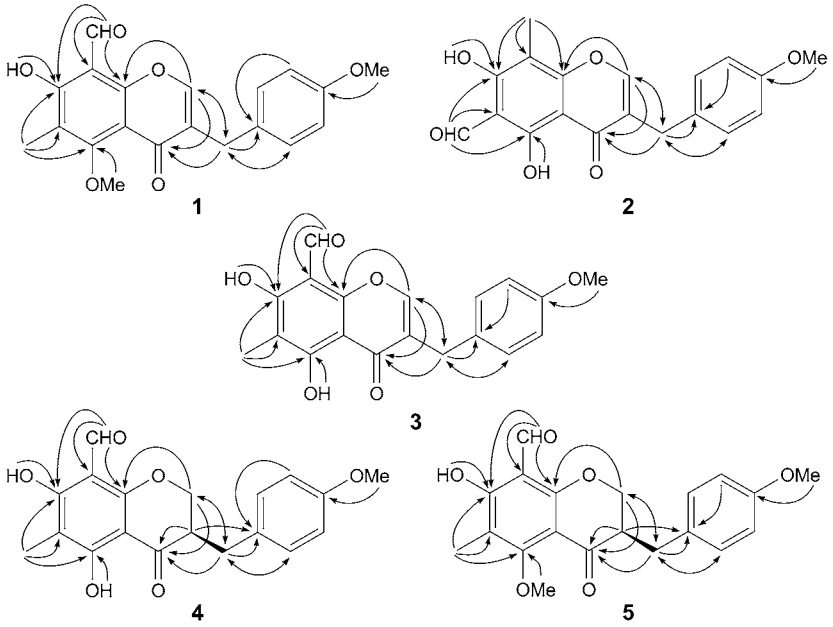


Fig. 2. *Selected HMBCs for compounds* **1**–**5**

HMBCs with two O-bearing C-atoms at δ(C) 164.7 (C(5)) and 166.6 (C(7)). The MeO group at δ(H) 3.91 (*s*) was positioned at C(5) by its HMBC with C(5). The above deduction allowed the determination of the structure of compound **1** as 8-formyl-7-hydroxy-5,4′-dimethoxy-6-methylhomoisoflavone[1]).

Compounds **2** and **3** showed slight differences in their ^{1}H- and ^{13}C-NMR spectra. The two OH signals appeared at δ(H) 14.04 and 12.62 (2*s*) for **2**, and correspondingly, at δ(H) 13.78 and 12.98 (2*s*) for **3** (*Table 1*). In the ^{13}C-NMR spectra, a aldehyde signal was observed at δ(C) 192.7 for **2** and δ(C) 189.7 for **3** (*Table 2*). Further 2D HSQC and HMBC experiments revealed that compound **2** is a 6-formyl-8-methyl homoisoflavonoid, while compound **3** is an 8-formyl-6-methyl-homoisoflavonoid (*Fig. 2*). Interestingly, the structure of **2**, '6-aldehydoisoophiopogonone B', has already been reported by *Zhu et al.* in 1987 [10], but the NMR data in [10] is identical to that of compound **3** in the current study. So herein we correct the formerly reported structure to 8-formylophiopogonone B[1]) (**3**) and provide new assignments for the two isomers.

Compound **4** showed a UV absorption maximum at 277 nm and an IR absorption band at 1634 cm^{-1} typical for 3-benzylchroman-4-one type homoisoflavonoids [11]. The molecular formula was determined to be $C_{19}H_{18}O_6$ by HR-ESI-MS. In the ^{1}H-NMR spectrum, two couples of geminal H-atoms resonating at δ(H) 4.44 (*dd*, J = 4.8, 11.6 Hz, 1 H) and 4.26 (*dd*, J = 7.6, 11.6 Hz, 1 H) and δ(H) 3.21 (*dd*, J = 4.4, 14.0 Hz, 1 H) and 2.73 (*dd*, J = 10.0, 14.0 Hz, 1 H), as well as an aliphatic H-atom at δ(H) 2.88–2.93 (*m*) revealed that compound **4** is a 3-benzylchroman-4-one type homoisoflavonoid. Two coupled signals at δ(H) 7.14 (*d*, J = 8.4 Hz, 2 H) and 6.88 (*d*, J = 8.4 Hz, 2 H) established the presence of a 1,4-disubstituted benzene ring. An OH signal at δ(H) 12.95 (*s*) and an aldehyde signal at δ(H) 10.06 (*s*) revealed the existence of an HO–C(5) and a CHO group, respectively. The ^{13}C-NMR data corroborated the above deduction with 17 signals, including two CO groups at δ(C) 197.4 and 191.1, twelve aromatic C-atoms (two overlapped), a MeO group at δ(C) 55.3, an O-bearing C-atom attributable to C(2) at δ(C) 70.0, and a Me group at δ(C) 6.0 (*Table 2*). The positions of the aldehyde, MeO, and Me groups were determined by the HMBC experiment (*Fig. 2*). The HMBCs CH_2(2)/C(10) and CHO–C(8)/C(10) suggested a linkage between the aldehyde group and C(8). The Me group was then located at C(6) by its HMBCs with C(5) and C(7). The HMBC cross-peaks MeO/C(4′) established their linkage. The structure of compound **4** was finally determined to be 8-formylophiopogonanone B[1]) (**4**).

Compound **5** showed similar UV and IR spectra to those of **4**. HR-ESI-MS revealed a molecular formula $C_{20}H_{20}O_6$, indicating that compound **5** might be the methylated derivative of **4**. Comparison of the ^{1}H-NMR spectra of the two compounds showed that **5** had an additional MeO group at δ(H) 3.86 (*s*) instead of the OH–C(5) signal at δ(H) 12.95 (*s*) in **4**. The above information suggested the structure of 8-formyl-7-hydroxy-5,4′-dimethoxy-6-methylhomoisoflavanone[1]) for compound **5**, and this deduction was confirmed by 2D HSQC and HMBC experiments (*Fig. 2*). Based on LC-MS^n data, the structure of **5** has already been published [9] but this is the first report of its spectroscopic data and full NMR assignment.

The absolute configurations of compounds **4** and **5** were determined on the basis of their specific optical rotation, ECD spectra, and TD DFT calculations. Compounds **4** and **5** showed small negative specific rotation values (−23 and −45) and negative

ECD absorptions around 290 nm, which are typical features for (3*R*) configuration [13][14]. To further confirm the above deduction, TD DFT calculations of their ECD spectra were carried out. Compared to the experimental ECD spectrum, the calculated one for **4** showed similar first positive, second negative, and third positive *Cotton* effects at 342 (+ 30), 284 (+ 3), and 247 (– 9) nm, which confirmed the absolute configuration (3*R*). For compound **5**, the calculated ECD spectrum also showed the same pattern as the experimental one, which allowed the assignment of the absolute configuration (3*R*) (*Fig. 3*).

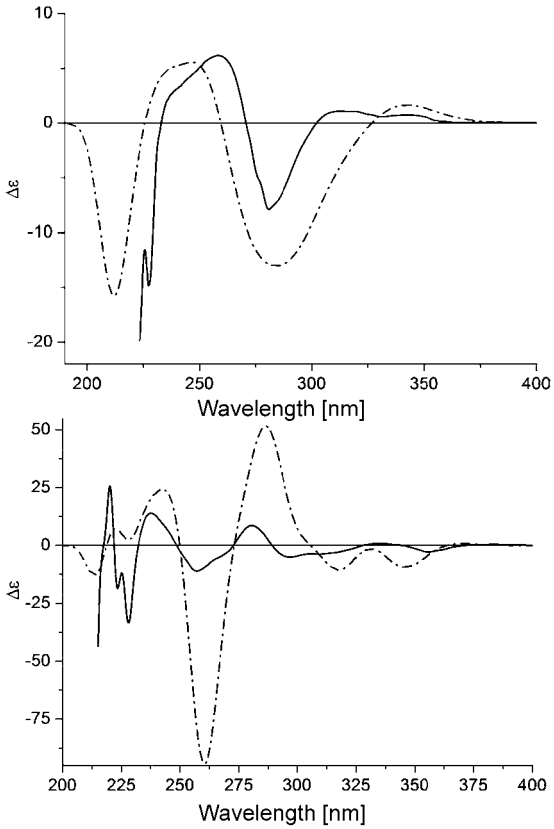


Fig. 3. *Experimental* (–) *and calculated* (·–·–) *ECD spectra of compounds* **4** (top) *and* **5** (bottom)

By comparing with the literature data, the structures of the remaining compounds were identified as ophiopogonone C (**6**) [15], methylophiopogonone B monomethyl ether (**7**) [6], ophiopogonanone B (**8**) [16], 5,7,4′-trihydroxy-3′-methoxy-6,8-dimethylhomoisoflavanone (**9**) [3], methylophiopogonanone B (**10**) [6], 8-formylophiopogonanone A (**11**) [15], methylophiopogonanone B monomethyl ether (**12**) [6], and methylophiopogonanone A (**13**) [6] (*Fig. 1*). These compounds have been previously reported from *O. japonicus.*

All compounds were evaluated against the human-lung-carcinoma (A549) cell line for their cytotoxicity. As shown in *Table 3*, the nine compounds **2**, **3** and **7**–**13** exhibited cytotoxic effects with IC_{50} values ranging from 0.84 to 32.76 μM. Analyses of the results indicated that a compound with a Me group instead of an aldehyde group showed better activity (**10** *vs.* **4**; **13** *vs.* **11**). Methylation of OH–C(5) or OH–C(7) reduced the cytotoxic activity (**1** *vs.* **3**; **12** *vs.* **10**). Compared to the reported cytotoxicities of compounds **10** ($IC_{50} = 0.25$ μg/ml) and **13** ($IC_{50} = 1.2$ μg/ml) against Hela S3 cells [17], of compounds **8** ($IC_{50} = 14.0$ μg/ml) and **10** ($IC_{50} = 6.0$ μg/ml) against Hela cells, as well as of compound **10** ($IC_{50} = 34.6$ μg/ml) against SMMC-7721 cells [18], the activity shown by these compounds against A549 cells in the current study was in the same range.

Table 3. *Cytotoxic Activities against the Human-Lung-Carcinoma A5A9 Cell Line* (IC_{50} values in [μM]) *of Compounds* **1**–**13**[a])

Compound	IC_{50}	Compound	IC_{50}
1	> 50	**8**	15.42 ± 1.19
2	16.76 ± 2.60	**9**	6.40 ± 1.20
3	10.01 ± 1.34	**10**	0.84 ± 0.13
4	> 50	**11**	19.01 ± 3.83
5	> 50	**12**	11.80± 2.88
6	> 50	**13**	1.66 ± 0.24
7	32.76 ± 4.21	Taxol [nM]	0.77 ± 0.12

[a]) Experiments were performed in triplicates; results are presented as mean ± s.d.

This work was financially supported by the *National Natural Science Foundation of China* (30801429) and the *Health Bureau of Zhejiang Province* (2012KYA065).

Experimental Part

General. Column chromatography (CC): silica gel *H60* (SiO_2; *Qing Dao Hai Yang Chemical Group Co., Ltd.*); C_{18} column (*Phenomenex 00G-4324-N0*; 10 μm, 10 mm (i.d.) × 25 cm); *MCI gel* (*Mitsubishi*, Japan); *D101* macroporous resin (*Tianjin Agricultural Chemical Co. Ltd.*, P. R. China). HPLC: *Agilent 1100* (*Agilent Technologies*, Santa Clara, CA, USA. TLC: precoated silica gel plates *HSGF 254* (*Yan Tai Jiang You Silica Gel Development Co., Ltd.*). Optical rotations: *Jasco-P-1010*, Polarimeter. UV Spectra: *Beckman DU-600* spectrometer; λ_{max} (log ε) in nm. ECD Spectra: *Jasco J-815 CD* spectrometer. IR Spectra: *Bruker-VECTOR-22* spectrophotometer; KBr pellets; in cm^{-1}. NMR Spectra: *Varian-Unity-INOVA-400/54* spectrometers: δ in ppm rel. to Me_4Si as internal standard, J in Hz. ESI-MS: *Micromass-Quattro* triple-quadrupole mass spectrometer equipped with an ESI source (*Micromass*, Manchester, UK); in m/z.

Plant Material. Root tubers of *O. japonicus* were obtained from the *Huqingyu Pharmaceutical Factory*, Hangzhou, Zhejiang Province, P. R. China, and further authenticated by one of us, (*J.-X. M.*). A voucher specimen has been deposited with the Institute of Modern Chinese Medicine, Zhejiang University (accession number OJ-2009-I).

Extraction and Isolation. The dried soft root tubers of *O. japonicus* (10 kg) were frozen at −26° and ground. The powder was then extracted at r.t. with 95% EtOH (3 × 50 l, for 7 d each time). After evaporation of the solvent, the crude extract (2100 g) was suspended in H_2O (2.5 l) and then partitioned successively with AcOEt and BuOH (3 × 2.0 l for each) to give the AcOEt (108 g) and BuOH fractions

(306 g). The AcOEt fraction was subjected to CC (*D101* macroporous resin, aq. EtOH, 0%, 25%, 50%, and 95% (*v*/*v*) each 10 l); homoisoflavonoid fraction (95% elution part; 92 g). The homoisoflavonoid fraction was further separated by CC (*MCI* gel, 30% to 95% aq. EtOH: *Fractions A–J*). *Fr. D* (7.6 g) was subjected to CC (*Sephadex LH-20* gel, 95% EtOH): *Frs. D1–D4*. *Fr. D3* (56 mg) was further separated by CC (SiO_2, petroleum ether/AcOEt 30:1): **8** (5 mg) and **9** (8 mg). *Fr. F* (7.6 g) was subjected to CC (*Sephadex LH-20* gel, 95% EtOH): *Frs. F1–F4*. Compounds **10** (89 mg) and **13** (21 mg) were obtained by recrystallization of *Fr. F3* (210 mg) and *Fr. F4* (95 mg) in AcOEt, resp. *Fr. F2* (190 mg) was further separated by CC (SiO_2, petroleum ether/AcOEt 20:1): *Fr. F2A* (105 mg) and *Fr. F2B* (50 mg), were each subjected to reversed-phase CC (*C18,* 60% and 55% aq. EtOH, resp.): **5** (54 mg) and **1** (7 mg). *Fr. H* (13.8 g) was purified on CC (SiO_2, petroleum ether/AcOEt 80:1): *Frs. H1–H7*. Compounds **7** (14 mg), **2** (5 mg), **4** (274 mg), and **3** (80 mg) were obtained by recrystallization of *Fr. H2*, *Fr. H3*, *Fr. H5*, and *Fr. H6*, resp. *Fr. H1* (60 mg) was further purified CC (*Sephadex LH-20* gel, 95% EtOH): **12** (12 mg). *Fr. I* (190 mg) was subjected to CC (SiO_2, petroleum ether/AcOEt 80:1): **2** (6 mg), **11** (9 mg), and **6** (10 mg).

8-Formyl-7-hydroxy-5,4′-dimethoxy-6-methylhomoisoflavone (= *7-Hydroxy-5-methoxy-3-[(4-methoxyphenyl)methyl]-6-methyl-4-oxo-4*H-*1-benzopyran-8-carboxaldehyde*; **1**): White amorphous powder. UV (MeOH): 232 (4.16), 258 (4.17), 298 (3.92). IR (KBr): 3422, 2923, 1645, 1622, 1588, 1485, 1466, 1250, 1195, 983, 926. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (pos.): 355 ($[M+H]^+$). ESI-MS (neg.): 353 ($[M-H]^-$). HR-ESI-MS (neg.): 353.1034 ($[M-H]^-$, $C_{20}H_{17}O_6^-$; calc. 353.1031).

6-Formylisoophiopogonone B (= *5,7-Dihydroxy-3-[(4-methoxyphenyl)methyl]-8-methyl-4-oxo-4*H-*1-benzopyran-6-carboxaldehyde*; **2**): White amorphous powder. UV (MeOH): 232 (3.84), 287 (4.01), 338 (3.29). IR (KBr): 3549, 3475, 3415, 2924, 1650, 1618, 1596, 1510, 1457, 1427, 1174. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (neg.): 339 ($[M-H]^-$). HR-ESI-MS (neg.): 339.0878 ($[M-H]^-$, $C_{19}H_{15}O_6^-$; calc. 339.0874).

8-Formylophiopogonanone B (= *(3*R*)-3,4-Dihydro-5,7-dihydroxy-3-[(4-methoxyphenyl)methyl]-6-methyl-4-oxo-2*H-*1-benzopyran-8-carboxaldehyde*; **4**): White amorphous powder. $[\alpha]_D^{20} = -23$ ($c = 2.93$, $CHCl_3$). CD ($CHCl_3$): 312 (+1.1), 281 (−7.9), 256 (+6.2). UV (MeOH): 277 (4.73), 281 (4.71). IR (KBr): 3410, 2922, 1634, 1511, 1474, 1325, 1246, 1152, 981. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (neg.): 341 ($[M-H]^-$). HR-ESI-MS (neg.): 341.1020 ($[M-H]^-$, $C_{19}H_{17}O_6^-$; calc. 341.1025).

8-Formyl-7-hydroxy-5,4′-dimethoxy-6-methylhomoisoflavanone (= *(3*R*)-3,4-Dihydro-7-hydroxy-5-methoxy-3-[(4-methoxyphenyl)methyl]-6-methyl-4-oxo-2*H-*1-benzopyran-8-carboxaldehyde*; **5**): White amorphous powder. $[\alpha]_D^{20} = -45$ ($c = 5.88$, $CHCl_3$). CD ($CHCl_3$): 356 (−2.8), 335 (+0.8), 297 (−5.1), 280 (+8.6), 257 (−11), 238 (+13.9). UV (MeOH): 260 (4.07), 349 (3.27). IR (KBr): 3400, 2925, 1681, 1633, 1580, 1510, 1469, 1377, 1309, 1131, 1037, 1006. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (neg.): 355 ($[M-H]^-$). HR-ESI-MS (neg.): 355.1192 ($[M-H]^-$, $C_{20}H_{19}O_6^-$; calc. 355.1187).

Quantum-Chemical TD DFT Calculations of ECD Spectra. Conformational searching was firstly carried out at the HF/3–21G level in the Spartan 08 software package [19]. Subsequently, the resulting conformers with relative energy within 2 kcal/mol, one (**4C1**) for compound **4** and two (**5C1** and **5C2**) for compound **5** (see *Supporting Information*[2])), were reoptimized by DFT calculations at the B3LYP/6-311++G(2d,2p) level with the PCM ($CHCl_3$) solvent model in the Gaussian 09 program [20]. The B3LYP-SCRF(PCM, $CHCl_3$)/6-311++G(2d,2p) harmonic vibrational frequencies were further calculated to confirm their stability. The energies, oscillator strengths, and rotational strengths of the first 25 electronic excitations of the conformers were calculated by the TD DFT methodology at the B3LYP-SCRF(PCM, $CHCl_3$)/6-311++G(2d,2p) level, and the ECD spectra were then simulated by the GaussSum 2.25 program [21] ($\sigma = 0.5$ eV for **4**; $\sigma = 0.3$ eV for **5**). To get the final spectra, the simulated spectra of the lowest-energy conformations were averaged according to their relative *Gibbs* free energy (ΔG).

Sulforhodamine B (SRB) Assay of Cytotoxic Activities. Suspended human-lung-tumor A549 cells were cultured in *RPMI 1640* (*HyClone*), and supplemented with 10% fetal bovine serum (*HyClone*), penicillin (100 IU/ml), and streptomycin (100 μg/ml) at 37° in a humidified atmosphere with 5% CO_2. The logarithmic phase cells (100 μl) were seeded onto 96-well plates at the concentration of $4 \cdot 10^3$ cells per well. After 24 h, different concentrations of the compounds, dissolved in DMSO, were added at 10 μl/

[2]) Supporting information is available from the corresponding author.

well, and 3 parallel wells for each concentration were tested. Control cells were treated with DMSO alone and positive controls with taxol. The cells were cultivated for 72 h and then fixed with 10% trichloroacetic acid for 1 h and washed with distilled water. SRB was dissolved at 4 mg/ml in PBS (phosphate-buffered saline) and 100 µl of the soln. were added to each well, and the cells were stained for 20 min. The supernatant was then removed, and 100 µl of *Tris* buffer (10 mM) was added into each well. The absorbance at 490 nm was measured with a microplate reader (*Thermo*). The inhibition rates were calculated with *OD* mean values, from inhibition rate = $(OD_{control} - OD_{sample})/OD_{control}$. The IC_{50} value was determined by using the *Bliss* method.

REFERENCES

[1] H. Wang, J. Qi, T. Xu, J. H. Liu, M. J. Qin, D. N. Zhu, B. Y. Yu, *J. Agric. Food. Chem.* **2010**, *58*, 5432.
[2] C.-L. Duan, Y.-J. Li, P. Li, Y. Jiang, J.-X. Liu, P.-F. Tu, *Helv. Chim. Acta* **2010**, *93*, 227.
[3] N. T. Hoang Anh, T. Van Sung, A. Porzel, K. Franke, L. A. Wessjohann, *Phytochemistry* **2003**, *62*, 1153.
[4] S. Wang, X. Lin, L. Y. Wang, K. F. Ruan, Y. Feng, X. Y. Li, *Int. J. Biol. Macromol.* **2012**, *50*, 734.
[5] J. Tao, H. Wang, H. Zhou, S. Li, *Life Sci.* **2005**, *77*, 3021.
[6] A. Tada, R. Kasai, T. Saitoh, J. Shoji, *Chem. Pharm. Bull.* **1980**, *28*, 1477.
[7] T. M. Hung, C. V. Thu, N. T. Dat, S. W. Ryoo, J. H. Lee, J. C. Kim, M. Na, H. J. Jung, K. Bae, B. S. Min, *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2412.
[8] L. G. Lin, H. Xie, H. L. Li, L. J. Tong, C. P. Tang, C. Q. Ke, Q. F. Liu, L. P. Lin, M. Y. Geng, H. L. Jiang, W. M. Zhao, J. Ding, Y. Ye, *J. Med. Chem.* **2008**, *51*, 4419.
[9] Y. N. Lin, D. N. Zhu, J. Qi, M. J. Qin, B. Y. Yu, *J. Pharm. Biomed. Anal.* **2010**, *52*, 757.
[10] Y. X. Zhu, K. D. Yan, G. S. Tu, *Phytochemistry* **1987**, *26*, 2873.
[11] Y. Watanabe, S. Sanada, Y. Ida, J. Shoji, *Chem. Pharm. Bull.* **1985**, *33*, 5358.
[12] B. M. Abegaz, J. Mutanyatta-Comar, M. Nindi, *Nat. Prod. Commun.* **2007**, *2*, 475.
[13] M. Adinolfi, G. Barone, M. M. Corsaro, L. Mangoni, R. Lanzetta, M. Parrilli, *Tetrahedron* **1988**, *44*, 4981.
[14] G. Amschler, A. W. Frahm, A. Hatzelmann, U. Kilian, D. Mueller-Doblies, U. Mueller-Doblies, *Planta Med.* **1996**, *62*, 534.
[15] J. M. Chang, C. C. Shen, Y. L. Huang, M. Y. Chien, J. C. Ou, B. J. Shieh, C. C. Chen, *J. Nat. Prod.* **2002**, *65*, 1731.
[16] N. Kaneda, H. Nakanishi, T. Kuraishi, T. Katori, *Yakugaku Zasshi* **1983**, *103*, 1133.
[17] S. Takatsuki, T. Narui, A. Maeyama, R. Asano, H. Abuki, Y. Hiraga, T. Okuyama, *Nat. Med.* **1998**, *52*, 145.
[18] K. W. Wang, H. Zhang, L. Q. Shen, W. Wang, *Carbohyd. Res.* **2011**, *346*, 253.
[19] Spartan 04; Wavefunction Inc., Irvine, CA.
[20] Gaussian 09, Revision A.1, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, Gaussian, Inc., Wallingford CT, 2009.
[21] N. M. O'Boyle, A. L. Tenderholt, K. M. Langner, *J. Comput. Chem.* **2008**, *29*, 839.

Received August 23, 2012