Five New Homoisoflavonoids from the Tuber of *Ophiopogon japonicus*

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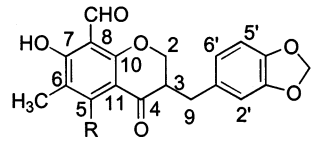
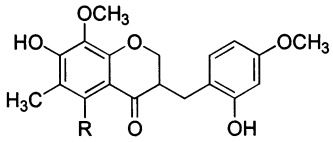
Five new homoisoflavonoids, ophiopogonanone C (**1**), ophiopogonanone D (**2**), ophiopogonone C (**3**), ophiopogonanone E (**4**) and ophiopogonanone F (**5**), and six known compounds were isolated from an ethanol extract of the tubers of *Ophiopogon japonicus* (Thunb) Ker-Gawl. Spectroscopic analyses were used to elucidate the structures of these compounds.

The tuber of *Ophiopogon japonicus* (Thunb) Ker-Gawl (known as Maidong in China) (Liliaceace) is sweet with a slightly bitter aftertaste. It is recommended for latent heat in the lungs due to 'yin'-asthenia, fever in consumptive disease or general debility, dehydration of febrile disease, and dry mouth.[1] Previous phytochemical studies of the tuber derived from *O. japonicus* resulted in the isolation of homoisoflavonoids,[2−4] saponins,[5−7] and amides.[7] In this study, we report the isolation of five new homoisoflavonoids and six known compounds from the tubers of *O. japonicus*.

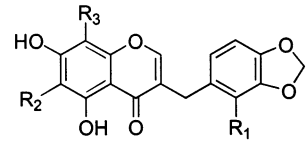
The dried tubers of *O. japonicus* were extracted with boiling H_2O to give a H_2O extract and residue. The residue was further refluxed with 95% EtOH to provide an EtOH extract. The H_2O extract was partitioned with EtOAc to give an EtOAc-soluble portion. The EtOH extract and the EtOAc-soluble portion were combined and chromatographed repeatedly using a Si gel column, a Sephadex LH-20 column, and PTLC to afford five new homoisoflavonoids (**1**−**5**); three known homoisoflavonoids, 6-aldehydoisoophiopogonone A (**6**),[8] 5,7,2′-trihydroxy-6-methyl-3-(3′,4′-methylenedioxybenzyl)chromone (**7**),[9] and 2′-hydroxymethylophiopogonone A (**8**);[4] and three known amides, *E*-*p*-coumaroyltyramine,[10,11] *E*-feruloyltyramine,[10,11] and *E*-*p*-coumaroyl-*β*-hydroxytyramine.[7]



1 R=OH
2 R=OCH_3



4 R=OH
5 R=OCH_3



3 R_1=H R_2=CH_3 R_3=CHO
6 R_1=H R_2=CHO R_3=CH_3
7 R_1=OH R_2=CH_3 R_3=H
8 R_1=OH R_2=CH_3 R_3=CH_3

Compound **1** was obtained as colorless needles with the molecular formula $C_{19}H_{16}O_7$, as established by HREIMS and ^1H and ^{13}C NMR spectra. The IR spectrum showed absorption bands at 3391 (OH) and 1638 (C═O) cm^{-1}. The ^1H NMR spectrum of **1** indicated the presence of a methylenedioxy group at δ 5.97 (2H, s), an aldehyde group at δ 10.07, and a methyl group attached to an aromatic nucleus at δ 2.03 (Table 1). These spectral properties are very similar to those of 6-aldehydoisoophiopogonanone A.[12] The ^1H NMR signals at δ 4.29 (1H, J = 8.0 and 11.5 Hz), 4.46 (1H, J = 4.5 and 11.5 Hz), and 2.92 (1H, m) showed the protons of the γ-dihydropyrone moiety of a homoisoflavanone.[2] In addition, two benzylmethylene protons appeared at δ 2.72 (1H, dd, J = 10.5 and 14.0 Hz) and 3.22 (1H, dd, J = 4.5 and 14.0), and three ABX aromatic protons appeared at δ 6.68 (1H, dd, J = 7.5 and 1.0 Hz), 6.73 (1H, d, J = 1.0 Hz), and 6.78 (1H, d, J = 7.5 Hz). The ^1H NMR spectrum of **1** also indicated the presence of two chelated hydroxy groups at δ 12.92 and 12.98 (each 1H, s). The substitutions on ring A of the homoisoflavanone were initially determined by the HMBC data. From the HMBC spectrum of **1**, diagnostic long-range correlations were observed for δ_H 12.92 (C_5-OH) to C-6 and C-11 as well as for δ_H 12.98 (C_7-OH) to C-6 and C-8, for the methyl group at δ_H 2.03 to C-5 and C-7, and for the aldehyde group at δ_H 10.07 to C-7 and C-10. In the NOE spectrum, when the methyl signal at δ 2.03 was irradiated, the δ 12.92 (C_5-OH) and 12.98 (C_7-OH) signals showed 2.9% and 1.4% enhancements, respectively. When the signal of the aldehyde proton at δ 10.07 was irradiated, the δ 12.98 (C_7-OH) signal showed 1.3% enhancement, but no NOE enhancement of the δ 12.92 (C_5-OH) signal was observed. All of the above results are compatible with the structure of **1** being 5,7-dihydroxy-6-methyl-8-aldehydo-3-(3′,4′-methylenedioxybenzyl)chroman-4-one, a new compound named ophiopogonanone C.

Compound **2** had the molecular formula $C_{20}H_{18}O_7$, a CH_2 unit more than that of compound **1**, as deduced from the HREIMS and ^1H and ^{13}C NMR spectra. The ^1H and ^{13}C NMR spectral data (Table 1) of **2** were very similar to those of **1**, except the 5-OH signal at δ_H 12.92 in **1** was replaced

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Table 1. ^{1}H and ^{13}C NMR Data of **1**, **2**, **3**, **4**, and **5**

	1 ($CDCl_3$)		**2** ($CDCl_3$)		**3** ($CDCl_3$)		**4** [$(CD_3)_2CO$]		**5** (CD_3OD)	
position	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C
2	4.46 (1H, dd, 4.5, 11.5) 4.29 (1H, dd, 8.0, 11.5)	70.2	4.45 (1H, dd, 5.0, 11.5) 4.26 (1H, dd, 8.0, 11.5)	69.9	8.21 (1H, s)	154.3	4.40 (1H, dd, 4.0, 11.0) 4.24 (1H, dd, 8.5, 11.0)	69.9	4.34 (1H, dd, 4.5, 11.0) 4.19 (1H, dd, 7.0, 11.0)	69.5
3	2.92 (1H, m)	46.5	2.80 (1H, m)	48.2		124.6	3.08(1H, m)	45.3	2.89 (1H, m)	47.1
4		197.4		189.6		181.3		199.0		193.2
5		167.4		168.1		165.7		157.3		155.5
6		105.7		114.2		107.5		103.7		112.8
7		168.5		166.8		167.3		157.7		156.4
8		104.0		106.8		102.7		127.9		131.8
9	3.22 (1H, dd, 4.5, 14.0) 2.72 (1H, dd, 10.5, 14.0)	32.7	3.20 (1H, dd, 4.5, 14.0) 2.67 (1H, dd, 10.5, 14.0)	32.8	3.77 (2H, s)	30.1	2.69 (1H, dd, 10.0, 14.0) 3.26 (1H, dd, 5.0, 14.0)	26.9	2.64 (1H, dd, 10.0, 14.0) 3.14 (1H, dd, 5.0, 14.0)	27.1
10		165.0		165.4		158.1		151.9		154.5
11		101.0		107.3		104.5		101.6		107.6
1′		131.1		131.8		132.5		117.1		117.2
2′	6.73 (1H, d, 1.0)	109.5	6.72 (1H, d, 1.5)	109.5	6.88 (1H, m)	109.4		156.4		156.4
3′		148.3		148.2		148.1	6.50 (1H, d, 2.5)	101.8	6.39 (1H, d, 2.0)	101.2
4′		146.9		146.7		146.6		160.1		159.9
5′	6.78 (1H, d, 7.5)	108.8	6.76 (1H, d, 8.0)	108.6	6.78 (1H, d, 7.5)	108.3	6.42 (H, dd, 2.5, 8.0)	104.9	6.37 (1H, dd, 2.0, 8.5)	104.5
6′	6.68 (1H, dd, 7.5, 1.0)	122.4	6.67 (1H, dd, 1.5, 8.0)	122.3	6.88 (1H, m)	122.1	7.07 (1H, d, 8.0)	131.8	6.98 (1H d, 8.5)	131.4
$-O-CH_2-O-$	5.97 (2H, s)	101.3	5.95 (2H, s)	101.3	5.96 (2H, s)	101.2				
5-OH	12.92 (1H, s)				13.02 (1H, s)		12.37 (H, s)			
6-CH_3	2.03 (3H, s)	6.2	2.08 (3H, s)	7.3	2.07 (3H, s)	5.75	2.01 (3H, s)	6.7	2.07 (3H, s)	7.1
7-OH	12.98 (1H, s)		12.95 (1H, s)		14.05 (1H, s)					
CHO	10.07 (1H, s)	191.4	10.18 (1H, s)	192.8	10.29 (1H, s)	190.8				
OCH_3			3.90 (3H, s)	62.0			3.74 (3H, s) 3.75 (3H, s)	54.7 60.7	3.72 (3H, s) 3.74 (3H, s) 3.78 (3H, s)	60.2 54.4 60.1

by a MeO signal (δ_H 3.90) in **2**. Therefore, it was assumed that **2** was the methylation product of **1** at C_5-OH. From the HMBC spectrum of **2**, characteristic long-range correlations were observed for δ_H 3.90 (C_5-OCH_3) to C-5 and for δ_H 12.95 (C_7-OH) to C-6 and C-8. In the NOE spectrum, when the methyl signal at δ_H 2.08 was irradiated, the δ_H 3.90 (C_5-OCH_3) and δ_H 12.95 (C_7-OH) signals showed 6.9% and 2.9% enhancements, respectively. When the signal of the aldehyde proton at δ 10.18 was irradiated, only the δ_H 12.95 (C_7-OH) signal showed 2.5% enhancement. Thus, compound **2** was determined to be 5-methoxy-6-methyl-7-hydroxy-8-aldehydo-3-(3′,4′-methylenedioxybenzyl)chroman-4-one, a new compound named ophiopogonanone D.

Compound **3** was obtained as red powder. The molecular formula of $C_{19}H_{14}O_7$ was supported by its HREIMS and ^{1}H and ^{13}C NMR spectra. The IR spectrum showed absorption bands at 3449 (OH) and 1644 (C═O) cm^{-1}. The ^{1}H, ^{13}C, and 2D NMR spectral data (Table 1) of **3** resembled those of **6**. It was assumed that **3** was the 2,3-didehydrogenated product of **1**. The HMBC correlation peaks of δ_H 8.21 to C-4, C-9, and C-10 also supported this assumption, and the substituent on rings A and B of **1** and **3** were the same. Consequently, **3** was established to be 5,7-dihydroxy-6-methyl-8-aldehydo-3-(3′,4′-methylenedioxybenzyl)-chromone, a new compound named ophiopogonone C.

Compound **4** was obtained as a colorless glue-like solid, and its molecular formula was determined as $C_{19}H_{20}O_7$ by HREIMS and ^{1}H and ^{13}C NMR spectra. The IR spectrum showed absorption bands at 3406 (OH) and 1637 (C═O) cm^{-1}. According to the ^{1}H and ^{13}C NMR spectral data (Table 1), **4** was presumed to be a homoisoflavonoid. From the HMBC spectrum of **4**, significant long-range correlations were observed for δ_H 12.37 (C_5-OH) to C-6 and C-11 and for the methoxy group at δ_H 3.75 to C-8. In ring B, three ABX aromatic protons appeared at δ_H 6.42 (1H, dd, J = 8.0 and 2.5 Hz), 6.50 (1H, d, J = 2.5 Hz), and 7.07 (1H, d, J = 8.0 Hz). The HMBC correlation peaks for the two methylene protons of the benzyl group [δ_H 2.69 (1H, dd) and 3.26 (1H, dd)] to δ_C 156.4 and for the methoxy protons [δ_H 3.74 (3H, s)] to δ_C 160.1 showed that a hydroxy group was attached to C-2′ and a methoxy group was attached to C-4′. On the basis of these results, **4** was concluded to be 5,7,2′-trihydroxy-6-methyl-8-methoxy-3-(4′-methoxybenzyl)chroman-4-one, a new compound named ophiopogonanone E.

Compound **5** had the molecular formula $C_{20}H_{22}O_7$ as determined by its HREIMS and ^{1}H and ^{13}C NMR spectra. The IR spectrum showed absorption bands at 3451 (OH) and 1638 (C═O) cm^{-1}. The ^{1}H NMR of **5** showed three methoxy signals at δ 3.72, 3.74, and 3.78 but no downfield hydroxy group. The other ^{1}H and ^{13}C NMR spectral data (Table 1) of **4** and **5** were analogous. Therefore, it was proposed that **5** was the C-5 methyl ether of **4**. From the HMBC spectrum of **5**, the diagnostic long-range correlations were observed for δ_H 3.72 to C-5, for δ_H 2.07 to C-5 and C-7, and for δ_H 3.78 to C-8. Thus, the structure of **5** was elucidated as 5,8,4′-trimethoxy-6-methyl-7,2′-dihydroxy-3-benzylchroman-4-one, a new compound named ophiopogonanone F.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanaco MP-I3 micro melting point apparatus and are uncorrected. Optical rotations were recorded on a JASCO DIP-370 polarimeter. IR spectra were taken on a

Nicolet AVATAR 320 FT-IR spectrometer, and UV spectra were acquired on a Hitachi U-3200 spectrophotometer. NMR spectra were run on a Varian INOVA 500 MHz NMR spectrometer. EIMS and HREIMS spectra were obtained using Finnigan MAT GCQ and Finnigan MAT 95S spectrometers, respectively.

Plant Material. The tubers of *Ophiopogon japonicus* were collected from a market in Taipei and identified by Mr. Jun-Chih Ou, National Research Institute of Chinese Medicine. A voucher specimen (NRICM-01-002) was previously deposited at the herbarium of the National Research Institute of Chinese Medicine, Taipei, Taiwan, Republic of China.

Extraction and Isolation. The dried tubers of *O. japonicus* were extracted with boiling H_2O to give a H_2O extract and residue. The residue was further refluxed with 95% EtOH to provide an EtOH extract, and the H_2O extract was partitioned with EtOAc to give an EtOAc-soluble portion. The EtOH extract and EtOAc-soluble portion were combined and then chromatographed on a Si gel column eluting with a gradient mixture of *n*-hexane−EtOAc (10:1 → 1:4) to give 42 fractions. Fractions 8−10 (*n*-hexane−EtOAc, 4:1) were combined and concentrated under reduced pressure to give a dark brown residue, which was further crystallized from $CHCl_3$-MeOH to yield **1** (5 mg). Fractions 11 and 12 (*n*-hexane−EtOAc, 3:1) were combined and rechromatographed on a Sephadex LH-20 column eluting with MeOH−H_2O (4:1) to yield **2** (2 mg) and **3** (3 mg). Fractions 13−19 (*n*-hexane−EtOAc, 2:1) were combined and further separated on a Si gel (230−400 mesh) column eluting with increasing polarity of *n*-hexane−$CHCl_3$ to give **6** (10 mg). Fractions 20−21 (*n*-hexane−EtOAc, 1:1) were combined and further chromatographed on a Si gel (230−400 mesh) column eluting with *n*-hexane−$CHCl_3$ (1:1) followed by PTLC ($CHCl_3$−EtOAc, 20:1) to afford **8** (6 mg). Fractions 23 and 24 (*n*-hexane−EtOAc, 1:2) were treated in the same manner to produce **7** (4 mg), **4** (1 mg), and **5** (2 mg). In similar fashion, three compounds, *E*-*p*-coumaroyltyramine (10 mg),[10,11] *E*-feruloyltyramine (8 mg),[10,11] and *E*-*p*-coumaroyl-*β*-hydroxytyramine (14 mg),[7] were collected from fractions 35−42 (*n*-hexane−EtOAc, 1:4).

Ophiopogonanone C (1): colorless needles ($CHCl_3$), mp 171−172 °C; UV (MeOH−$CHCl_3$, 1:5) λ_{max} 273 nm; IR (KBr) 3391, 2923, 2839, 1638, 1502, 1318, 1241 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* (%) 356 $[M]^+$ (100), 324 (4), 294 (5), 135 (89); HREIMS *m*/*z* $[M^+]$ 356.0864 (calcd for $C_{19}H_{16}O_7$, 356.0896).

Ophiopogonanone D (2): yellow glue-like solid, mp 65−68 °C; $[\alpha]^{20}_D$ −10.0° (*c* 0.2, MeOH); UV (MeOH) λ_{max} 260.6 nm; IR (KBr) 3438, 2917, 2844, 1677, 1629, 1571, 1477 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* (%) 370 $[M]^+$ (100), 356 (44), 235 (39), 209 (47), 161 (45), 135 (64), 121 (36), 104 (14), 91 (8); HREIMS *m*/*z* $[M^+]$ 370.1051 (calcd for $C_{20}H_{18}O_7$, 370.1052).

Ophiopogonone C (3): red powder, mp 147−149 °C; UV (MeOH−$CHCl_3$, 1:5) λ_{max} 273.4 nm; IR (KBr) 3438, 2923, 2844, 1644, 1582, 1435 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* (%) 354 $[M]^+$ (100), 340 (5), 232 (12), 195 (32); HREIMS *m*/*z* $[M^+]$ 354.0718 (calcd for $C_{19}H_{14}O_7$, 354.0739).

Ophiopogonanone E (4): colorless glue-like solid, mp 62−64 °C; UV (MeOH−$CHCl_3$, 1:5) λ_{max} 296.4 nm; IR (KBr) 3406, 2922, 2849, 1637, 1514, 1472, 1372 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* (%) 360 $[M]^+$ (70), 324 (100), 327 (29), 299 (9), 236 (12), 224 (18), 209 (12), 137 (17); HREIMS *m*/*z* $[M^+]$ 360.1216 (calcd for $C_{19}H_{20}O_7$, 360.1209).

Ophiopogonanone F (5): dark red glue-like solid, mp 75−78 °C; $[\alpha]^{20}_D$ +300° (*c* 0.01, MeOH); UV (MeOH) λ_{max} 286.4 nm; IR (KBr) 3451, 1638 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* (%) 374 $[M]^+$ (62), 356 (100), 341 (29), 237 (36), 169 (11); HREIMS *m*/*z* $[M^+]$ 374.1372 (calcd for $C_{20}H_{22}O_7$, 374.1366).

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