New Isoflavones and Flavanol from *Iris potaninii*

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Two new isoflavones, 6,3′,4′-trimethoxy-7,8,5′-trihydroxyisoflavone (1), 7,4′-dimethoxy-8,3′,5′-trihydroxy-6-*O*-β-D-glucopyranosylisoflavone (2), and 5,3,3′-trihydroxy-7,4′-dimethoxyflavanone (3) have been isolated from the underground parts of *Iris potaninii* along with known isoflavones (4—8) and iriflophenone (9). The structures of the new compounds were determined using NMR and mass spectroscopic methods.

Key words *Iris potaninii*; Iridaceae; isoflavone; flavanone

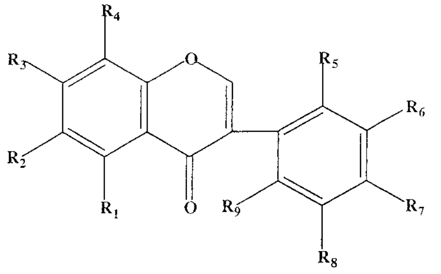
Iris potaninii Mazim. (family Iridaceae) has been used in traditional Mongolian medicine for the treatment of various diseases, such as bacterial infections, cancer, and inflammation. Some benzoquinones isolated from *Iris* species have been used as anticancer agents in modern Chinese medicine.[1,2] In our previous papers we reported that 10 new flavones, a new isoflavone, two new quinones, and five new peltogynoids were isolated and their structures elucidated from other species of the genus.[3—5] This paper describes the structure elucidation of two isoflavones (**1**, **2**) and one flavanone (**3**) isolated from the roots of this plant.

Results and Discussion

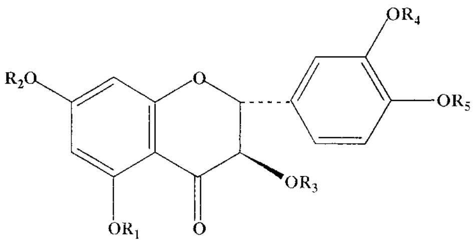
The methanolic extract of the underground parts of *I. potaninii* was subjected to repeated chromatography on columns of silica gel, Labor RP-18, and Sephadex LH-20 and preparative thin-layer and HPLC to obtain compounds **1**—**9**. Compound **1** was isolated as a colorless crystal. The high-resolution electron impact mass spectrum (HR-EI-MS) of **1** showed the $[M]^+$ at *m/z*: 360.0845, in agreement with the molecular formula $C_{18}H_{16}O_8$ (Calcd 360.0831). The ^{1}H-NMR spectrum of **1** showed signals for three aromatic protons, and one olefinic proton along with signals for three methoxy groups. The broad-band decoupled ^{13}C-NMR spectrum showed resonances for all 18 carbons in molecule **1**, which included three methyl, four methine, and 11 quaternary carbons. A singlet at δ: 8.28 (H-2) was coupled with C-2 (δ: 154.3) in the heteronuclear multiple quantum coherence (HMQC) spectrum and H-2 showed long-range couplings with C-3 (δ: 123.6), C-4 (δ: 181.4), and C-1′ (δ: 123.8) (heteronuclear multiple bond correlation (HMBC), supporting information), indicating the isoflavone nature of compound **1** singlet at δ: 6.78 (H-5) was coupled with C-10 (δ: 106.1), C-6 (δ: 153.9) and C-4 (δ: 181.4), doublet at δ 7.03 (H-2′) was coupled with C-2′ (δ: 123.4), C-1′ (δ: 123.8), and C-3′ (δ: 149.9) singlet at δ: 3.98 or 60.5 (OCH_3-4′) was coupled with C-4′ (δ: 149.6).

The UV spectrum by the application of diagnostic shift reagents showed absorbance at λ_{max} (MeOH) (nm): 214, 260, 292, 375 sh, (+$AlCl_3$) 216, 264, 296 sh, 388 (unchanged), (+NaOAc) 272, 392. The bathochromic shift with NaOAc (260→272) indicates the presence of a free hydroxyl group at position C-7,[6] while an absence of large bathochromic shifts in band-I with $AlCl_3$ and NaOMe indicates that the C-5 and C-4′ positions are free or substituted.[6]

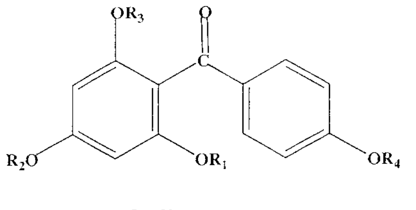
The MS, ^{1}H-, ^{13}C-NMR, HMQC, HMBC, and UV spectra of **1** and their comparison with reference values indicate that compound **1** is 7,8,5′-trihydroxy-6,3′,4′-trimethoxyisoflavone, a new natural product.



1 $R_1 = R_5 = R_9 = H$, $R_2 = R_6 = R_7 = OMe$, $R_3 = R_4 = R_8 = OH$
2 $R_1 = R_5 = R_9 = H$, $R_3 = R_7 = OMe$, $R_4 = R_6 = R_8 = OH$ $R_2 = O$-glc
4 $R_4 = R_5 = R_9 = H$, $R_1 = R_6 = R_7 = R_8 = OMe$, $R_2 = R_3 = OCH_2O$
5 $R_4 = R_5 = R_8 = R_9 = H$, $R_1 = R_7 = OH$. $R_6 = OMe$, $R_2 = R_3 = OCH_2O$
6 $R_4 = R_5 = R_9 = H$, $R_6 = R_7 = OMe$, $R_1 = R_8 = OH$, $R_2 = R_3 = OCH_2O$
7 $R_4 = R_5 = R_8 = R_9 = H$, $R_1 = R_7 = OMe$, $R_6 = OH$, $R_2 = R_3 = OCH_2O$
8 $R_4 = R_5 = R_6 = R_8 = R_9 = H$, $R_1 = OMe$, $R_7 = OH$, $R_2 = R_3 = OCH_2O$



3 $R_1 = R_3 = R_4 = H$, $R_2 = R_5 = Me$



9 $R_1 = R_2 = R_3 = R_4 = H$

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Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Compounds **1**, **2**, and **3**

Carbon	**1**		**2**		**3**	
	^{1}H-NMR δ (ppm) C_5D_5N	^{13}C-NMR δ (ppm) C_5D_5N	^{1}H-NMR δ (ppm) C_5D_5N	^{13}C-NMR δ (ppm) C_5D_5N	^{1}H-NMR δ (ppm) CD_3OD	^{13}C-NMR δ (ppm) CD_3OD
2	8.28 (s, 1H)	154.3	8.16 (s, 1H)	154.2	4.57 (d, 1H, J=12.0)	83.6
3		123.6		124.2	5.01 (d, 1H, J=12.0)	72.4
4		181.4		181.6		195.9
5	6.78 (s, 1H)	117.1	6.86 (s, 1H)	117.7	11.19 (s, OH)	163.6
6		153.9		161.6	6.12 (d, 1H, J=2.1)	96.1
7		150.1		154.5		168.8
8		135.6		135.6	6.06 (d, 1H, J=2.1)	94.7
9		159.1		152.0		162.9
10		106.1		107.7		100.8
1′		123.8		124.6		127.8
2′	7.03 (d, 1H, $J_{2'6'}$=2.1)	123.4	7.06 (d, 1H, J=2.1)	123.1	7.07 (d, 1H, J=2.1)	109.7
3′		149.9		144.5		145.5
4′		149.6		148.2		146.6
5′		135.8		145.6	6.99 (d, 1H, J=6.8)	114.6
6′	7.37 (d, 1H, $J_{6'2'}$=2.1)	135.4	6.99 (d, 1H, J=2.1)	112.4	7.06 (dd, 1H, J=6.8, 2.1)	121.1
3′-MeO	3.88 (s, 3H)	60.3				
4′-MeO	3.98 (s, 3H)	60.5	3.79 (s, 3H)	56.0	3.95 (s,3H)	55.9
6-MeO	3.93 (s, 3H)	56.1				
7-MeO			4.06 (s, 3H)	60.8	3.82 (s, 3H)	55.8
1″			5.09 (d, 1H, J=7.8)	102.0		
2″				74.6		
3″				79.4		
4″				71.2		
5″				78.6		
6″				62.5		

Compound **2** was analyzed for $C_{23}H_{24}O_{14}$ which was supported by $[M+1]^+$ at *m/z*: 525. UV (214, 272, 322 nm) and ^{1}H-NMR (δ: 8.2, 1H, H-2) spectra established that **2** is an isoflavone glucoside.[7] ^{1}H-NMR (C_5D_5N) displayed, in addition to signals for two methoxyl groups and H-2 of an isoflavone nucleus, two doublets at 7.06 (d, 1H, J=2.1 Hz, H-2′) and 6.99 (d, 1H, J=2.1 Hz, H-6′), a singlet at δ: 6.86 (s, 1H, H-5), and a doublet at δ: 5.09 (J=7.8 Hz) integrating for a single proton was assigned to H-1″ of a glucose, indicating a β-linkage. UV shifts with $AlCl_3$, $AlCl_3$+HCl, and NaOAc, characteristic of the presence of a 5,7-dihydroxyl grouping were not observed, indicating an absence or substitution at positions C-5 and C-7.[6] The attachment of glucose at the 6-position is possible, according to UV, ^{1}H-, ^{13}C-NMR, HMQC, and HMBC spectra. All data were compared with reported data.[4—10] ^{13}C-NMR spectrum of **2** showed resonances for all 23 carbons in the molecule (Table 1). The distortionless enhancement by polarization transfer (DEPT) spectrum revealed the presence of two methyl, one methylene, nine methine of which five are sugar residues, and 11 quaternary carbons. The ^{1}H–^{1}H and ^{1}H–^{13}C correlations were determined by two-dimensional (2D) correlation spectroscopy (COSY-45) and HMQC experiments, respectively.

All ^{1}H- and ^{13}C-NMR assignments were further confirmed by HMBC. The most important connectivities in the HMBC spectra were H-2 (δ: 8.16) with C-2 (δ: 154.2), C-3 (δ: 124.3), and C-4 (δ: 181.6) of the C-ring. The aromatic proton of the A-ring also showed the following couplings: H-5 (δ: 6.86) with C-5 (δ: 117.7), C-10 (δ: 107.7), C-6 (δ: 161.6), C-4 (181.6), and H-2′ (δ: 7.06, d, 1H, J=2.1 Hz) with C-2′ (δ: 123.1), C-1′ (δ: 124.6), and C-3′ (δ: 144.5). H-6′ (δ: 6.99, d, 1H, J=2.1 Hz) proton of the B-ring was found to be coupled with C-6′ (δ: 112.4), C-1′ (δ: 124.6), and C-5′ (δ:145.6). Finally, the methoxyl protons (δ: 3.79) were found to be coupled with C-4′ (δ: 148.2) and the methoxyl protons (δ: 4.06) were coupled with C-7 (δ: 154.5), respectively. On the basis of the above studies, the structure 7,4′-dimethoxy-8,3′,5′-trihydroxy-6-*O*-β-D-glucopyranosylisoflavone was deduced for compound **2**, another new isoflavone.

Compound **3** was the minor component of *I. potaninii.* The presence of two doublets in the ^{1}H-NMR spectrum, at 4.57 (d, 1H, J=12.0 Hz, H-2) and at 5.01 (d, 1H, J=12.0 Hz, H-3) indicated that **3** has a flavanone skeleton, which was supported by 2D spectra. HR-MS established the molecular formula of **3** to be $C_{17}H_{16}O_7$, $[M]^+$ at *m/z* 332.0889. Many flavanones with a dihydroxy B-ring, 2′,4′-dihydroxy, 3′,4′-dihydroxy, 2′,6′-dihydroxy, 3′,5′-dihydroxy, and 2′,3′-dihydroxy, are known from our previous paper,[9] but 3′-hydroxy-4′-methoxy substituents on the B-ring are very rare and have not been found in conjunction with a 5-hydroxy-7-methoxy A-ring. The large coupling constant showed a *trans*-diaxial relationship and indicated a flavanonol structure for **3**. The 2*R*:3*R* configuration was confirmed by the CD measurement.[9,11,12] From these data, **3** was determined to be 5,3′,3-trihydroxy-7,4′-dimethoxyflavanone. The positions of two methoxyl signals (3.82, 3.94) were determined by HMBC. However, compound **3** is a new natural product. Compounds **4**—**8** were identified as 3′,4′,5′,5-tetramethoxy-6,7-methylenedioxyisoflavone,[10,13,14] 3′-methoxy-4′,5-dihydroxy-6,7-methylenedioxyisoflavone,[10,11,15] 3′,4′-dimethoxy-5′,5-dihydroxy-6,7-methylenedioxyisoflavone,[16,17] 4′,5-dimethoxy-3-hydroxy-6,7-methylenedioxyisoflavone,[18,19] and 5-methoxy-4′-hydroxy-6,7-methylenedioxyisoflavone[10] on the basis of their NMR spectral data and by comparison of their physical properties with those reported in the literature.

Compound **9** was identified as iriflophenone $C_{13}H_{10}O_5$, (M^+ 246) by comparison of NMR spectral data with reference values.[11] Compounds **4**—**9** were isolated from *I. potaninii* for the first time.

Experimental

General The following instruments were used to obtain physical data: NMR spectra were recorded on a JEOL JNM-A500 spectrometer with tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a JEOL JMS-DX300 spectrometer. Silica gel PSQ-100 (Fuji Silysia Chemical Ltd., 100 mesh), Lichroprep. Labor RP-18 (Merck, 40—63 μm) and Sephadex LH-20 (Pharmacia Biotech) were used for column chromatography. Silica gel $60F_{254}$ and RP-18 (Merck) were used for TLC.

Plant Material The underground part (rhizomes and roots) of *I. potaninii* MAXIM. were collected in September 2001 near Ulaanbaatar, Mongolia. It was identified by Dr. E. Ganbold at the Herbarium of the Institute of Botany, Mongolian Academy of Sciences, where voucher specimens of the plant have been deposited.

Extraction and Isolation Air-dried underground parts (0.5 kg) of *I. potaninii* were extracted three times successively with MeOH. After evaporation of the methanol extract, it was dried to afford 52 g of the crude solid. It was further dissolved in a methanol–water mixture (1 : 1) and partitioned with chloroform, ethyl acetate, and *n*-butanol, respectively, to give chloroform extract (15 g), ethyl acetate extract (10 g), and *n*-butanol extract (20 g). The chloroform extract (15 g) was subjected to silica gel column chromatography with chloroform : methanol (1 : 0→10 : 1). Fractions (10 ml) were collected and those containing the same component were checked on silica gel TLC using the same solvent system (chloroform : methanol) and then combined, evaporated, and recryst alized from MeOH or $CHCl_3$. NMR spectral data of new compounds are given in Table 1.

7,8,5′-Trihydroxy-6,3′,4′-trimethoxyisoflavone (**1**): Amorphous powder, UV λ_{max} (MeOH) (nm): 214, 260, 292 sh, 375 sh; $+AlCl_3$ 216, 264, 296 sh, 388 (unchanged), +NaOAc 272, 392 (H_3BO_3 regenerated the MeOH spectrum), +NaOMe (MeOH spectrum unchanged). ^{1}H- and ^{13}C-NMR (500 MHz, pyridin-d_5) data see: Table 1. EI-MS *m/z*: 360 (100), HR-EI-MS *m/z*: 360.0845 (Calcd 360.0831).

7,4′-Dimethoxy-8,3′,5′-trihydroxy-6-*O*-β-D-glucopyranosylisoflavone (**2**): Amorphous solid, UV λ_{max} (MeOH) (nm): 214, 272, 322, $+AlCl_3$-210, 274, 328, +NaOAc-214, 272, 324. The pseudo molecular ion $[M+1]^+$ was deduced from the positive fast atom bombardment mass spectrum (+ve FAB) at *m/z*: 525. HR-FAB-MS, $C_{23}H_{24}O_{14}$ 524.1473 (Calcd 524.1318). ^{1}H- and ^{13}C-NMR (500 MHz, pyridin-d_5, see Table 1).

5,3′,3-Trihydroxy-7,4′-dimethoxyflavanone (**3**): Yellow amorphous powder, $C_{17}H_{16}O_7$, EI-MS *m/z*: 332 $[M]^+$, HR-EI-MS *m/z*: 332.0889 (Calcd 332.0875). CD curve $[\theta]_{330}$ +7314 (max), $[\theta]_{284}$ −16026 (min), $[\theta]_{256}$ +6576 (max), $[\theta]_{240}$ +4852. ^{1}H- and ^{13}C-NMR (500 MHz, CD_3OD, see Table 1).

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