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Four New Flavones and a New Isoflavone from *Iris bungei*

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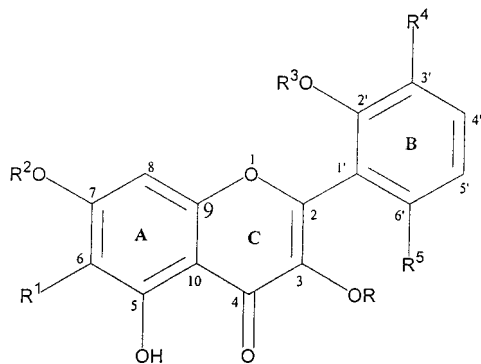
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Four new irisflavones A–D (**1**–**4**) and irilin D (**5**) have been isolated from the underground parts of *Iris bungei* along with known isoflavones, irilins A–B (**6**–**7**) and tlatancuayin (**8**). The structures of the new compounds were determined using NMR and mass spectroscopic methods and were found to be 2',5,7-trihydroxy-3,6-dimethoxyflavone (**1**), 2',5-dihydroxy-3,6,7-trimethoxyflavone (**2**), 2',5,6'-trihydroxy-3,6,7-trimethoxyflavone (**3**), 3,3',5-trihydroxy-2',7-dimethoxyflavone (**4**), and 3',4',5,7-tetrahydroxy-6-methoxyisoflavone (**5**). The structures of irisflavones **1**, **3**, and **4** were confirmed by single-crystal X-ray diffraction studies.

Iris bungei Maxim. (Iridaceae) has been used in Mongolian traditional medicine for the treatment of diseases such as cancer, inflammation, and bacterial infections. Some benzoquinones isolated from several *Iris* species have been used as anticancer agents in modern Chinese medicine.^{1–5} A number of flavones^{6,7} and isoflavones^{8–11} have been isolated from other species of this genus. In our continued phytochemical studies on *I. bungei*,¹² we report here the isolation of four new flavones and an isoflavone.

Results and Discussion

Methanolic extracts of the underground parts of *I. bungei* were subjected to repeated column and thin-layer chromatography to obtain compounds **1**–**8**. Irisflavone A (**1**) was isolated as yellow needles with the molecular formula C₁₇H₁₄O₇, indicating 11 degrees of unsaturation. Strong IR absorptions at 1655 (aromatic C=C) and 1632 cm^{–1} (C=O) along with UV bands at 353, 275, and 202 indicated that the compound had a flavone skeleton with oxygen at C-3.^{13,14}



- 1 R₂ = R₃ = R₄ = R₅ = H, R = CH₃, R₁ = OCH₃
- 2 R₃ = R₄ = R₅ = H, R = R₂ = CH₃, R₁ = OCH₃
- 3 R₃ = R₄ = H, R = R₂ = CH₃, R₁ = OCH₃, R₅ = OH
- 4 R = R₁ = R₅ = H, R₂ = R₃ = CH₃, R₄ = OH

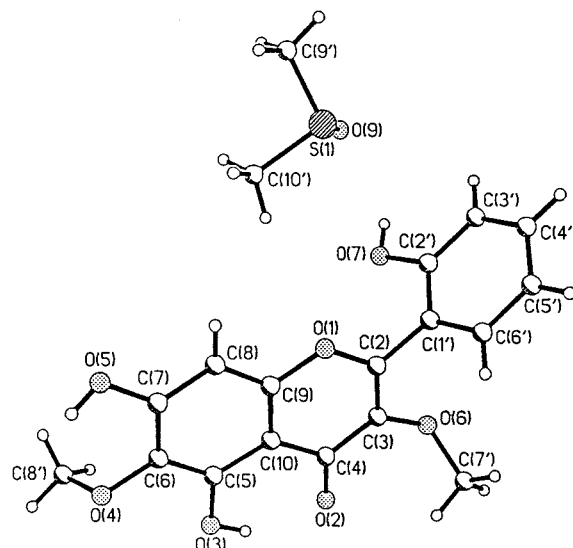


Figure 1. Computer-generated ORTEP drawing of **1**.

The ¹H NMR spectrum of **1** showed signals for five aromatic protons, along with signals for two methoxy groups. The broad-band decoupled ¹³C NMR spectrum showed resonances for all 17 carbons in the molecule **1**. The heteronuclear multiple-bond connectivity (HMBC, Supporting Information)¹⁵ spectrum supported the new structure **1** (2',5,7-trihydroxy-3,6-dimethoxyflavone) for irisflavone A. A single-crystal X-ray diffraction analysis unambiguously confirmed structure **1** for this compound. Hydrogen bonding between the phenolic group at C-5 (ring A) and the oxygen of the C-4 ketonic carbonyl was also apparent in the X-ray structure (Figure 1).

Irisflavone B (**2**), C₁₈H₁₆O₇, exhibited an ¹H NMR spectrum closely resembling that of **1**. The major difference was the presence of an additional methoxy singlet at δ 3.88. The ¹³C NMR spectrum of **2** also had one more methoxy carbon signal at δ 56.4. The downfield chemical shift of C-7 (δ 159.3), as compared to C-7 of compound **1** at δ 155.4, indicated that the third methoxy group was substituted at C-7 of ring-A. The position of the third methoxy group was confirmed by the HMBC experiment. Irradiation of the methoxy signals (δ 3.88) resulted in a significant nuclear Overhauser enhancement (NOE) of the H-8 (δ 6.50). Thus, compound **2** was deduced to be 2',5-dihydroxy-3,6,7-trimethoxyflavone.

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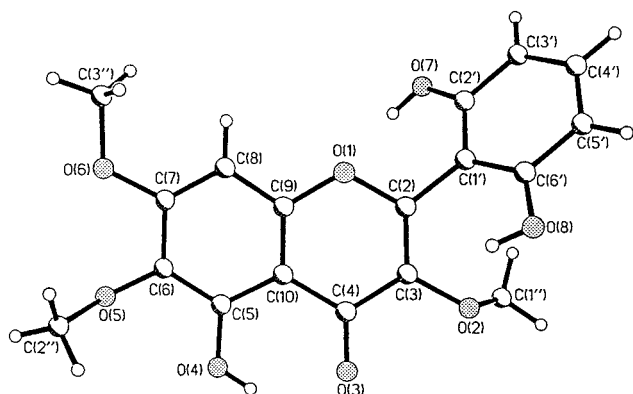


Figure 2. Computer-generated ORTEP drawing of **3**.

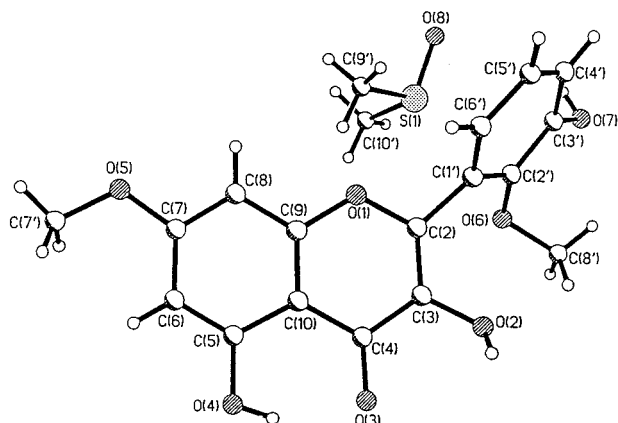
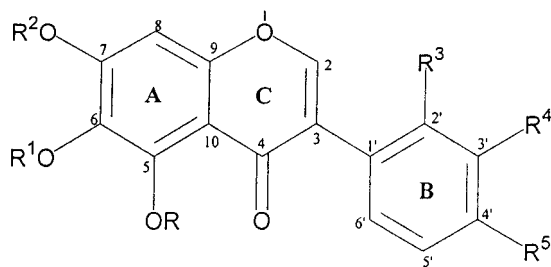


Figure 3. Computer-generated ORTEP drawing of **4**.

Irisflavone C (**3**), $C_{18}H_{16}O_8$, was obtained as yellow needles. The structure was determined by EIMS and single-crystal X-ray diffraction analysis (Figure 2). The structure of **3** was found to be 2',5,6'-trihydroxy-3,6,7-trimethoxyflavone, another new flavonoid.

The 1H NMR spectrum of compound **4** (irisflavone D, $C_{17}H_{14}O_7$) showed signals for five aromatic protons, along with two methoxy group signals. Two one-proton doublets *meta* coupled to each other were assigned to H-6 and H-8 in ring A. A two-proton overlapping multiplet at δ 7.02 was assigned to H-4' and H-6', whereas a broad triplet at δ 6.91 (br t, $J_{5/6,4} = 8.1$ Hz) was due to H-5' of ring B. Irradiation of the methoxy signal at δ 3.70 resulted in NOE enhancements of H-6 and H-8 signals, whereas irradiation of the other methoxy signal had no effect on any aromatic proton. These spectroscopic observations indicated that compound **4** was 3,3',5-trihydroxy-2',7-methoxyflavone. Single-crystal X-ray diffraction analysis confirmed this structure, also a new flavonoid (Figure 3).



- 5 $R = R_2 = R_3 = H$, $R_1 = CH_3$, $R_4 = R_5 = OH$
 6 $R = R_2 = R_4 = R_5 = H$, $R_1 = CH_3$, $R_3 = OH$
 7 $R = R_4 = R_5 = H$, $R_1 = R_2 = CH_3$, $R_3 = OH$
 8 $R_4 = R_5 = H$, $R = CH_3$, $R_1 + R_2 = -CH_2-$, $R_3 = OCH_3$

The 1H NMR spectrum of irilin D (**5**) (Table 1), $C_{16}H_{12}O_7$, showed signals for four aromatic protons, one olefinic proton, and one methoxy methyl. A total of 16 carbons appeared in the ^{13}C NMR spectrum, which included one methyl, five methine, and 10 quaternary carbons. A singlet at δ 8.27 (H-2), was coupled with C-2 (δ 155.3) in the HMQC spectrum, and H-2 showed long-range couplings with C-3 (δ 120.8), C-4 (δ 180.1), and C-1' (δ 120.5) (HMBC, Supporting Information),¹⁵ indicating the isoflavone nature of compound **5**. The MS, 1H NMR, ^{13}C NMR, HMQC, and HMBC spectra of **5** indicated that **5** is 3',4',5,7-tetrahydroxy-6-methoxyisoflavone, a new natural product. This compound was earlier reported as a synthetic product.¹⁶

The known compounds irilins A–B (**6–7**) and tlatan-cuayin (**8**) were isolated for the first time from this plant. These compounds have been previously reported from other species of the genus *Iris*.^{10,17} The structures were identified by comparison with reported data.

Experimental Section

General Experimental Procedures. The UV spectra were measured on a Hitachi U-3200 spectrophotometer. The IR spectra were recorded on a JASCO A-302 spectrophotometer. The 1H NMR spectra were recorded on Bruker AM 400 and AMX 500 NMR spectrometers using a UNIX data system at 400 and 500 MHz, respectively, while ^{13}C NMR spectra were recorded at 100 and 125 MHz on the same instruments using DMSO- d_6 and $CDCl_3$ as solvents, respectively. The LREI and HREIMS were recorded on a JMS HX 110 mass spectrometer with the data system DA 5000. X-ray diffraction experiments were performed on a Nicolet P₄ instrument using monochromated Cu K α radiation ($\lambda = 1.5417$ Å). The crystallographic data of compounds **1**, **3**, and **4** have been deposited at the Cambridge Crystallographic Data Center.¹⁸

Plant Material. The underground parts of *Iris bungei* Maxim. were collected in September 1997 from Choir Somone, Dornogov area of Mongolia. A herbarium specimen of this plant (Voucher No. N659) was deposited at the Botanical Institute of Mongolian Academy of Sciences, Ulaanbaatar, Mongolia.

Extraction and Isolation. The underground dried plant material (100 g) was extracted with MeOH at room temperature for 2 weeks, and the resulting extract was concentrated to a gum (8 g). This gum was loaded onto a Si gel (150 g) column and eluted with $CHCl_3$ –*n*-hexane (8:2) to afford pure irisflavone A (**1**) (21.3 mg, $R_f = 0.532$, $CHCl_3$ –*n*-hexane (9:1) along with a yellow fraction (235 mg). This fraction was again loaded onto a Si gel column (30 g), and the column was again eluted with $CHCl_3$ –*n*-hexane (5:5). This yielded two subfractions, which were then subjected to repeated preparative TLC in $CHCl_3$ –*n*-hexane (7:3) to obtain irisflavone B (**2**) (9.4 mg, $R_f = 0.641$), irisflavone C (**3**) (1.2 mg, $R_f = 0.592$), irisflavone D (**4**) (16.8 mg, $R_f = 0.603$), irilin D (**5**) (12.3 mg, $R_f = 0.586$), irilin A (**6**) (22.1 mg, $R_f = 0.623$), irilin B (**7**) (18.2 mg, $R_f = 0.662$), and irilin C (**8**) (25.8 mg, $R_f = 0.689$).

Irisflavone A (1): yellow needles; UV λ_{max} MeOH (log ϵ) 353 nm (3.96), 275 nm (3.98), 202 nm (4.18); IR ν_{max} KBr cm^{-1} 3235 (OH), 2932 (CH), 1655 (C=O), 1632 (C=C), 1576 (aromatic C=C), 1201 (C–O); 1H and ^{13}C NMR, Table 1; EIMS m/z 330 (100), 315 (68), 299 (36), 167 (42), and 121 (39); HREIMS m/z 330.0785 ($C_{17}H_{14}O_7$, calcd 330.0739).

Crystal Data of Irisflavone A (1). Irisflavone A (**1**) was crystallized from dimethyl sulfoxide (DMSO). A light yellow prismatic crystal with dimensions 0.25 \times 0.33 \times 0.30 mm was selected for crystallographic measurements. Molecular formula = $C_{17}H_{14}O_7$, molecular mass = 330.0582 amu, crystal system = orthorhombic, space group = $P2_12_1$, unit cell dimensions $a = 7.8600(10)$ Å, $b = 10.5380(10)$ Å, $c = 23.255(5)$ Å; volume = 1926.2(5) Å³, density = 1.408 mg/m³, $F(000) = 856$, λ (Cu K α) = 1.5417 Å. Unit cell dimensions were determined by least-squares fit of 20 strong reflections measured at room temperature. The intensity data within 2θ range of 3.5–135°

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

carbon no.	1		2		4		5	
	^1H NMR δ (ppm) CDCl_3	^{13}C NMR δ (ppm) CDCl_3	^1H NMR δ (ppm) CDCl_3	^{13}C NMR δ (ppm) CDCl_3	^1H NMR δ (ppm) $\text{DMSO}-d_6$	^{13}C NMR δ (ppm) $\text{DMSO}-d_6$	^1H NMR δ (ppm) $\text{DMSO}-d_6$	^{13}C NMR δ (ppm) $\text{DMSO}-d_6$
2		155.8		155.4		156.6	8.27 (s)	155.3
3		137.2		137.4		137.2		120.8
4		175.4		175.4		176.5		180.1
5		151.8		152.8		150.4		152.8
6		130.3		132.7	6.37 (d, $J_{6,8} = 2.1$ Hz)	97.4		131.5
7		155.4		159.3		160.6		155.4
8	6.56 (s)	93.5	6.50 (s)	90.5	6.60 (d, $J_{8,6} = 2.1$ Hz)	92.0	6.30 (s)	93.9
9		153.0		153.1		148.2		153.1
10		106.2		106.6		104.5		104.2
1'		118.2		118.2		118.8		120.5
2'		155.5		155.6		164.6	6.62 (d, $J_{2',6'} = 2.2$ Hz)	115.4
3'	7.10 (br d, $J_{3',4'} = 8.3$ Hz)	120.8	7.11 (br d, $J_{3',4'} = 8.3$ Hz)	120.8		145.8		145.4
4'	7.47 (br dt, $J_{4'/3',5'} = 8.3$ Hz, $J_{4',6'} = 2.2$ Hz)	133.3	7.45 (br d, $J_{4'/3',5'} = 8.3$ Hz, $J_{4',6'} = 2.2$ Hz)	133.4	7.02 (m)	123.6		143.8
5'	7.09 (br t, $J_{5'/6',4'} = 8.3$ Hz)	119.7	7.07 (br t, $J_{5'/6',4'} = 8.3$ Hz)	119.8	6.91 (br t, $J_{5'/6',4'} = 8.1$ Hz)	120.8	6.64 (d, $J_{5',6'} = 8.3$ Hz)	118.6
6'	7.67 (dd, $J_{6',5'} = 8.3$, $J_{6',4'} = 2.2$ Hz)	129.5	7.66 (dd, $J_{6',5'} = 8.3$, $J_{6',4'} = 2.2$ Hz)	129.5	7.02 (m)	124.8	6.80 (d, $J_{6',5'} = 8.3$ Hz)	121.8
3-OCH ₃	3.87 (s)	62.2	3.88 (s)	62.3				
6-OCH ₃	4.04 (s)	60.1	3.94 (s)	60.8			3.76 (s)	60.2
7-OCH ₃			3.91 (s)	56.8	3.82 (s)	56.0		
2'-OCH ₃					3.70 (s)	60.2		
5-OH	12.63 (s)		12.71		12.73		12.59	

were collected at 293(2) K. A total of 2552 reflections were recorded, of which 2365 reflections were judged on the basis of $I > 2\sigma(I)$. The structure was solved by direct methods using the SHELXTL program and refined by the full matrix least-squares calculation on F^2 . The final R and R_w factors were 0.0526 and 0.1351, respectively.¹⁸

Irisflavone B (2): yellow needles, mp 205–206 °C; UV λ_{max} MeOH (log ϵ) 349 nm (3.78), 270 nm (3.99), 202 nm (4.21); IR ν_{max} KBr cm^{-1} 3242 (OH), 2905 (CH), 1651 (C=O), 1628 (C=C), 1597 (aromatic C=C), 1205 (C–O); ^1H and ^{13}C NMR, Table 1; EIMS m/z 344 (100), 329 (45), 313 (42), 301 (41), 152 (54), and 121 (48); HREIMS m/z 344.0813 ($\text{C}_{18}\text{H}_{16}\text{O}_7$, calcd 344.0896).

Irisflavone C (3): yellow needle; UV λ_{max} MeOH (log ϵ) 355 nm (3.63), 278 nm (3.88), 212 nm (3.96), 202 nm (4.03); IR ν_{max} KBr cm^{-1} 3315 (OH), 2935 (CH), 1638 (C=O), 1625 (C=C), 1596 (aromatic C=C), 1212 (C–O); EIMS m/z 360 (100), 345 (48), 329 (91), 152 (51), and 137 (47); HREIMS m/z 360.0791 ($\text{C}_{18}\text{H}_{16}\text{O}_8$, calcd 360.0845).

Crystal Data of Irisflavone C (3). Irisflavone C (**3**) was crystallized from CHCl_3 solution by slow solvent evaporation. A light yellow prismatic crystal with dimensions $0.25 \times 0.33 \times 0.30$ mm was selected for crystallographic measurements. Molecular formula = $\text{C}_{18}\text{H}_{16}\text{O}_8$, molecular mass = 360.31 amu, crystal system = monoclinic, space group = C_2/c , unit cell dimensions $a = 13.047(4)$ Å, $b = 20.817(8)$ Å, $c = 12.797(4)$ Å; volume = $3147(2)$ Å³, density = 1.141 mg/m³, $F(000) = 1128$; $\lambda(\text{Cu K}\alpha) = 1.5417$ Å. Unit cell dimensions were determined by least-squares fit of 20 strong reflections measured at room temperature using Cu K α radiation. The intensity data within 2θ range of 3.5 – 135° were collected at 293(2) K. A total of 2791 reflections were recorded, of which 2310 reflections were judged on the basis of $I > 2\sigma(I)$. The structure was solved by direct methods using the SHELXTL program and refined by the full matrix least-squares calculation on F^2 . The final R and R_w factors were 0.0833 and 0.2497, respectively.¹⁸

Irisflavone D (4): amorphous powder; UV λ_{max} MeOH (log ϵ) 361 nm (3.44), 272 nm (3.61), 212 nm (3.84); IR ν_{max} KBr cm^{-1} 3262 (OH), 2909 (CH), 1644 (C=O), 1629 (C=C), 1585 (aromatic C=C), 1208 (C–O); ^1H and ^{13}C NMR, Table 1; EIMS

m/z 330 (100), 229 (42), 167 (44); HREIMS m/z 330.0801 ($\text{C}_{17}\text{H}_{14}\text{O}_7$, calcd 330.0739).

Crystal Data of Irisflavone D (4). Irisflavone D (**4**) was crystallized from DMSO, which yielded yellow prismatic crystals. A crystal with dimensions $0.25 \times 0.33 \times 0.30$ mm was selected for the crystallographic measurements. Molecular formula = $\text{C}_{17}\text{H}_{14}\text{O}_7$, molecular mass = 362.34 amu, crystal system = triclinic, space group = $P1$, unit cell dimensions $a = 6.960(3)$ Å, $b = 9.961(3)$ Å, $c = 13.539(8)$ Å; volume = $914.2(7)$ Å³, density = 1.316 mg/m³, $F(000) = 424$; $\lambda(\text{Cu K}\alpha) = 1.5417$ Å. Unit cell dimensions were determined by least-squares fit of 20 strong reflections measured at room temperature using Cu K α radiation. The intensity data within 2θ range of 3.5 – 135° were collected at 293(2) K. A total of 4048 reflections were recorded, of which 3174 reflections were judged on the basis of $I > 2\sigma(I)$. The structure was solved by direct methods using the SHELXTL program and refined by the full matrix least-squares calculation on F^2 . The final R and R_w factors were 0.0578 and 0.1587, respectively.¹⁸

Irilin D (5): amorphous powder; UV λ_{max} MeOH (log ϵ) 341 nm (3.25), 264 nm (3.52), 203 nm (4.01); IR ν_{max} KBr cm^{-1} 3150 (OH), 2905 (CH), 1725 (C=O), 1680 (C=C), 1490 (aromatic C=C), 1305 (C–O); ^1H and ^{13}C NMR, Table 1; EIMS m/z 316 (100), 301 (79), 298 (40), 182 (31), 167 (91), 135 (40); HREIMS m/z 316.0468 ($\text{C}_{16}\text{H}_{12}\text{O}_7$, calcd 316.0583).

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Supporting Information Available: HMBC data of compounds **1** and **5**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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