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FLAVANONES FROM *IRIS TENUIFOLIA*

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Key Word Index—*Iris tenuifolia*; Iridaceae; flavanones; HMBC; X-ray crystal structure.

Abstract—From the underground part of *Iris tenuifolia* several flavonoids have been isolated. Six new flavanones have been characterized by high resolution mass spectrometry (HR-MS), and ^1H and ^{13}C NMR as 5,2′,3′-trihydroxy-6,7-methylenedioxyflavanone, 5,2′-dihydroxy-6,7-methylenedioxyflavanone, 5,2′,3′-trihydroxy-7-methoxyflavanone, 5,3′-dihydroxy-7,2′-dimethoxyflavanone, 3,5,2′,3′-tetrahydroxy-7-methoxyflavanone and 3,5,3′-trihydroxy-7,2′-dimethoxyflavanone. The crystal and molecular structure of the first flavanone has been established by X-ray crystallography. Copyright © 1997 Published by Elsevier Science Ltd

INTRODUCTION

Iris tenuifolia Pall. (Iridaceae) occurs in Olzitt Somon of the middle Gobi district of Mongolia (Central Asia). Flavonoids have been reported from other *Iris* species [1–5], including several with a 6,7-methylenedioxy moiety from *I. missouriensis* [5] and *I. nigricans* [3]. The present paper deals with the isolation and the structural elucidation of four novel 2*S*-flavanones (**1–4**) and two new 2*R*,3*R*-dihydroflavonols (**5**, **6**).

RESULTS AND DISCUSSION

HPLC of the crude ethanolic extract of the dry underground part of *I. tenuifolia* showed six main phenolic constituents at 254 nm, which were separated by column chromatography and purified by recrystallization.

Compound **1** was the major component of *I. tenuifolia*. The presence of three double doublets in the ^1H NMR, at 2.73 (1H, J = 3.1, 17.7 Hz), at 3.24 (1H, J = 13.4, 17.7 Hz) and at 5.73 (1H, J = 3.1, 13.4 Hz), indicated that **1** had a flavanone skeleton, which was indicated also in the ^1H NMR of **2**. The ^1H NMR of **1** and **2** showed a doublet at 6.07 (2H, J = 7.3 Hz) and 6.08 (2H, J = 8.5 Hz), respectively, which is evidently assignable to a methylenedioxy group. A singlet at 6.30 and 6.32 for **1** and **2**, respectively, could be assigned to one aromatic proton of the A-ring. The HR-MS established the molecular formula of **1** as $C_{16}H_{12}O_7$, and that of **2** as $C_{16}H_{12}O_6$. In the mass spectra of both **1** and **2**, the peak (m/z, 180) due to a fragment ion $C_8H_4O_5$ via the retro-Diels–Alder pathway [6] was observed. These results indicated that **1** and **2** have one hydroxyl group and one methylenedioxy group attached to the A-ring and the number of hydroxyl groups attached to the B-rings of **1** and **2** was two and one, respectively. The position of two hydroxyl groups in the B-ring of **1** was easily determined by the ^1H NMR and the splitting pattern of the aromatic three protons on the B-ring was characteristic of *ortho*- and *meta*-disubstitution. Similarly, **2** had an *ortho*-substitution pattern. All the hydroxyl groups and methylenedioxy groups assignments were confirmed by ^{13}C NMR and 2D-NMR (COSY, HMBC) (see Fig. 1). These results indicated that the hydroxyl group attached to the A-ring of **1**

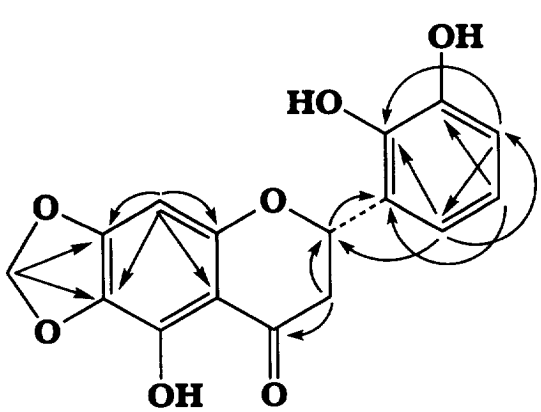

Fig. 1. HMBC spectrum (H → C) of **1**.

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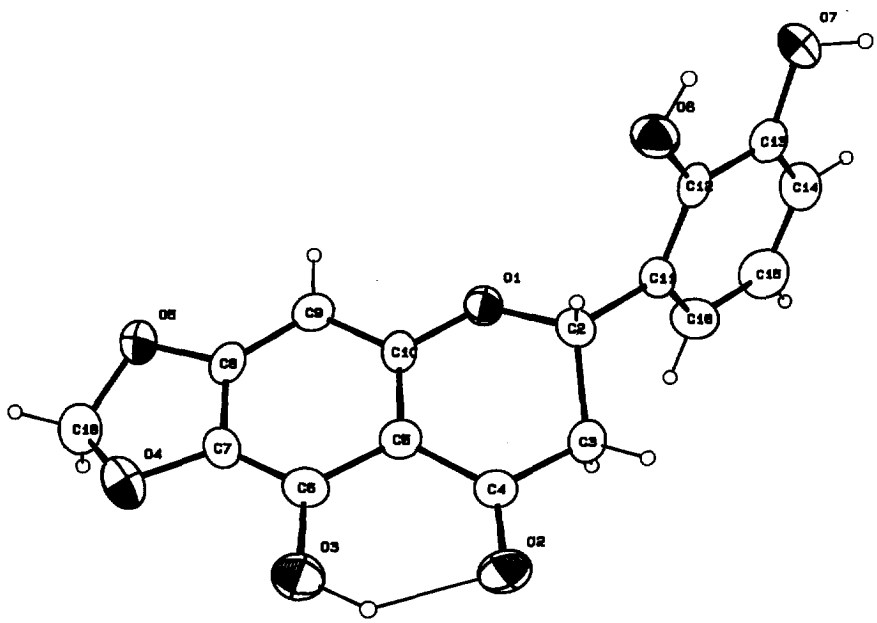


Fig. 2. Perspective view and atom labelling scheme of **1**.

and **2** was located at the 5-position, and that the methylenedioxy group was present at the 6- and 7-positions. The CD spectra of **1** and **2** were strongly negative, indicating that the absolute configuration of 2-position of **1** and **2** was S [7, 8]. The molecular structure of **1** was confirmed by single crystal X-ray diffraction (see Fig. 2), which supports perfectly the above structural elucidations by NMR and MS for **1**. The selected bond lengths of C—O for **1** given in Table 1 are characteristic of the bond nature. Thus, **1** and **2** were determined to be 5,2′,3′-trihydroxy-6,7-methylenedioxyflavanone and 5,2′-dihydroxy-6,7-methylenedioxyflavanone, respectively.

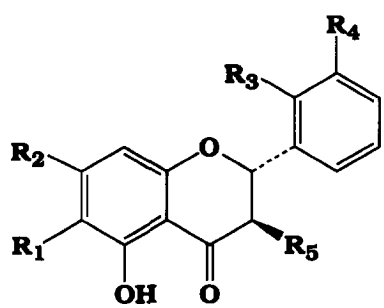
The basic structural similarity of the A- and B-rings for **3**, **4**, **5** and **6** was suggested by the mutual comparison of ^1H and ^{13}C NMR spectral data. The aromatic protons for all four compounds were recorded as *meta*-related doublets for H-6 and H-8 on the A-ring and for H-4′, H-5′ and H-6′ on the B-ring.

Compound **3**, $C_{16}H_{14}O_6$ from HR-MS, differed from **1** in the absence of a methylenedioxy moiety and the presence of one methyl group (3.80 at ^1H NMR). The location of the methoxyl group at the C-7 position

Table 1. Selected bond distances of C—O for **1**

atm 1	atm 2	Distance	atm 1	atm 2	Distance
01	C10	1.361(6)	04	C18	1.444(9)
01	C2	1.460(6)	05	C8	1.365(6)
02	C4	1.242(7)	05	C18	1.452(8)
03	C6	1.357(7)	06	C12	1.358(6)
04	C7	1.389(7)	07	C13	1.367(7)

Numbers in parentheses are estimated standard deviations in the least significant digits.



1 R_1, R_2=OCH_2O, R_3=R_4=OH, R_5=H

2 R_1, R_2=OCH_2O, R_3=OH, R_4=R_5=H

3 R_1=H, R_2=OMe, R_3=R_4=OH, R_5=H

4 R_1=H, R_2=R_3=OMe, R_4=OH, R_5=H

5 R_1=H, R_2=OMe, R_3=R_4=R_5=OH

6 R_1=H, R_2=R_3=OMe, R_4=R_5=OH

was confirmed by the ^{13}C NMR and 2D-NMR (COSY, HMBC). Thus, **3** was determined to be 5,2′,3′-trihydroxy-7-methoxyflavanone. Compound **4**, $C_{17}H_{16}O_6$ from HR-MS, revealed the presence of two methoxyl groups at the 7- and 2′-positions at 3.79 and 3.76 in the ^1H NMR spectrum, respectively. The characterization of **4** by 2D-NMR supported its structure assignment as 5,3′-dihydroxy-7,2′-dimethoxyflavanone. Both **3** and **4** are new flavanones having the absolute configuration of 2S as suggested by

the CD spectra [7, 8]. Compound **5**, showed signals of two protons appearing as an AB-quartet at 5.53 and 4.77 ($J = 10.7$ Hz) assignable to a C-2-H and a C-3-H, respectively. The large coupling constant showed a *trans*-diaxial relationship and indicated a flavanonol structure for **5**. The 2*R*:3*R* configuration was confirmed by the CD measurement [7–9]. From these data, **5** was determined to be 3,5,2′,3′-tetrahydroxy-7-methoxyflavanone. Compound **6** was identified as 3,5,2′-trihydroxy-7,3′-methoxyflavanone from the observation of two methoxyl signals (3.74 and 3.79) and by HMBC.

Many flavanones with a dihydroxy B-ring: 2′,4′-dihydroxy [10], 3′,4′-dihydroxy [11], 2′,6′-dihydroxy [12] and 3′,5′-dihydroxy [13], are known. However, the isomers with the 2′,3′-dihydroxy configuration on the B-ring are very rare, and have not been found in conjunction with a 5-hydroxy-7-methoxy A-ring.

EXPERIMENTAL

General. Mps were uncorr. NMR was measured with TMS as int. standard. The chemical shifts are reported in the ppm scale. TLC and CC were carried out using Merck kiesel gel. HPLC was recorded on a Shimadzu 6A system, (column: ϕ4.6 mm × 15 cm, solvent: 40% aqueous acetonitrile, flow rate: 1 ml/1 min, wave length: 254 nm, column temp. 40°, R_ts: **1**/14.5 min, **2**/25.2 min, **3**/18.2 min, **4**/27.3 min, **5**.8.9 min, **6**/12.2 min).

Plant material. The underground part (rhizomes and roots) of *I. tenuifolia* Pall. were collected in August 1993 from Olzitt Somon of middle Gobi district of Mongolia. It was identified by Dr Ts. Tsebat in the Herbarium of the Institute of Traditional Medicine, Ministry of Health, Mongolia, where voucher specimens of the plant have been deposited.

Extraction and isolation. Air-dried underground parts (250 g) of *I. tenuifolia* were extracted successively with 96% EtOH (× 3). After evaporation of the ethanolic extract, 16 g of the crude solid was subjected to the silica gel CC with $CHCl_3$:MeOH (100:2.5). Fractions (10 ml) were collected and fractions containing the same component, checked by silica gel TLC using $CHCl_3$:MeOH (100:2.5) were combined, evaporated and recryst. from MeOH or $CHCl_3$:MeOH (100:2.5).

X-ray structure determination and refinement. X-ray quality single crystals of **1** were obtained by slow evaporation from soln in BuOH and H_2O. The crystal was examined on an Enraf-Nonius CAD4 Kappa goniometer using Mo-*Kα* radiation. The determination of crystal parameters and intensity collection were routine. Intensity data were corrected for Lorentz and polarization effects but not for absorption ($\mu = 1.19\ cm^{-1}$).

The structure was solved by the direct method and refined by difference Fourier and full-matrix least-squares techniques. The most non-hydrogen atomic positions could be located in an initial E-map. The final model, utilizing anisotropic thermal parameters for all non-hydrogen atoms and fixed parameters for idealized hydrogen atoms, was carried to convergence. Final difference Fourier synthesis for the molecule was judged to be essentially featureless. Complete lists of crystal data, atomic co-ordinates, individual bond lengths and angles, and temp. factors were deposited in the CCDC format.

5,2′,3′-*Trihydroxy*-6,7-*methylenedioxyflavanone* (**1**). *Mp* 225°. $[\alpha]_{21}^{D} -14.6°$ (MeOH; *c* 1.0). CD curve $[\theta]_{343} +4956$ (max), $[\theta]_{313} -7607$ (min), $[\theta]_{303} -6493$ (max), $[\theta]_{283} -17945$ (min). ^{1}H NMR (500 MHz, DMSO *d6*) *δ*: 6.89 (1H, *dd*, $J = 1.2, 7.9$ Hz, H-6′), 6.80 (1H, *dd*, $J = 1.2, 7.9$ Hz, H-4′), 6.69 (1H, *t*, $J = 7.9$ Hz, H-5′), 6.30 (1H, *s*, H-8), 6.07 (2H, *d*, $J = 7.3$ Hz), $-CH_2-$), 5.73 (1H, *dd*, $J = 3.1, 13.4$ Hz, H-2), 3.24 (1H, *dd*, $J = 13.4, 17.7$ Hz, H-3a), 2.73 (1H, *dd*, $J = 3.1, 17.7$ Hz, H-3b). ^{13}C NMR (500 MHz, DMSO *d6*) *δ*: 74.7, 41.2, 197.9, 143.1, 127.4, 155.8, 90.4, 159.4, 103.6, 125.2, 142.6, 145.2, 115.3, 119.1, 117.1, (C-2 ~ C-6′), 102.4 ($-CH_2-$). EIMS (*m/z*): 316 $[M]^+$, 298 $[M-H_2O]^+$, 180 $[M-C_8H_8O_2]^+$. High-resolution EIMS, calcd for $C_{16}H_{12}O_7$ $[M]^+$: 316.0583; Found: 316.0586.

5,2′-*Dihydroxy*-6,7-*methylenedioxyflavanone* (**2**). *Mp* 195°. $[\alpha]_{21}^{D} -51.4°$ (MeOH; *c* 0.5). CD curve $[\theta]_{343} +5219$ (max), $[\theta]_{296} -13012$ (min), $[\theta]_{259} -999$ (max), $[\theta]_{254} -1268$ (min). ^{1}H NMR (500 MHz, DMSO *d6*) *δ*: 7.44 (1H, *dd*, $J = 1.2, 7.9$ Hz, H-6′), 7.20 (1H, *dd*, $J = 1.2, 7.9$ Hz, H-4′), 6.89–6.86 (2H, *m*, H-3′, H-5′), 6.32 (1H, *s*, H-8), 6.08 (2H, *d*, $J = 8.5$ Hz, $-CH_2-$), 5.73 (1H, *dd*, $J = 3.1, 12.8$ Hz, H-2), 3.29 (1H, *dd*, $J = 3.1, 17.1$ Hz, H-3a), 2.75 (1H, *ξdd*, $J = 3.1, 17.1$ Hz, H-3b). ^{13}C NMR (500 MHz, DMSO *d6*) *δ*: 74.7, 41.0, 197.8, 143.0, 127.3, 155.6, 90.3, 159.3, 103.5, 124.3, 154.2, 115.4, 129.4, 119.0, 127.0 (C-2 ~ C-6′), 102.3 ($-CH_2-$), EIMS (*m/z*): 300 $[M]^+$, 282 $[M-H_2O]^+$, 180 $[M-C_8H_8O]^+$. High-resolution EIMS, calcd for $C_{16}H_{12}O_6$ $[M]^+$. 300.0634; Found: 300.0625.

5,2′,3′-*Trihydroxy*-7-*methoxyflavanone* (**3**). *Mp* 190°. $[\alpha]_{21}^{D} -29.7°$ (MeOH; *c* 2.9). CD curve $[\theta]_{330} +6856$ (max), $[\theta]_{283} -32085$ (min), $[\theta]_{249} +3609$ (max), $[\theta]_{242} +2023$ (min). ^{1}H NMR (500 MHz, DMSO-d_6) *δ*: 6.90 (1H, *dd*, $J = 1.2, 7.9$ Hz, H-6′), 6.80 (1H, *dd*, $J = 1.2, 7.9$ Hz, H-4′), 6.70 (1H, *t*, $J = 7.9$ Hz, H-5′), 6.12, (1H, *d*, $J = 2.4$ Hz, H-8), 6.09 (1H, *d*, $J = 2.4$ Hz, H-6), 5.75 (1H, *dd*, $J = 3.1, 12.8$ Hz, H-2), 3.23 (1H, *dd*, $J = 12.8, 17.1$ Hz, H-3a), 2.64 (1H, *dd*, $J = 3.1, 17.7$ Hz, H-3b). ^{13}C NMR (500 MHz, DMSO *d6*) *δ*: 74.2, 41.1, 196.9, 163.2, 94.6, 167.4, 93.7, 163.1, 102.5, 125.3, 142.6, 145.1, 115.2, 119.0, 117.0 (C-2 ~ C-6′). EIMS (*m/z*): 302 $[M]^+$, 284 $[M-H_2O]^+$, 167 $[M-C_8H_7O_2]^+$. High-resolution EIMS, calcd for $C_{16}H_{14}O_6$ $[M]^+$: 302.0790; Found: 302.0792.

5,3′-*Dihydroxy*-7,2′-*dimethoxyflavanone* (**4**). *Mp* 145°. $[\alpha]_{21}^{D} -8.9°$ (MeOH; *c* 1.0). CD curve $[\theta]_{329} +7788$ (max), $[\theta]_{286} -31166$ (min), $[\theta]_{249} +2702$ (max), $[\theta]_{242} +1791$ (min). ^{1}H NMR (500 MHz, DMSO-d_6) *δ*:

6.99–6.92 (2H, *m*, H-5′ H-6′), 6.90 (1H, *dd*, $J = 3.7$, 6.1 Hz, H-4′), 6.11 (1H, *d*, $J = 2.4$ Hz, H-8), 6.10 (1H, *d*, $J = 2.4$ Hz, H-6), 5.76 (1H, *dd*, $J = 3.1$, 12.8 Hz, H-2), 3.30–3.25 (1H, *m*, H-3a), 2.70 (1H, *dd*, $J = 3.1$, 17.1 Hz, H-3b). ^{13}C NMR (500 MHz, DMSO-d_6) δ: 74.1, 41.5, 196.8, 163.3, 94.7, 167.4, 93.8, 162.9, 102.6, 131.8, 145.2, 150.2, 117.3, 124.1, 117.3 (C-2 ~ C-10). EIMS (*m/z*): 316 $[M]^+$, 167 $[M-C_9H_7O_2]^+$. High-resolution EIMS, calcd for $C_{17}H_{16}O_6$ $[M]^+$: 316.0947; Found: 316.0947.

3,5,2′,3′-*Tetrahydroxy*-7-*methoxyflavanone* (**5**). *Mp* 213°. $[\alpha]_{21}^{D}$ 48.2° (MeOH; *c* 2.5). CD curve $[\theta]_{330}+7314$ (max), $[\theta]_{284}-16026$ (min), $[\theta]_{251}+6546$ (max), $[\theta]_{244}+5054$ (min). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 6.83 (1H, *dd*, $J = 1.2$, 7.9 Hz, H-6′), 6.80 (1H, *dd*, $J = 1.2$, 7.9 Hz, H-4′), 6.66 (1H, *t*, $J = 7.9$ Hz, H-5′), 6.11 (1H, *d*, $J = 2.4$ Hz, H-6), 6.08 (1H, *d*, $J = 2.4$ Hz, H-8), 5.53 (1H, *d*, $J = 10.7$ Hz, H-2), 4.77 (1H, *d*, $J = 10.7$ Hz, H-3). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 78.1, 70.4, 198.2, 163.1, 94.8, 167.6, 93.6, 162.7, 101.3, 123.2, 144.4, 145.2, 115.3, 118.7, 118.7 (C-2 ~ C-10). EIMS (*m/z*): 318 $[M]^+$, 300 $[M-H_2O]^+$, 167 $[M-C_8H_7O_3]^+$. High-resolution EIMS, calcd for $C_{16}H_{14}O_7$ $[M]^+$: 318.0740; Found: 318.0744.

3,5,3′-*Trihydroxy*-7,2′-*dimethoxyflavanone* (**6**). *Mp* 186°. $[\alpha]_{21}^{D}$ 47.1° (MeOH; *c* 2.5). CD curve $[\theta]_{331}+7542$ (max), $[\theta]_{294}-16454$ (min), $[\theta]_{251}+5813$ (max), $[\theta]_{246}+4972$ (min). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 7.04–6.95 (2H, *m*, H-5′ H-6′), 6.93 (1H, *dd*, $J = 1.8$, 6.1, 11.6 Hz, H-4′), 6.13 (1H, *d*, $J = 2.4$ Hz, H-6), 6.08 (1H, *d*, $J = 2.4$ Hz, H-8), 5.46 (1H, *d*, $J = 11.6$ Ha, H-2), 4.76 (1H, *dd*, $J = 6.1$ Hz, H-3). ^{13}C NMR (500 MHz, DMSO-d_6) δ: 77.6, 70.6, 198.3, 163.0, 94.8, 167.5, 93.7, 162.4, 101.2, 130.3, 146.6, 150.1, 117.1, 123.7, 118.5 (C-2 ~ C-10). EIMS (*m/z*): 332 $[M]^+$, 167 $[M-C_9H_9O_3]^+$. High-resolution EIMS, calcd for $C_{17}H_{16}O_6$ $[M]^+$: 332.0896; Found 332.0919.

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