Isoflavonoid Glycosides from the Rhizomes of *Iris germanica*

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Four isoflavone glycosides were isolated from the rhizomes of *Iris germanica*. Compounds 1 and 2 are new, while compounds 3 and 4 are known isoflavone glycosides. These compounds were identified as iriskashmirianin 4′-*O*-*β*-D-glucoside (1), nigricin 4′-*O*-*β*-D-glucoside (2), irilone 4′-*O*-*β*-D-glucoside (3) and iridin (4). Their structures were determined with the help of spectroscopic methods.

Key words *Iris germanica*; Iridaceae; isoflavone glycosides; iriskashmirianin 4′-*O*-*β*-D-glucoside; nigricin 4′-*O*-*β*-D-glucoside

Plants of the genus *Iris* (Iridaceae) have been previously recognized as rich sources of secondary metabolites,[1—4] and comprise over 300 species. Some of these are ornamental, and among them, 16 species are found in Pakistan.[5,6] Previous phytochemical investigations of the *Iris* plants have resulted in the isolation of a variety of compounds including flavonoids, isoflavonoides and their glycosides, benzoquinones, triterpenoids and stilbene glycosides.[1,5,7] The compounds isolated from these species were reported to have piscicidal, antineoplastic, antioxidant, anti-tumor, antiplasmodial, and antituberculosis properties.[8—10]

Iris germanica L. is widely distributed in most parts of the world, and is also cultivated as an ornamental plant. Rhizomes of the plant yield an essential oil which is used in perfumes and cosmetics, while the leaves are a rich source of ascorbic acid and vitamins.[6] A number of secondary metabolites from *I. germanica* have been reported earlier.[8,9,11]

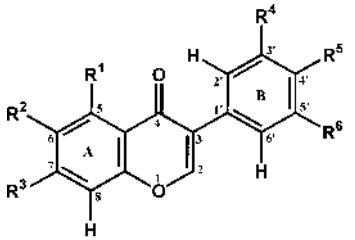
Results and Discussion

The present work on the methanolic extracts of the rhizomes of *Iris germanica* of Turkish origin has resulted in the isolation and characterization of two new compounds **1** and **2**, along with two known compounds **3** and **4**.[11,12]

Germanaism A (**1**) was isolated as a white amorphous solid. A pseudo-molecular ion peak was observed at *m*/*z* 505.1342 (M^++H) in the high resolution Fast Atom Bombardment (HR-FAB)-MS (+ve), consistent with the formula $C_{24}H_{25}O_{12}$ (Calcd $C_{24}H_{25}O_{12}$: 505.1345). FAB-MS also showed an ion at *m*/*z* 343 ($M^+-C_6H_{11}O_5$) resulting from the loss of a hexose moiety. The electron impact (EI)-MS showed fragments at *m*/*z* 147 and 194 due to retro Diels–Alders cleavage of the aglycone part of compound **1**. Strong IR absorptions at 1649 (C=O), 1559 (aromatic C=C), 3328 (OH) and 940 (OCH_2O) cm^{-1}, along with a broad C–O stretching band in the region 1045—1267 cm^{-1} suggested the presence of a sugar moiety. Characteristic UV absorptions at 263 (band II) and 319 (band I) nm indicated the presence of a 5,4′-isoflavone skeleton.[13]

The ^{1}H- and ^{13}C-NMR spectra (DMSO-d_6, 500 MHz) of **1** showed a number of signals characteristic of sugar and isoflavone moieties.[14,15] The ^{1}H-NMR spectrum showed singlets at δ 7.89 (H-2) and 6.52 (H-8) characteristic of a benzopyrane moiety. H-2′ appeared as a doublet at δ 7.07 (d, $J_{2',6'}$=2.0 Hz), showing *meta* coupling with H-6′ which resonated at δ 6.83 (dd, $J_{6',5'}$=8.5 Hz, $J_{6',2'}$=2.0 Hz). H-6′ was *ortho* coupled to H-5′ at δ 6.93 (d, $J_{5',6'}$=8.5 Hz). Two $-OCH_3$ singlets at δ 3.94 and 3.91, along with a 2H singlet for a methylenedioxy at δ 6.10, appeared in the ^{1}H-NMR spectrum. On irradiating the signal at δ 7.07 (H-2′), a nuclear Overhauser effect (NOE) enhancement was observed at δ 3.91 (3′-OCH_3) which indicated that the methoxyl group is adjacent to the C-2′ proton. The ^{1}H-NMR spectrum of the aglycone obtained after the hydrolysis of compound **1** resembled the known compound iriskashmirianin.[16] The anomeric proton signal of the *β*-D-glucose moiety appeared as a doublet at δ 4.73 ($J_{1'',2''}$=7.5 Hz) along with other characteristic signals.

The broad-band (BB) decoupled ^{13}C-NMR spectrum of **1** showed resonances for all twenty-four carbon atoms. A comparative study of the ^{13}C-NMR data with the reported data again indicated that the aglycone part of **1** is the known iriskashmirianin.[16] The presence of *β*-D-glucose was evident from the presence of an anomeric carbon signal at δ 100.1 along with signals for hydroxyl-bearing methines at δ 73.2 (C-2″), 77.0 (C-3″), 71.2 (C-4″) and 76.8 (C-5″), and a methylene carbon at δ 60.6 (C-6″).[17,18] Direct one-bond ^{1}H–^{13}C connectivities were determined from the ^{1}H-detected heteronuclear multiple quantum (HMQC) spectrum. The anomeric δ 4.73 (H-1″) showed HMQC interactions with a signal at δ 100.1 (C-1″). The structure of **1** was finally confirmed by the heteronuclear multiple bond connectivity (HMBC) technique (Fig. 1). The interaction of the anomeric proton (δ 4.73) with C-4′ (δ 146.3) indicated that the sugar unit is connected to C-4′, and this was further confirmed by the interactions of H-2′ (δ 7.07) with C-4′ and C-3′ (δ 148.5). The identity of the sugar as *β*-D-glucose was confirmed through comparison of



1 R^1 R^2 OCH_3, R^3 R^4 OCH_2O, R^5 OGlu, R^6=H
2 R^1=OCH_3, R^2=R^3=OCH_2O, R^5= OGlu, R^4=R^6=H,
3 R^1=OH, R^2=R^3= OCH_2O, R^5= OGlu, R^4=R^6=H
4 R^1–R^4=OH, R^2=R^5=R^6=OCH_3, R^3=OGlu

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the chemical shifts of its carbons with standard reference data.[17,18] The nature of the sugar and aglycone part was further confirmed by hydrolysis (see experimental) and TLC comparsion with the authentic samples of D-glucose and iriskashmirianin, respectively. On the basis of the above spectral and chemical evidences, the structure of **1** was deduced to be 5-methoxy-3-(3′-methoxy-4′-*O*-β-D-glucopyranosyl)-6,7-methylenedioxy-4*H*-1-benzo-pyran-4-one.

Germanaism B (**2**) was isolated as an amorphous solid. The exact molecular ion was measured by FAB-HR-MS (*m/z*: 475.1235, $M+H^+$), which corresponded to the formula $C_{23}H_{23}O_{11}$ (Calcd for $C_{23}H_{23}O_{11}$: 475.1240). The fragment at *m/z* 313 ($M^+-C_6H_{11}O_5$) which appeared in the HR-FAB-MS (+ve) resulted from the loss of a sugar unit from the molecule. The ^{1}H- and ^{13}C-NMR spectra of compound **2** closely resembled those of compound **1**, the only difference being the appearance of two 2H doublets at δ 7.14 ($J_{2',3'}$=9.0 Hz) and 7.43 ($J_{5',6'}$=9.0 Hz). A comparative study of the ^{1}H- and ^{13}C-NMR with the reported data indicated that the aglycone in compound **2** is the known nigricin.[19,20]

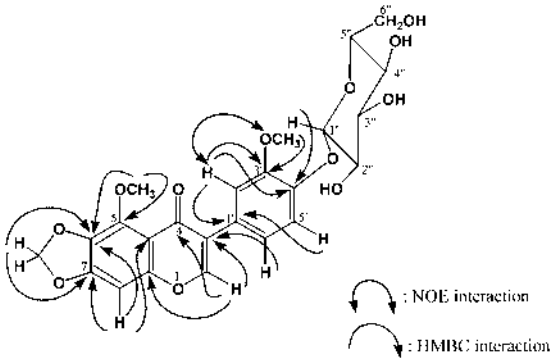

Fig. 1. Selected HMBC and NOE Correlations of **1**

^{1}H–^{1}H and ^{1}H–^{13}C connectivities were established by COSY and HMQC experiments. The structure of **2** was finally deduced on the basis of HMBC connectivities in which the anomeric proton at δ 4.90 (H-1″) showed an interaction with C-4′ (δ 158.9) of aglycon nigricin. This was also confirmed by the NOE difference measurement, where C-5 methoxy protons (δ 4.03) and the H-8 (δ 6.77) signals afforded no mutual NOE effects, indicating their *para* disposition to each other in ring A, separated by a methylenedioxy moiety. The identity of the sugar as β-D-glucose was established through comparison of the NMR chemical shifts with the reference data,[17,18] and by eo-TLC after the hydrolysis of **2** with authentic samples. This confirmed that in compound **2** the aglycone was nigricin[21,22] and the glycone was β-D-glucose.

On the basis of the above spectral and chemical data, compound **2** was identified as 5-methoxy-3-(4′-*O*-β-D-glucopyranosyl)-6,7-methylenedioxy-4*H*-1-benzo-pyran-4-one.

Experimental

The UV spectra were measured on a Hitachi U-3200 spectrophotometer. The IR spectra were recorded on a Jasco A-302 spectrophotometer. Optical rotations were measured with a Schmidt+Haensch Polartronic D polarimeter. The melting points were determined in glass capillary tubes using a Buchi 535 melting point apparatus. The ^{1}H-NMR spectra were recorded on Bruker AM 400 and AMX 500 NMR spectrometers using the UNIX data

Table 1. ^{1}H- and ^{13}C-NMR Chemical Shifts (ppm, *J* in Hz) of Compounds **1**—**4**

C. No.	**1** (DMSO-d_6)		**2** (CD_3OD)		**3** (C_5D_5N)		**4** (CD_3OD)	
2	7.89 (1H, s)	151.7	8.00 (1H, s)	153.2	8.10 (1H, s)	153.6	8.21 (1H, s)	156.1
3	—	123.9	—	126.3	—	123.7	—	124.5
4	—	173.9	—	177.5	—	181.5	—	182.5
5	—	140.5	—	142.4	—	154.5	—	154.7
6	—	135.9	—	136.6	—	135.1	—	134.7
7	—	152.6	—	155.4	—	154.0	—	158.0
8	6.52 (1H, s)	93.6	6.77 (1H, s)	94.0	6.64 (1H, s)	89.5	6.98 (1H, s)	95.6
9	—	153.9	—	156.4	—	158.6	—	154.6
10	—	113.2	—	114.4	—	108.5	—	108.5
1′	—	125.7	—	127.2	—	124.7	—	127.8
2′	7.07 (1H, d, $J_{2',6'}$=2.0)	113.8	7.14 (2H, d, $J_{2',3'}$=9.0)	131.5	7.65 (2H, d, $J_{2',3'}$=9.0)	130.7	6.70 (1H, d, $J_{2',6'}$=2.8)	106.1
3′	—	148.5	7.43 (2H, d, $J_{3',2'}$=9.0)	117.5	7.44 (2H, d, $J_{3',2'}$=9.0)	116.7	—	151.6
4′	—	146.3	—	158.9	—	143.0	—	138.0
5′	6.93 (1H, d, $J_{5',6'}$=8.5)	121.4	7.43 (2H, d, $J_{5',6'}$=9.0)	117.5	7.44 (2H, d, $J_{5',6}$=9.0)	116.7	—	154.5
6′	6.83 (1H, dd, $J_{6',5'}$=8.5, $J_{6',2'}$=2.0)	115.1	7.14 (2H, d, $J_{6',5'}$=9.0)	131.5	7.65 (2H, d, $J_{6',5'}$=9.0)	130.7	6.69 (1H, d, $J_{6',2'}$=2.8)	111.1
1″	4.73 (1H, d, $J_{1'',2''}$=7.5)	100.1	4.90 (1H, d, $J_{1'',2''}$=7.5)	102.0	5.10 (1H, d, $J_{1'',2''}$=7.5)	101.9	5.10 (1H, d, $J_{1'',2''}$=7.3)	102.0
2″	3.41 (1H, t, J=8.0)	73.2	3.45 (1H, m)	74.5	4.39 (1H, m)	74.9	3.44 (1H, m)	71.2
3″	3.49 (1H, t, J=8.0)	77.0	3.46 (1H, m)	78.1	4.44 (1H, m)	78.9	3.51 (1H, m)	78.5
4″	3.34 (1H, t, J=8.5)	71.2	3.41 (1H, m)	74.9	4.36 (1H, m)	71.2	3.55 (1H, m)	74.7
5″	3.30 (1H, m)	76.8	3.39 (1H, m)	77.9	4.16 (1H, m)	78.4	3.47 (1H, m)	77.9
6″a	3.75 (1H, dd, $J_{6'',5''}$=3.0, $J_{6''a,6''b}$=12.0)	60.6	3.90 (1H, dd, $J_{6'',5''}$=2.0, $J_{6''a,6''b}$=12.0)	62.5	4.57 (1H, dd, $J_{6'',5''}$=2.0, $J_{6''a,6''b}$=10.0)	62.3	3.93 (1H, dd, $J_{6'',5''}$=4.4, $J_{6''a,6''b}$=12.0)	62.4
6″b	3.54 (1H, dd, $J_{6'',5''}$=5.0, $J_{6''b,6''a}$=12.0)		3.71 (1H, dd, $J_{6'',5''}$=5.5, $J_{6''b,6''a}$=12.0)		4.55 (1H, dd, $J_{6'',5''}$=2.5, $J_{6''b,6''a}$=10.0)		3.72 (1H, dd, $J_{6'',5''}$=5.7, $J_{6''b,6''a}$=12.0)	
5-OCH_3	3.94 s	55.9	4.03 s	61.3	—	—	—	—
6-OCH_3	—	—	—	—	—	—	3.80 s	61.4
3′-OCH_3	3.91 s	60.7	—	—	—	—	—	—
4′-OCH_3	—	—	—	—	—	—	3.84 s	61.0
5′-OCH_3	—	—	—	—	—	—	3.88 s	56.5
6,7-(OCH_2O)	6.10 s	102.3	6.10 s	104.0	6.12 s	103.3	—	—

system at 300 and 500 MHz, respectively, while the ^{13}C-NMR spectra were recorded at 75, 100 and 125 MHz, respectively, on the same instruments using DMSO-d_6, CD_3OD and pyridine-d_5 as solvents. The FAB and HR-EI-MS were recorded on Jeol JMS 600 and HX 110 mass spectrometers, respectively, with the data system DA 5000. Column chromatography (CC) was carried out on silica gel, 70—230 mesh. TLC purification was carried out on pre-coated TLC plates (silica gel F-254, 0.5 mm, E. Merck). Compounds on the TLC plates were detected at 254 and 366 nm and by ceric sulphate as a spraying reagent.

Plant Material The rhizomes of *Iris germanica* L. were collected from Reyhanli, Hatay, Turkey, in July of 1999, and were air-dried. Voucher specimen (GUE# 2229) was deposited in the Herbarium of the Faculty of Pharmacy, Gazi University, Ankara, Turkey. The plant was identified by one of the authors (B.S.).

Extraction and Isolation Air-dried roots of *I. germanica* (1 kg) were extracted with methanol (15 l) for 15 d at 25 °C. After evaporation of the solvent, an extract (130.2 g) was obtained which was dissolved in distilled water and defatted with hexane. The defatted aqueous extract was further fractionated with $CHCl_3$ (5 l) and then with EtOAc (5 l). On evaporation of the two fractionts, 10.2 and 13.3 g of extracts were obtained, respectively. The EtOAc extract (13.3 g) was subjected to CC on silica gel (70—230 mesh size) and eluted with gradients of pet. ether : $CHCl_3$ and methanol (0—100%, each 3 l) to afford 10 fractions. Repeated column chromatography of the EtOAc fractions on elution with $CHCl_3$: MeOH (92 : 8) afforded compounds **1** (12 mg), **2** (16 mg), **3** (30 mg), and **4** (7.5 mg).

Compounds **1** and **2** were dissolved in a mixture of methanol (2 ml) and 3% H_2SO_4 (2 ml), and heated at 100 °C for 2 h. The solution was neutralized with Na_2CO_3 and extracted with EtOAc. The EtOAc soluble compounds were identified as aglycones of **1** and **2**, as iriskashmirianin and nigricin, respectively, and the sugar in the aqueous phase was identified as glucose by co-TLC with authentic samples of glucose using the solvent system EtOAC : BuOH : H_2O (2 : 7 : 1).[21)]

Germanaism A (**1**): White amorphous solid, (12.0 mg, 1.2×10^{-3}% yield); $[\alpha]_D^{24}$ +61.6° (c=0.83, MeOH); mp 187 °C; IR ν_{max}^{KBr} cm^{-1}: 3328 (OH), 1649 (C=O), 1559 (aromatic. C=C) and 940 (OCH_2O); UV λ_{max} (MeOH) (log ε) nm: 319 (3.423), 263 (3.986); *Rf*: 0.1, $CHCl_3$: MeOH (92 : 8); ^{1}H- and ^{13}C-NMR (DMSO-d_6): Table 1; EI-MS *m/z* (%): 342 (100), 327 (10), 194 (28) and 147 (15). HR-FAB-MS (+ve) *m/z*: 505.1342 (Calcd $C_{24}H_{25}O_{12}$ 505.1345).

Germanaism B (**2**): Amorphous solid, (16.0 mg, 1.6×10^{-3}% yield); $[\alpha]_D^{24}$ +50.2° (c=0.57, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3345 (OH), 1665 (C=O), 1565 (aromatic, C=C) and 935 (OCH_2O); UV λ_{max} (MeOH) (log ε) nm: 319 (3.808), 262 (3.287); *Rf*: 0.14, $CHCl_3$: MeOH (92 : 8); ^{1}H- and ^{13}C-NMR (CD_3OD): Table 1; EI-MS *m/z* (%): 312 (89), 298 (100), 194 (20), 117 (15); HR-FAB-MS (+ve) *m/z*: 475.1235 (Calcd for $C_{23}H_{23}O_{11}$: 475.1240).

Irilone-4′-β-D-glucopyranoside (**3**): Amorphous solid, (30 mg, 3.0×10^{-3}% yield); $[\alpha]_D^{24}$ +47.6° (c= 0.63, MeOH); mp: 154 °C; IR ν_{max}^{KBr} cm^{-1}: 3340 (OH), 1674 (C=O), 1570 (C=C) and 923 (OCH_2O); UV λ_{max} (MeOH) (log ε) nm: 271 (3.556); *Rf*: 0.17, $CHCl_3$: MeOH (92 : 8); ^{1}H- and ^{13}C-NMR data (C_5D_5N): Table 1; EI-MS *m/z* (%): 460 (10), 298 (100), 281 (20), 180 (35), 117 (29); HR-FAB-MS (+ve) *m/z*: 461.1063 (Calcd $C_{22}H_{21}O_{11}$ 461.1083).

Iridin (**4**): White amorphous solid, (7.5 mg, 7.5×10^{-4}% yield); $[\alpha]_D^{24}$ +52.1° (c=0.73, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3335 (OH), 1646 (C=O), 1558 (C=C); UV λ_{max} (MeOH) (log ε) nm: 263 (3.494), 322 (3.523); *Rf*: 0.16 ($CHCl_3$: MeOH (92 : 8); ^{1}H- and ^{13}C-NMR data (CD_3OD): Table 1; EI-MS *m/z* (%): 360 (100), 345 (85), 182 (15), 177 (20); HR-FAB-MS (+ve) *m/z*: 523.1445 (Calcd 523.1451).

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