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THREE ISOFLAVONOIDS FROM *IRIS GERMANICA*

A. A. ALI, N. A. EL-EMARY, M. A. EL-MOGHAZI, F. M. DARWISH and A. W. FRAHM*

Pharmacognosy Department Faculty of Pharmacy, Assiut University, Assiut, Egypt; *Institute of Pharmaceutical Chemistry, Kreuzbergweg 26, 5300 Bonn 1, University of Bonn, West Germany

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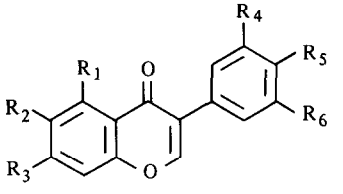
Key Word Index—*Iris germanica*; Iridaceae; 5,3′-dihydroxy-4′,5′-dimethoxy-6,7-methylenedioxyisoflavone; 5-hydroxy-6,4′-dimethoxyisoflavone 7-glucoside and 5,3′-dihydroxy-4′,5′-dimethoxyisoflavone 7-glucoside.

Abstract—From the rhizome of *Iris germanica* one new hexaoxygenated isoflavone, two new polyoxygenated isoflavone glucosides, the known isoflavonoids irisolidone, irigenin and iridin and acetovanillone, sitosterol, α-amyrin and β-amyrin were isolated. The structures of the new compounds were established by chemical and spectroscopic means and by correlation with known constitutents.

INTRODUCTION

Iris germanica L. var. *alba*, is a common ornamental plant in upper Egypt. The chemistry of some related species has been reported [1, 2], but no publications could be traced concerning this variety. The present report† deals with the isolation and structure elucidation of one aglycone (**1**) and two glycosides (**2** and **3**), from the rhizome of the plant. Three known isoflavonoids irisolidone, irigenin [2, 4] and iridin [5, 6] were also characterized, together with acetovanillone [2], sitosterol, α-amyrin and β-amyrin.



	R_1	R_2	R_3	R_4	R_5	R_6
Irigenin	OH	OMe	OH	OH	OMe	OMe
Irisolidone	OH	OMe	OH	H	OMe	H
Iridin	OH	OMe	OGlc	OH	OMe	OMe
1	OH	-O-CH_2-O-		OH	OMe	OMe
2	OH	OMe	OGlc	H	OMe	H
3	OH	H	OGlc	OH	OMe	OMe

RESULTS AND DISCUSSION

The first unknown (**1**), formula $C_{18}H_{14}O_8$ derived from high resolution mass spectrometry, exhibited UV absorption at 270 and 340 nm characteristic of an isoflavonoid [7]; IR absorption bands were found at 1660 (C–O), 1610 (C=C) and 940 cm^{-1} (methylenedioxy). The presence of an aromatic methylenedioxy moiety was supported by the positive Labat test [8] (green colour) and by a sharp two proton singlet at δ6.2 in the ^1H NMR spectrum, which showed additionally two low field singlets at δ9.1 and 12.9 characteristic for the hydroxy protons at position 3′ and 5 respectively. Shifts of two singlet signals for the H-2 and H-8 protons fell in the normal shift region for the isoflavonoid nucleus, which was confirmed by studying the UV spectra with different complexing reagents [7]. The 3′4′5′ oxygenation pattern of ring C could be derived from two *meta* coupled doublets at δ6.55 and 6.70. Methylation of the free hydroxyl groups gave a product with mp and ^1H NMR data identical with those reported for irisflorentin [9] (5,3′,4′,5′-tetramethoxy-6,7-methylenedioxyisoflavone) and the mmp showed no depression. Therefore the structure of **1** is confirmed as 5,3′-dihydroxy-4′,5′-dimethoxy-6,7-methylenedioxyisoflavone.

The second unknown (**2**), showed UV maxima at 268 and 336 nm. The ^1H NMR spectrum revealed the presence of two methoxy groups and a characteristic distorted signal at δ5.1 for one glucosyl proton (ruling out 3-glycosylation and suggesting 7-glycosylation) [10] as well as a characteristic signal for the 5-hydroxy proton of the isoflavonoid nucleus at δ12.8 which disappeared on deuterium exchange. Acid hydrolysis of **2** gave irisolidone [2, 4], which was identified by co-chromatography, mmp and UV and ^1H NMR spectral measurements, and glucose. Thus **2** is identified as irisolidone 7-glucoside (5-hydroxy-6,4′-dimethoxyisoflavone 7-*O*-glucoside). The third unknown compound (**3**) showed UV absorption maxima at 268 and 340 (sh) nm, again indicating an isoflavonoid structure and confirmed by the ^1H NMR spectrum which exhibited a singlet at δ8.4 typical for the H-2 proton. Also it revealed two exchangeable proton signals at δ9.1 (OH-3′) and 12.8 (OH-5) and two methoxy groups. A broad multiplet integrating for six protons at δ3.1–3.3 and a broad signal (1H) at δ5.2, were assigned to the aliphatic protons and the anomeric proton of the sugar moiety, respectively. Acid hydrolysis gave 5,7,3′-trihydroxy-4′,5′-dimethoxyisoflavone and glucose. Thus,

†For previous report see ref. [3].

the structure of **3** could be determined as 5,3′-dihydroxy-4′,5′-dimethoxyisoflavone 7-*O*-glucoside. Because **3** was only present in small amounts complete characterization was not possible but this will be reported in a subsequent paper.

EXPERIMENTAL

All mps are uncorr. The IR spectra were determined from KBr pellets, UV spectra were measured in MeOH, ^{1}H NMR spectra were measured in DMSO using TMS as int. standard. Si gel refers to Si gel (Merck) for CC and Si gel G and Whatman 3 MM for TLC and PPC, respectively. The plant material was obtained from rhizomes cultivated in the Experimental Station of Medicinal Plants of the Faculty of Pharmacy, Assiut University.

Extraction and isolation of the isoflavonoids. The air-dried rhizome (1 kg) was extracted with MeOH and evaporated. The residue was then extracted with Et_2O, EtOAc and then with *n*-BuOH. Each extract was separately dried over dry Na_2SO_4 and concd under red. pres. before chromatography over an SiO_2 column. The Et_2O fraction was eluted with $CHCl_3$ and subsequently with mixture of $CHCl_3$–MeOH gradient up to 20% MeOH to afford: α-amyrin, β-amyrin, sitosterol, acetovanillone, irisolidone, irigenin and **1**. The EtOAc fraction was eluted with $CHCl_3$ then with a gradient of $CHCl_3$–MeOH up to 40% MeOH to afford **2** and iridin. The *n*-BuOH fraction was eluted with $CHCl_3$–MeOH mixtures ending with pure MeOH to afford iridin [5, 6] and **3**.

Characterization of known compounds. Acetovanillone (50 mg) crystallized from C_6H_6 as colourless needles, mp 114–115°; irisolidone (80 mg) crystallized from MeOH as yellow needles, mp 186–187°; irigenin (100 mg) crystallized from MeOH as pale yellow needles, mp 183°; iridin (300 mg) crystallized from MeOH as an amorphous pale yellow powder, mp 207–208°.

The ^{13}C NMR data of irigenin and iridin are given in Table 1, and are almost identical in the range of sp^2-carbon absorptions. An additional six lines for the glucose moiety in iridin appear in the range δ60–100. The doublet signal at δ155 is typical for an unsubstituted C-2 position of the flavon system. The low field shift of the carbonyl carbon at δ180 indicates the C-5 hydroxylation. The unsubstituted C-8 position is identified via the two bond coupling constant of $J = 4$ Hz of the C-8a doublet signal at δ153, which is only found for an oxygenated carbon coupled to a hydrogen atom, which is butressed by two oxygen functions [11]. Signal identification has been done by means of signal multiplicities before and after D_2O exchange, application of shift additivity rules [12] and comparison with partial structures of Caviunin [13] and 3′,4′,5′-trimethoxyisoflavon [14].

Characterization of new compounds. 5,3′-*Dihydroxy*-4′,5′-*dimethoxy*-6,7-*methylenedioxyisoflavone* (**1**). Compound **1** (100 mg) from the Et_2O fraction, was eluted from the SiO_2 column with $CHCl_3$–MeOH (10:1) and recrystallized from MeOH as yellow needles, mp 255–256°, green colour with $FeCl_3$, rose red colour with Mg–HCl. PC R_f 0.63 (15% HOAc), 0.89 (TBA 3:1:1). UV: λ_{max}^{MeOH} (nm): 270, 340 sh. MS *m/z* 358.0711 ($C_{18}H_{14}O_8$, required 358.3136 base peak), 343 $[M-Me]^+$ (53.95), 315 $[M-C_2H_3O]^+$ (26.5), 312 $[M-C_2H_6O]^+$ (7.65), 311 $[M-C_2H_7O]^+$ (6.25), 272 $[M-C_4H_8O_2]^+$ (5.34), 244 $[M-C_5H_6O_3]^+$ (14.12), 181 $[M-C_{10}H_9O_3]^+$ (15.56). ^{1}H NMR (d_6 – DMSO): δ3.6 (3H, *s*, OMe), 3.7 (3H, *s*, OMe), 6.2 (2H, *s*, CH_2<O/O>), 6.6 (2H, 2*d*, H-2′, H-6′), 6.8 (1H, *s*, H-8), 8.4 (1H, *s*, H-2), 9.1 (1H, *s*, exchangeable, OH-3′) and 12.9 (1H, *s*, exchangeable, OH-5).

Methylation of **1** with ethereal CH_2N_2 gave a product with colourless needles, mp 174°; MS *m/z* 386 $[M]^+$. IR, UV and NMR data are identical to that reported for irisflorentin [9] (5,3′,4′,5′-tetramethoxy-6,7-methylenedioxyisoflavone).

Table 1. 20 MHz ^{13}C NMR spectra of irigenin and iridin

	Irigenin		Iridin		Glycoside
Carbon	[δ(ppm)]	Multiplicity	[δ(ppm)]	Multiplicity	[δ(ppm)]
OMe-5′	56.03	*q*	55.95	*q*	—
OMe-4′, OMe-6	60.14	2*q*	59.98	2*q*	—
C-8	94.12	*d*	94.20	*d*	—
C-2′, C-4a	104.88	*dd* + *sd*	104.83	*d*	—
C-6′	110.48	*dd*	110.50	*d*	—
C-3	122.02	*s*	122.11	*s*	—
C-1′	126.29	*s*	125.89	*s*	—
C-6	131.61	*s*	130.88	*s*	—
C-4′	136.57	*s*	136.68	*s*	—
C-3′	150.28	*s*	150.31	*s*	—
C-5′	152.83	*s*	152.44	*s*	—
C-5, C-8a	153.07	2*s*	153.14	2*s*	—
C-2	154.86	*d*	155.41	*d*	—
C-7	157.50	*s*	156.76	*s*	—
C-4	180.41	*s*	178.20	*s*	—
C-1″	—		—		100.32
C-3″*	—		—		77.38
C-5″*	—		—		76.80
C-2″	—		—		73.24
C-4″	—		—		69.80
C-6″	—		—		60.77

*Tentative assignment.

5-Hydroxy-6,4'-dimethoxyisoflavone 7-glucoside (**2**). Compound **2** (65 mg) from the EtOAc fraction was eluted from the SiO_2 column with $CHCl_3$–MeOH (10:3) and crystallized from MeOH as amorphous yellow powder, mp 215–216°. UV λ_{max}^{MeOH} nm: 268, 336 sh. ^1H NMR (d_6-DMSO): δ 3.6 and 3.62 (2 × 3H, 2*s*, 2 OMe groups), 6.9 (1H, *s*, H-8), 7.0 (2H, *d*, 8.5 Hz, H-3', H-5'), 7.5 (2H, *d*, 8.5 Hz, H-2', H-6'), 8.5 (1H, *s*, H-2), 5.1 (1H, *d*, 6.5 Hz, anomeric proton), 3.1–3.5 (6H, *m*, six glucosyl protons), 12.8 (1H, *s*, exchangeable, OH-5). Acid hydrolysis in 10% H_2SO_4 at 100° for 1 hr gave an aglycone which was extracted with EtOAc and crystallized from MeOH to afford yellow needles, mp 186–187°. PC: R_f 0.36 (15% HOAc), 0.93 (TBA 3:1:1). UV λ_{max}^{MeOH} nm: 268, 338 sh. MS *m/z* (rel. int.): 314.0807 ($C_{17}H_{14}O_6$, base peak), 299 $[M-Me]^+$ (50.5), 296 $[M-H_2O]^+$ (44.9), 271 $[M-C_2H_3O]^+$ (34.7). ^1H NMR spectrum was superimposable with that of irisolidone [2, 4] and mmp showed no depression. Sugars were chromatographed with standard markers on Whatman 3 MM paper using EtOAc–pyridine–H_2O (12:5:4) and detected with aniline-hydrogen phthalate.

5,3'-Dihydroxy-4',5'-dimethoxyisoflavone 7-glucoside (**3**). Compound **3** (50 mg) from the *n*-BuOH fraction was eluted from the SiO_2 column with $CHCl_3$–MeOH (1:1) and recrystallized from MeOH to give a pale yellow amorphous powder, mp 213–215°. PC: R_f 0.84 (15% HOAc), 0.54 (TBA 3:1:1). UV λ_{max}^{MeOH} nm: 268, 340 sh. ^1H NMR (d_6-DMSO): δ 3.78 and 3.8 (3H, each, 2*s*, 2 OMe groups), 7.0 (4H, 2*d*, H-8, H-6, H-2', H-6'), 8.4 (1H, *s*, H-2), 5.2 (1H, *d*, anomeric proton), 3.1–3.5 (6H, *m*, six glucosyl protons), 9.1 (1H, *s*, exchangeable, OH-3'), 12.8 (1H, *s*, exchangeable, OH-5). The aglycone was recrystallized from MeOH to afford yellow needles, mp 190°, MS *m/z* 330 $[M]^+$, calculated for $C_{17}H_{14}O_7$, corresponding to 5,7,3'-trihydroxy-4',5'-dimethoxyisoflavone.

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