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Isoflavonoids and flavone glycosides from rhizomes of *Iris carthaliniae*

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Abstract

Two new isoflavonoid biosides, tectorigenin 4′-glucosyl(1→6)glucoside and iristectorigenin B 7-glucosyl(1→6)glucoside, a new isoflavonoid monoside, 4′-methyltectorigenin 7-glucoside and a new flavone glucoside, 6,4′-dimethoxy-5-hydroxyflavone 7-glucoside, together with tectoridin and tectorigenin 4′-glucoside were isolated from rhizomes of *Iris carthaliniae*. The structures of the isolated compounds were determined by NMR spectral analysis.

Keywords: *Iris carthaliniae*; Iridaceae; Isoflavonoid and flavone glycosides; Tectorigenin 4′- and iristectorigenin B 7-glucosyl(1→6)glucoside; 6,4′-dimethoxy-5-hydroxyflavone 7-glucoside

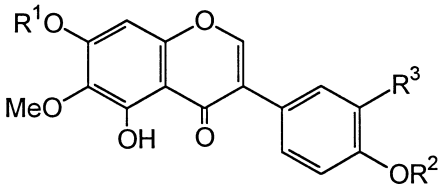
1. Introduction

The genus *Iris* is represented in Egypt by three species of rare occurrence (El Hadidi & Fayed, 1995). Most irises now growing in Egyptian gardens are introduced species or hybrids cultivated mainly for ornamental purposes because of their beautifully colored flowers.

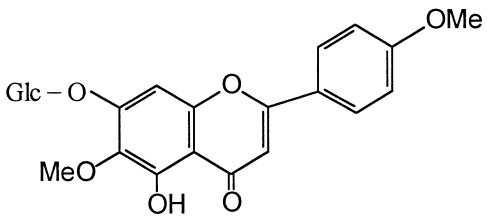
Iris species are known to be rich in isoflavonoids and flavonoids (Arisawa & Morita, 1976; Shawl & Kumar, 1992), in addition to *C*-glycosylxanthones (Arisawa, Morita, Kondo, & Takemoto, 1973; Fujita & Inoue, 1982; Wong, Pezzuto, Fong, & Farnsworth, 1986) and quinones (Wong, Pezzuto, Fong, & Farnsworth, 1985) in some taxa. Other species cultivated in the Assiut area have been the subject of previous phytochemical studies in our laboratory (El-Emary, Kobayashi, & Ogihara, 1980; El-Moghazi, Ali, El-Emary, & Darwish, 1980; Ali, El-Emary, El-Moghazi, Darwish, & Frahm, 1983; Ali, El-Emary, & Darwish, 1993).

2. Results and discussion

Compound **1** had the molecular formula $C_{28}H_{32}O_{16}$, which was established from the FAB-mass spectrum $[M+1]^+$, m/z 625. The ^1H NMR spectrum of **1** displayed



R_1	R_2	R_3
H	Glc – Glc	H
Glc – Glc	H	OMe
Glc	Me	H
Glc	H	H
H	Glc	H



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a characteristic one proton singlet at δ 8.0 for H-2 of an isoflavonoid nucleus, in addition to a sharp singlet (3H) for a methoxyl group at δ 4.0 and a one proton singlet at δ 6.7 for H-8. The spectrum also showed a pair of doublets at δ 7.7 and 7.5 characteristic of a *p*-substituted benzene ring (each 2H, $J=8.8$ Hz). The glucosidic nature of **1** was confirmed from the appearance of two anomeric proton doublets at δ 5.6 and 5.1 ($J=7.3$ Hz) and the high *J* values indicated a β-linkage of the sugar (Markham & Geiger, 1994). The signals for H-6″(a, b) were shifted downfield to δ 4.7 (1H, dd, $J=2.2$, 11.7 Hz) and δ 4.3 (1H, dd, $J=4.4$, 11.7 Hz), respectively, suggesting the presence of a glucosidic linkage at position 6 of the sugar, while the rest of the sugar protons appeared as a multiplet (10 H) between δ 3.8-4.4. The C-2 and C-3 shifts in the ^{13}C NMR spectrum, were in good agreement with the reported values for isoflavonoid compounds and were in accord with the known substitution effects (Markham, Chari, & Mabry, 1982; Agrawal, 1989). The ^{13}C NMR chemical shifts of the sugar carbons were consistent with the corresponding data for two glucose units (Breitmaier & Voelter, 1987). The ^{13}C NMR shifts of the sugar carbons C-6″ and C-1‴, which appeared at δ 69.6 and 105.1, respectively, suggested that the interglucosidic linkage was (1→6).

The HMBC spectrum of **1** showed cross peaks between H-8/C-6/C-7/C-9 (δ 6.7/132.9/154.3/153.9). The proton signal of the methoxyl group was correlated with C-6 confirming its 6 position (δ 3.98/132.9). The proton signal of H-2 showed cross peaks with C-3, C-4 and C-9 respectively (δ 8.0/122.5/180.9/153.9). The proton signals at δ 7.5 and 7.7 were correlated with C-4′ (δ 158.4). The signal of H-1″ at δ 5.5 was correlated with C-4′ (δ 158.4), while H-1‴ (δ 5.1) correlated with C-6″ (δ 69.6) confirming the bioside linkage to be glucose (1→6) glucose. The presence of free hydroxyl groups at C-5 and C-7, was confirmed through application of diagnostic shift reagents (Mabry, Markham, & Thomas, 1970).

Acid hydrolysis of **1** with 5% HCl in methanol, gave glucose and tectorigenin, which were identified by co-PC and co-TLC, with authentic samples.

On the basis of these data, we concluded that **1** is tectorigenin 4′-*O*-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside, a new isoflavonoid bioside.

Compound **2** analyzed for $C_{29}H_{34}O_{17}$ which was supported by its FAB mass spectrum $[M+1]^+$, *m/z* 655. Its ^{1}H NMR spectrum indicated an isoflavonoid nucleus by the appearance of the H-2 signal at δ 8.1. It also displayed signals for two methoxyl groups at δ 3.8 and 4.0, and a characteristic one proton signal for H-8 at δ 6.8. The presence of three aromatic protons was confirmed through an ABX-type coupling at δ 7.3 (1H, dd, $J=8.8$ and 2 Hz, H-6′), δ 7.4 (1H, d, $J=2$ Hz, H-2′) and δ 7.7 (1H, d, $J=8.8$ Hz, H-5′). In addition, the two anomeric protons at δ 5.6 (1H, d, $J=7.3$ Hz, H-1″) and 5.1 (1H, d, $J=7.3$ Hz, H-1‴) indicated that **2** was a bioside with β configuration for the sugar. The ^{13}C NMR spectral data of **2** were almost identical to those of **1** except for the shifts for C-1′∼C-6′. Supporting evidence for the structure of **2** was provided by the HMBC spectral data, which showed the correlation of both H-6′ (δ 7.3) and H-2 (δ 7.4) with C-4′ (δ 147.9). The proton signal of H-5′ (δ 7.7) showed cross peaks with C-3′ (δ 150) and C-1′ (δ 125.8). The H-2 proton signal (δ 8.1) showed correlation with C-3 (δ 122.7), C-9 (δ 153.6) and C-4 (δ 180.9). Also, H-8 (δ 6.8) showed cross peaks with the carbons at position 6 (δ 132.9), 9 (δ 153.6) and 10 (δ 105.5). The protons of the methoxyl groups at δ 3.8 and 4.0 showed HMBC correlation with the carbons at position 3′ and 6, respectively. The HMBC spectrum confirmed the glucosidic linkage at C-7 and the (1→6) interglucosidic linkage. The ^{1}H and ^{13}C NMR data of the aglycone are in good agreement with those reported for iristectorigenin B (Agrawal, 1989). Thus, **2** is characterized as iristectorigenin B 7-*O*-β-D-glucopyranosyl (1→6)-β-D-glucopyranoside, which is reported here for the first time from a plant source.

Compound **3** had the molecular formula $C_{23}H_{24}O_{11}$, which was supported by the FAB mass spectrum, $[M+1]^+$, *m/z* 477. The ^{1}H NMR spectrum established that **3** was an isoflavonoid glucoside (δ 8.1, s, 1H, H-2). It exhibited two singlets (each three protons) at δ 4.04 and 4.06 for two methoxyl groups and a singlet (1H) at δ 7.1 representing H-8. Also, it displayed an A_2B_2 pattern typical of 4′ substituted isoflavones with two doublets at δ 7.3 and 7.7 ($J=8.4$ Hz), which could be assigned to the 3′,5′ and 2′,6′ protons, respectively. A doublet at δ 5.7 (1H, $J=7.3$ Hz) was assigned to H-1″ of a glucose indicating a β-linkage (Markham & Geiger, 1994). ^{13}C NMR spectral data for **3** were in complete accord with the proposed structure. In the HMBC spectrum, the H-8 signal showed distinct cross peaks with the C-7 (δ 157.2), C-9 (δ 153) and C-6 (δ 135.4) signals, which correlated with the methoxyl group signal at δ 4.06. The proton signal at δ 7.7 (H-2′, H-6′) showed a correlation with C-4′ (δ 158.8), which in turn correlated with the proton signal of the methoxyl group at δ 4.04. The H-2 signal (δ 8.1) showed HMBC correlations with C-3 (δ 121.8), C-4 (δ 181.3) and C-9 (δ 153). The glycosidic linkage at position 7 was confirmed by the HMBC correlation of H-1″ (δ 5.7) with C-7 (δ 157.2). Other assignments of **3** were based on previously published spectral data and on comparison with closely related compounds (Markham, Mabry, & Swift, 1970; Devon & Scott, 1975). From the above data, **3** can be identified as 4′-*O*-methyltectorigenin 7-*O*-β-D-glucopyranoside, a new isoflavone glucoside.

Compounds **4** and **5** were identified as tectoridin (the 7-*O*-β-D-glucoside of tectorigenin) and tectorigenin 4′-*O*-β-D-glucoside, respectively. They were identified by comparison of their spectral properties with literature data (Shawl & Kumar, 1992), and by cochromatography with authentic samples.

Compound **6** gave a $[M+1]^+$, *m/z* 477 in its FAB

mass spectrum corresponding to the molecular formula $C_{23}H_{24}O_{16}$. Its ^{1}H NMR spectrum indicated it was a flavone glucoside (δ 6.9, 1H, s, H-3). It showed two methoxyls at δ 4.03 (6H, s), a singlet at δ 7.3 (1H) assignable to H-8 and a pair of doublets at δ 7.9 and 7.2 (each 2H, $J=8.1$ Hz) assignable to the protons located at 2′,6′ and 3′,5′. The anomeric proton H-1″ appeared at δ 5.8 (1H, d, $J=7.3$ Hz), suggesting a β-glucose linkage. The ^{13}C NMR chemical shifts for C-2 and C-3 were in complete accord with those reported for flavones (Markham et al., 1982; Agrawal, 1989). The HMBC spectrum confirmed the placement of the methoxyl groups at positions 6 and 4′ and the glucose unit at position 7. Also, H-3 (δ 6.9) showed a cross peak with C-2 (δ 164.9), confirming the structure of **6** to be 6,4′-dimethoxy-5-hydroxyflavone 7-*O*-β-D-glucopyranoside which is a new compound.

3. Experimental

3.1. General

^{1}H, ^{13}C and 2-D-NMR (HH-COSY, HSQC and HMBC) were measured in pyridine-d_5 using a JEOL α-600 spectrometer. FAB-MS spectra were obtained on a JEOL DX-110 spectrometer.

3.2. Plant material

Iris carthaliniae was grown at the Experimental Station of Medicinal Plants, Assiut University from seeds supplied by the American Iris Society (SIGNA) in July 1991. A voucher specimen has been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Assiut University. The rhizomes were collected in January 1997.

3.3. Extraction and isolation

The fresh rhizomes (2 kg) were cut into small pieces and extracted by maceration in EtOH (90%) at room temp. The residue left after evaporation of the solvent was diluted with H_2O and successively extracted with Et_2O, EtOAc and BuOH.

The BuOH soluble fraction (3 g) was chromatographed over silica gel using $CHCl_3$–MeOH gradients. The fraction eluted with $CHCl_3$–MeOH (8:2) was rechromatographed over Sephadex LH-20 using MeOH. Further purification by PTLC using $CHCl_3$–MeOH–H_2O (13:6:1) yielded pure **1** and **2**.

The EtOAc soluble fraction (5 g) was chromatographed over silica gel using $CHCl_3$–MeOH mixtures. The fraction eluted with $CHCl_3$–MeOH (9:1) was rechromatographed over Sephadex LH-20 using MeOH, and subsequent PTLC using $CHCl_3$–MeOH–H_2O (75:23:2), gave **4** and **5**. The fraction eluted from the column using $CHCl_3$–MeOH (85:15) was subjected to PTLC using $CHCl_3$–MeOH–H_2O (75:23:2) to obtain pure compounds **3** and **6**.

3.4. Compound *1*

Yellow amorphous powder (8 mg), FAB ms $[M+1]^+$, m/z 625 calculated for $C_{28}H_{32}O_{16}$; UV λ_{max}^{MeOH}: 216, 272, 320; $+AlCl_3$: 282, 325 (sh); $+$NaOAc: 280, 315 (sh), 348 nm. ^{1}H NMR spectrum: δ 3.8–4.3 (10 H, m, sugar protons), δ 4.0 (3H, s, OMe), δ 4.3 (1H, dd, $J=4.4$, 11.7 Hz, H-6″b), δ 4.7 (1H, dd, $J=2.2$, 11.7 Hz, H-6″a), δ 5.1 (1H, d, $J=7.3$ Hz, H-1‴), δ 5.5 (1H, d, $J=7.3$ Hz, H-1″), δ 6.7 (1H, s, H-8), δ 7.5 (2H, d, $J=8.8$ Hz, H-3′,5′), δ 7.7 (2H, d, $J=8.8$ Hz, H-2′,6′), δ 8.0 (1H, s, H-2). ^{13}C NMR spectrum: δ 60.3 (q, OMe), δ 62.5 (t, C-6‴), δ 69.6 (t, C-6″), δ 71.0 (d, C-4″), δ 71.5 (d, C-4‴), δ 74.7 (d, C-2″, C-2‴), δ 75.2 (d, C-3‴), δ 77.6 (d, C-3″), δ 78.1 (d, C-5″, C-5‴), δ 95.1 (d, C-8), δ 102.2 (d, C-1″), δ 105.0 (s, C-10), δ 105.1 (d, C-1‴), δ 117.1 (d, C-3′, C-5′), δ 122.5 (s, C-3), δ 125.5 (s, C-3, C-1′), δ 130.8 (d, C-2′, C-6′), δ 132.9 (s, C-6), δ 153.6 (d, C-2), δ 153.9 (s, C-5, C-9), δ 154.3 (s, C-7), δ 158.4 (s, C-4′) and δ 180.9 (s, C-4).

3.5. Compound *2*

Yellow amorphous powder (6 mg), FAB ms $[M+1]^+$, m/z 655 calculated for $C_{29}H_{34}O_{17}$; UV λ_{max}^{MeOH}: 265, 335; $+AlCl_3$: 275, 330; $+$NaOAc: 265, 335 nm. ^{1}H NMR spectrum: δ 3.8 (3H, s, 4″-OMe), δ 3.8–4.3 (10 H, m, sugar protons), δ 4.0 (3H, s, 6-OMe), δ 4.3 (1H, dd, $J=4.4$, 11.7 Hz, H-6″b), δ 4.7 (1H, dd, $J=2.2$, 11.7 Hz, H-6″a), δ 5.1 (1H, d, $J=7.3$ Hz, H-1‴), δ 5.6 (1H, d, $J=7.3$ Hz, H-1″), δ 6.8 (1H, s, H-8), δ 7.3 (1H, dd, $J=8.8$, 2 Hz, H-6′), δ 7.4 (1H, d, $J=2$ Hz, H-2′), δ 7.7 (1H, d, $J=8.8$, H-5′), δ 8.1 (1H, s, H-2). ^{13}C NMR spectrum: δ 56.0 (q, 3′-OCH_3), δ 60.3 (q, 6-OCH_3), δ 62.5 (t, C-6‴), δ 69.5 (t, C-6″), δ 71.0 (d, C-4″), δ 71.5 (d, C-4‴), δ 74.6 (d, C-2″, C-2‴), δ 75.2 (d, C-3‴), δ 77.6 (d, C-3″), δ 78.1 (d, C-5″, C-5‴), δ 95.1 (d, C-8), δ 102.2 (d, C-1″), δ 105.1 (d, C-1‴), δ 105.5 (s, C-10), δ 114.4 (d, C-2′), δ 116.8 (d, C-5′), δ 122.2 (d, C-6′), δ 122.7 (s, C-3), δ 125.8 (s, C-1′), δ 132.9 (s, C-6), δ 147.9 (s, C-4′), δ 150.0 (s, C-3′), 153.6 (s, C-9), δ 153.9 (s, C-5), δ 154.0 (d, C-2), δ 159.7 (s, C-7), δ 180.9 (s, C-4).

3.6. Compound *3*

Yellow amorphous powder (10 mg); FAB ms $[M+1]^+$, m/z 477 calculated for $C_{23}H_{24}O_{11}$; UV_{max}^{MeOH}: 263, 335; $+AlCl_3$: 270, 330; $+$NaOAc: 263, 335 nm. ^{1}H NMR spectrum: δ 4.04 (s, 3H, OMe), δ 4.06 (s, 3H, OMe), δ 4.22 (2H, m, H-4″,5″), δ 4.28 (1H, dd, $J=4.4$, 11.7 Hz, H-6″b), δ 4.34 (1H, dd, $J=8.8$, 9.5 Hz, H-2″), δ 4.4 (1H,

t, $J=9.5$ Hz, H-3″), δ 4.53 (1H, dd, $J=2.2$, 11.7 Hz, H-6″a), δ 5.7 (1H, d, $J=7.3$ Hz, H-1″), δ 7.1 (1H, s, H-8), δ 7.3 (2H, d, $J=8.8$ Hz, H-3′,5′), δ 7.7 (2H, d, 8.8 Hz, H-2′,6′), δ 8.1 (1H, s, H-2). ^{13}C NMR spectrum: δ 60.7 (q, 6-OMe), δ 63.0 (q, 4′-OMe), δ 62.0 (t, C-6″), δ 70.8 (d, C-4″), δ 74.2 (d, C-2″), δ 77.8 (d, C-3″), δ 78.8 (d, C-5″), δ 94.6 (d, C-8), δ 101.6 (d, C-1″), δ 107.3 (s, C-10), δ 116.0 (d, C-3′, C-5′), δ 121.8 (s, C-3), δ 123.1 (s, C-1′), δ 135.4 (s, C-6), δ 153.0 (s, C-9), δ 153.7 (s, C-5), δ 153.8 (d, C-2), δ 157.2 (s, C-7), δ 158.8 (s, C-4′) and δ 181.3 (s, C-4).

3.7. Compound **6**

Yellow amorphous powder (12 mg); FAB ms $[M+1]^+$, m/z 477 calculated for $C_{23}H_{24}O_{11}$; UV_{max}^{MeOH}: 246, 269, 334; $+AlCl_3$: 257, 278, 349, 381; $+AlCl_3+HCl$: 257, 279, 346, 381 nm. ^{1}H NMR spectrum: δ 4.03 (6H, s, OMe), δ 4.22 (2H, m, H-4″,5″), δ 4.28 (1H, dd, $J=4.4$, 11.7 Hz, H-6′b), δ 4.36 (1H, dd, $J=8.8$, 9.5 Hz, H-2″), δ 4.4 (1H, t, $J=9.5$ Hz, H-3″), δ 4.55 (1H, dd, $J=2.2$, 11.7 Hz, H-6″a), δ 5.8 (1H, d, $J=7.3$ Hz, H-1″), δ 6.9 (1H, s, H-3), δ 7.2 (2H, d, $J=8.1$ Hz, H-3′,5′), 7.3 (1H, s, H-8) and δ 7.9 (2H, d, $J=8.1$ Hz, H-2′,6′). ^{13}C NMR: δ 60.7 (q, OMe), δ 62 (t, C-6″), δ 70.8 (d, C-4″), δ 74.3 (d, C-2″), δ77.9 (d, C-3″), δ 78.8 (d, C-5″), δ 95.1 (d, C-8), δ 101.6 (d, C-1″), δ 103.2 (d, C-3), δ 116.1 (d, C-3′,5′), δ 121.7 (s, C-10), δ 123.1 (s, C-1′), 128.8 (d, C-2′, C-6′), δ 133.4 (s, C-6), δ 149.7 (s, C-9), δ 152.9 (s, C-5), δ 157.1 (s, C-7), δ 162.5 (s, C-4′), δ 164.9 (s, C-2) and δ 182.9 (s, C-4).

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