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NEW ISOFLAVONES FROM *IRIS NIGRICANS*

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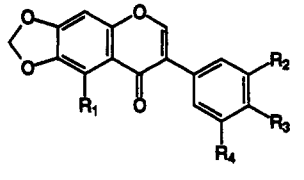
ABSTRACT.—From the rhizomes of *Iris nigricans* (Iridaceae), two new isoflavones, 4′-hydroxy-5-methoxy-6,7-methylenedioxyisoflavone [**1**] (nigricin) and 4′-hydroxy-5,3′-dimethoxy-6,7-methylenedioxyisoflavone [**3**] (nigricanin), were isolated and characterized, along with 5,4′-dihydroxy-6,7-methylenedioxyisoflavone [**4**] (irilone), 5,3′-dihydroxy-4′,5′-dimethoxy-6,7-methylenedioxyisoflavone [**5**], acetovanillone, ferulic acid, β-sitosterol and sucrose. The new structures were established by spectroscopic and chemical methods.

Iris nigricans Dinsmore (Iridaceae) is a perennial herb with a stout and compact rhizome. It is endemic in the fallow fields and steppe habitats of Jordan and is known by the common name "sawsan aswad," which refers to the dark brown-purple color of the flower (1,2). There have been no reports on the chemical constituents of this species. The genus, however, is rich in isoflavonoid compounds (3–6). Isoflavonoids have drawn considerable attention, because they have anti-mutagenic activity (7), and inhibitory effects on protein tyrosine kinase (8) and on pulmonary tumor promotion (9). In addition, cytotoxic compounds have been isolated from *I. missouriensis* (3,4). This paper deals with the isolation and characterization of two new isoflavones, **1** and **3**.

RESULTS AND DISCUSSION

Compound **1**, $C_{17}H_{12}O_6$ from hrms, was obtained as yellow needles and showed significant eims ion peaks at *m/z* 194 and 118 arising from the A and B rings by retro-Diels-Alder fission (10), and the number of carbons was confirmed from its ^{13}C-nmr spectrum. The uv spectrum exhibited absorption maxima at 268 and 344 (sh) nm which are characteristic for a trioxygenated A-ring of an isoflavone derivative (11). The absorption maxima showed no shifts on addition of $AlCl_3$, indicating the absence of a free OH group at C-5 (11); this was also confirmed by the lack of a low-field singlet signal in the ^1H-nmr spectrum (10–13 ppm) (10,11). Ir absorption bands were found at 3220 (free OH), 1668 (C=O), and 940 cm^{-1} (OCH_2O) (5,6).

The presence of a methylenedioxy moiety in **1** was supported by a sharp, two-proton singlet at δ 6.12 in the ^1H-nmr spectrum (12). The ^1H-nmr spectrum also revealed the presence of one MeO group at δ 3.91 and two singlet signals for H-2 and H-8 at δ 8.11



	R_1	R_2	R_3	R_4
1	OCH_3	H	OH	H
2	OCH_3	H	OCH_3	H
3	OCH_3	OCH_3	OH	H
4	OH	H	OH	H
5	OH	OH	OCH_3	OCH_3

and δ 6.71, which fell in the normal chemical shift region for the isoflavone nucleus (12–15, 19). A typical quartet of two doublets (each J=8.74 Hz) was observed at δ 7.13 and δ 7.45 and was assignable to H-3′,5′ and H-2′,6′ with the B-ring oxygenated at C-4′ (6,11). The placement of the MeO group at the C-5 position was confirmed by the ^{13}C-nmr spectrum; methylation of the C-5 OH group shifted the carbonyl carbon (C-4) resonance to its normal position (δ 173.9–175.4) (16,17). Due to the presence of the 5-MeO group, the methylenedioxy group could only be located at either C-6 and C-7 or C-7 and C-8. The chemical shifts of the hydrogen and carbon signals (δ 6.71 and 94.8, respectively) for the A-ring of **1** were compatible with those at C-8 (δ 6.5–6.9 and 90–96) but not at C-6 (δ 6.2–6.4 and 97–100) in the spectra of related flavonoids (12,15). Further confirmation was made by direct comparison with the spectral data of irilone [**4**]. Thus, the structure of **1** was determined as 4′-hydroxy-5-methoxy-6,7-methylenedioxyisoflavone, which was given a trivial name, nigricin. Compound **1** was methylated with ethereal CH_2N_2 to give a monomethyl ether **2** with M^+ *m/z* 326 which represented a difference of 14 mass units between the molecular ions of **1** and **2**. A significant ion peak at *m/z* 132, arising from the B-ring by retro-Diels-Alder fission (10), further indicated that the free OH group must be at C-4′. Compound **2** has been isolated from the bulbs of *Iris tingitana* (18).

Compound **4** was characterized as 5,4′-dihydroxy-6,7-methylenedioxyisoflavone. However, compound **4** was previously isolated from *Trifolium pratense* roots (19) and was named irilone. Our spectral data (uv, ^{1}H nmr, ^{13}C nmr, and ms) supported the structure of **4**. Treatment of **4** with CH_2N_2 gave a dimethyl ether which was identical with **2** in every respect (mp, uv, ms and co-tlc). This is the first report of the isolation of irilone from an *Iris* species. The flavone, kanzakiflavone-2 (5,4′-dihydroxy-6,7-methylenedioxyflavone), has been isolated from an ethereal extract of *Iris unguicularis* (20).

A molecular formula of $C_{18}H_{14}O_7$ from hrms for **3** was suggested by the molecular ion at *m/z* 342, and the number of carbons was confirmed by the ^{13}C-nmr spectrum. The presence of an OH group, an unsaturated carbonyl group, and a methylenedioxy group (3250, 1670, 940 cm^{-1}) was indicated from the ir spectrum (5,6). These data, together with the mass fragment ions at *m/z* 194 and 148, resulting from retro-Diels-Alder fission (10), and uv absorption maxima at 268 and 334 (sh) nm which showed no shifts on addition of $AlCl_3$ reagent, suggested that **3** did not possess a free OH group at C-5 and could be characterized as an isoflavone with a trioxygenated A-ring (11).

The ^{1}H-nmr spectrum of **3** revealed the presence of two MeO groups at δ 3.87 and δ 3.96, a doublet at δ 6.86 (J=8.79 Hz) due to ortho-coupling with H-6′, a double doublet at δ 7.03 (J=8.79, 2.20 Hz) ortho- and meta-coupled with H-5′ and H-2′, and a doublet at δ 7.20 (J=2.20 Hz) meta-coupled with H-6′ (12,15). The ^{13}C-nmr spectrum showed a carbonyl group at C-4 (δ 174.9) which resonated at its normal position and indicated that there was no free OH group at C-5 and that the OH group at C-5 is methylated (16,17). The structural similarity between compounds **1** and **3** in ring A was revealed by comparison of the uv, ^{1}H-nmr and ^{13}C-nmr spectral data. The position of the free OH group at C-4′ and another MeO group at C-3′ was confirmed with the aid of the nOe experiments on **3**. These data suggested that the structure of **3** is 4′-hydroxy-5,3′-dimethoxy-6,7-methylenedioxyisoflavone. This conclusion was further substantiated by ^{1}H-nmr (at δ 8.04) and ^{13}C-nmr (at δ 151.3) signals, which are typically assigned to H-2 and C-2 in isoflavones (δ 7.8–8.1 and 150.6–155.5) (12,14,15). Compound **3** was given the trivial name, nigricanin.

Compound **5** was characterized as 5,3′-dihydroxy-4′,5′-dimethoxy-6,7-methylenedioxyisoflavone by direct comparison with literature data (mp, uv, ^{1}H nmr, and ms) (5). The ^{13}C-nmr spectral data also supported the structure of **5** (14,15).

Compound **5** was first isolated from *Iris germanical* L. var. *alba* (5), and this is the second report of its isolation from an *Iris* species.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Stuart melting point apparatus and are uncorrected. Ir spectra were determined on KBr pellets on a Jasco ir-810 spectrophotometer. Uv spectra were determined on a Unikon 810 spectrophotometer. ^{1}H- and ^{13}C-nmr spectra were taken on a JEOL GX-270 spectrometer using TMS as internal standard. Low-resolution ms were recorded on a Varian MAT 311 instrument. The high-resolution eims were obtained on a Varian MAT, Model CH-5 spectrometer. Si gel (Kieselgel 60-Merck) was used for cc, and Kieselgel 60F_{254} (Merck) was employed for tlc. Anhydrous Na_2SO_4 was routinely used for drying solvent, and all solvents were evaporated under reduced pressure at 40°.

PLANT MATERIAL.—The plant material was collected in the vicinity of Madaba, 40 km south of Amman, Jordan, in April 1990, and identified by Prof. D. Al-Eisawi of the Faculty of Science, Department of Biological Sciences. A herbarium specimen was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, University of Jordan.

EXTRACTION AND FRACTIONATION.—The air-dried and chopped rhizomes (6.4 kg) of *I. nigricans* were extracted by percolation with EtOH (28 liters). After the solvent was evaporated, a syrupy residue (816 g) was obtained. The residue was suspended in H_2O (1.5 liters) and extracted with $CHCl_3$ (1 liter×3) (fraction A, 116.3 g), and with *n*-BuOH (1 liter×3) (fraction B, 178.1 g), respectively.

COLUMN CHROMATOGRAPHY OF FRACTION A.—Fraction A (116.3 g) was chromatographed over a Si gel (325 g) column and eluted with varying proportions of petroleum ether, $CHCl_3$, and MeOH.

ACETOVANILLONE.—Elution of the column (column A) with petroleum ether-$CHCl_3$ (3:7) (1 liter) and (1:4) (0.5 liter) afforded a solid residue (62 mg), which was recrystallized with Me_2CO to give acetovanillone (48 mg), mp 115–116°. The structure was identified by comparison with literature data (mp, ir, ^{1}H nmr, and ms).

4′-HYDROXY-5-METHOXY-6,7-METHYLENEDIOXYISOFLAVONE (NIGRICIN) [**1**].—Continued elution of the column (column A) with $CHCl_3$-MeOH (49:1) (1 liter) and $CHCl_3$-MeOH (4:1) (0.5 liter) afforded a residue (35 mg), which on treatment with MeOH furnished yellow needles of **1** (nigricin) (26 mg): mp 227–229°; uv (MeOH) λ max (log ε) 268 (3.94), 334 (sh) (3.58) nm; ir ν max 3320, 1668, 1618, 1600, 1520, 1492, 1271, 1050, 940, 755 cm^{-1}; ^{1}H nmr (Me_2CO-d_6) δ 3.91 (3H, s, OCH_3), 6.12 (2H, s, OCH_2O), 6.71 (1H, s, H-8), 7.13 (2H, d, J=8.76 Hz, H-3′, 5′), 7.45 (2H, d, J=8.76 Hz, H-2′, 6′), 8.11 (1H, s, H-2); ^{13}C nmr see Table 1; eims *m/z* 312 (M^+, 100) (measured 312.1141, calcd 312.1131 for $C_{17}H_{12}O_6$), 297 (14), 281 (6), 194 (16), 166 (3), 118 (2).

5,4′-DIMETHOXY-6,7-METHYLENEDIOXYISOFLAVONE [**2**].—Compound **1** (9 mg) was treated with ethereal CH_2N_2 for 24 h at room temperature. After removing the solvent, the residue was chromatographed over a small column of Si gel (5 g) with $CHCl_3$-MeOH (9:1) to give a monomethyl ether (**2**) as colorless needles: mp 180–181° (lit. 180–183°) (18); uv (MeOH) λ max 271, 332 (sh) nm; ir ν max 1670, 1616, 1600, 1520, 1270, 1050, 940, 760 cm^{-1}; ^{1}H nmr (Me_2CO-d_6) δ 3.82 (3H, s, MeO), 3.93 (3H, s, MeO), 6.11 (2H, s, OCH_2O), 6.69 (1H, s, H-8), 7.09 (2H, d, J=8.74 Hz, H-3′, 5′), 7.46 (2H, d, J=8.74 Hz, H-2′, 6′), 8.09 (1H, s, H-2); eims *m/z* 326 (M^+, 100), 311 (14), 194 (16), 166 (3), 132 (2).

4′-HYDROXY-5,3′DIMETHOXY-6,7-METHYLENEDIOXYISOFLAVONE (NIGRICANIN) [**3**].—Continued elution of the column (column A) with $CHCl_3$-MeOH (47:3) (0.5 liter) and (23:2) (1.5 liters) yielded a residue (29 mg), which was recrystallized with MeOH to afford **3** (nigricanin) (21 mg): mp 114–115°; uv (MeOH) λ max (log ε) 268 (4.03), 334 (sh) (3.16) nm; ir ν max 3250, 1670, 1620, 1600, 1520, 1495, 1460, 1280, 1220, 1118, 1060, 940, 840, 760 cm^{-1}; ^{1}H nmr (Me_2CO-d_6) δ 3.87 (3H, s, MeO), 3.96 (3H, s, MeO), 6.17 (2H, s, OCH_2O), 6.78 (1H, s, H-8), 6.86 (1H, d, J=8.79 Hz, H-5′), 7.03 (1H, dd, J=8.79, 2.20 Hz, H-6′), 7.20 (1H, d, J=2.20 Hz, H-2′), 8.04 (1H, s, H-2); ^{13}C nmr see Table 1; eims *m/z* 342 (M^+, 100) (measured 342.1131, calcd 342.1120 for $C_{18}H_{14}O_7$), 327 (16), 314 (40), 296 (47), 194 (13), 155 (24), 148 (8), 141 (18), 133 (20).

5,4′-DIHYDROXY-6,7-METHYLENEDIOXYISOFLAVONE (IRILONE) [**4**].—The elution of the column (column A) with $CHCl_3$-MeOH (9:1) (1.5 liters), (22:3) (1 liter) and (17:3) (1.5 liters) gave a residue (84 mg), which showed two spots on tlc. The residue was rechromatographed over Si gel (45 g) (column B) in EtOAc-MeOH (24:1) (0.5 liter) and (47:3) (1 liter) and afforded a residue (23 mg) which was recrystallized from MeOH to give yellow needles of **4** (irilone) (17 mg): mp 221–223°: uv (MeOH) λ max (log ε) 272 (4.24), 334 (sh) (3.38) nm; +$AlCl_3$/HCl 284 (4.21), 332 (sh) (3.73), 374 (sh) (3.38) nm; +NaOMe 282 (4.31), 370

TABLE 1. ^{13}C-Nmr Data of Compounds **1**, **3–5** (in Me_2CO-d_6).

Carbon	Compounds			
	1	**3**	**4**	**5**
C-2	152.1	151.3	154.6	155.1
C-3	123.7	124.7	121.3	122.0
C-4	174.1	174.9	181.6	180.5
C-5	153.4	153.7	153.7	153.9
C-6	141.1	140.1	142.1	141.3
C-7	154.2	155.5	157.1	156.3
C-8	94.8	96.6	93.3	95.3
C-9	149.9	151.3	153.3	157.6
C-10	112.1	115.3	108.6	107.3
C-1′	122.9	125.9	121.5	125.7
C-2′	129.2	113.9	130.9	104.4
C-3′	115.5	147.7	115.2	150.8
C-4′	157.3	148.0	157.2	136.3
C-5′	115.5	115.6	115.2	152.8
C-6′	129.2	122.7	130.9	110.3
CH_2O_2	103.4	130.7	102.9	102.8
C-5–MeO	60.1	61.3	—	—
C-3′–MeO	—	56.3	—	—
C-4′–MeO	—	—	—	59.8
C-5′–MeO	—	—	—	55.7

(sh) (3.38) nm; ^{1}H nmr (Me_2CO-d_6) 6.09 (2H, s, OCH_2O), 6.49 (1H, s, H-8), 6.89 (2H, d, J=8.30 Hz, H-3′, 5′), 7.41 (2H, d, J=8.30 Hz, H-2′, 6′), 7.88 (1H, s, H-2), 12.76 (1H, s, OH-5); ^{13}C nmr see Table 1; eims *m/z* 298 (M^+, 100) (measured 298.1021, calcd 298.1013 for $C_{16}H_{10}O_6$), 281 (7), 181 (36), 180 (42), 152 (24), 118 (11).

CONVERSION OF **4** TO **2**.—Compound **4** (3.5 mg) was treated with ethereal CH_2N_2 for 24 h at room temperature. Evaporation of the solvent yielded a white solid, which was the same as **2** in every respect (mp, ir, ms, and co-tlc).

5,3′-DIHYDROXY-4′,5′-DIMETHOXY-6,7-METHYLENEDIOXYISOFLAVONE [**5**].—The elution of the column (column B) with EtOAc-MeOH (9:1) (1 liter) and (43:7) (0.5 liter) afforded a residue (36 mg), which was treated with MeOH to furnish yellow needles of **5** (31 mg): mp 253°; uv (MeOH) λ max (log ε) 272 (4.10), 340 (sh) (3.02) nm; +$AlCl_3$ 284 (4.04), 320 (sh) (3.62), 380 (sh) (3.14) nm; ir ν max 3400, 1680, 1622, 1600, 1580, 1515, 1462, 1360, 1210, 1040, 940, 810 cm^{-1}; ^{1}H nmr (Me_2CO-d_6) δ 3.79 (3H, s, MeO), 6.17 (2H, s, OCH_2O), 6.68 (2H, d, J=1.75 Hz, H-2′, 6′), 6.78 (1H, s, H-8), 8.27 (1H, s, H-2), 13.00 (1H, s, OH-5); ^{13}C nmr see Table 1.

COLUMN CHROMATOGRAPHY OF FRACTION B.—Fraction B (178.1 g) was adsorbed on Si gel (30 g) and chromatographed over a Si gel (220 g) column (column C) eluted with varying proportions of $CHCl_3$ and MeOH.

Elution of the column (column C) with $CHCl_3$-MeOH (19:1) (1.2 liters) and $CHCl_3$-MeOH (27:24) (1 liter) yielded ferulic acid (32 mg); continued elution of column C with $CHCl_3$-MeOH (82:15) (1 liter) and $CHCl_3$-MeOH (4:1) (2 liters) gave β-sitosterol (167 mg). Finally, the continued elution of column C with $CHCl_3$-MeOH (7:3) (1 liter) gave sucrose (109 mg). All these compounds were identified by comparison (mp, ir, uv, and ms) with literature data (5,21) and with authentic samples which are available in our laboratory (S.A. and D.A.).

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