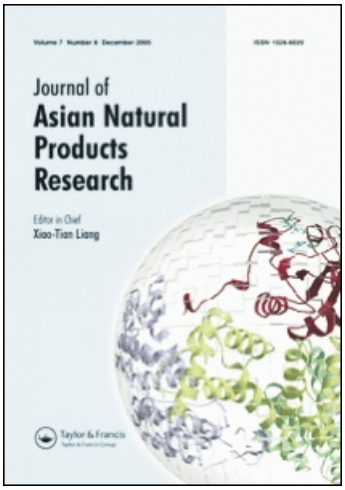

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New isoflavones from *Iris kashmiriana*

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Phytochemical investigation of the rhizomes of *Iris kashmiriana* (Iridaceae) led to the isolation of three isoflavones characterized by 1D and 2D NMR, IR, UV, and MS as 4′-hydroxy-8-methoxy-6,7-methylenedioxyisoflavone (isonigricin, **1**), 5,6-dihydroxy-4′,7-dimethoxyisoflavone (isoirisolidone, **2**), and 5,7-dihydroxy-4′,6-dimethoxyisoflavone (irisolidone, **3**). Compound **1** is a new isoflavone, while **2** is reported for the first time from a natural source.

Keywords: *Iris kashmiriana*; Iridaceae; isoflavones; isonigricin

1. Introduction

Plants of the genus *Iris* comprise over 300 species in the world, known for their ornamental relevance and medicinal value. These species have been introduced as diuretic and expectorant at low doses while as a strong purgative and emetic at high doses, in addition to being useful for pulmonary asthma, cancer, inflammation, liver, and uterus diseases, as well as haemorrhoid and gripe [1,2]. Phytochemical investigation of *Iris* species has resulted in the isolation of a variety of compounds including quinones, triterpenoids, flavonoids, isoflavonoids, and stilbene glycosides [3].

Flavonoids are prominent plant secondary metabolites with variable phenolic structures and are consumed by humans as dietary constituents. Although not considered nutrients and thus essential for life, flavonoid ingestion may play a significant role in health and disease [4,5]. Numerous studies have shown an association between isoflavone-rich dietary consumption and reduced cancer risk, particularly breast and prostate cancers [6–8]. It has been shown that some isoflavones can act as inhibitors of the multidrug resistance transporter MRP1 by influencing the biophysical properties of membranes [9]. The preventive role of isoflavones in cancer [6,10,11], cardiovascular diseases, osteoporosis, and menopausal symptoms in addition to their antioxidant [12,13], antimicrobial [14], anti-inflammatory, and estrogenic activities [7,8] has been largely documented. The estrogenic activities of genistein, daidzen, and equol are currently being extensively investigated at the molecular, preclinical, and clinical levels to determine their potential for the treatment of chronic diseases such as hormone-dependent cancer, cardiovascular diseases, and osteoporosis [15].

The wide spectrum of pharmacological activities associated with the isoflavones prompted us to undertake the phytochemical investigation of *I. kashmiriana*, which is reported to be a rich source of isoflavones

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[16]. The present work deals with the isolation and structure elucidation of three isoflavones **1**–**3** from the rhizomes of *I. kashmiriana.*

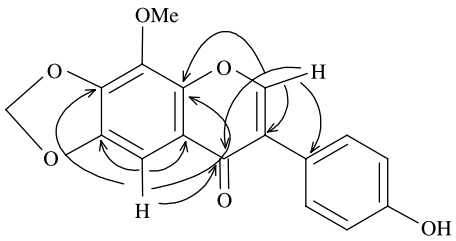
2. Results and discussion

Petroleum ether (60°–80°) extracts and methanolic extracts of the rhizomes of *I. kashmiriana* were subjected to column chromatography on silica gel to obtain a new isoflavone isonigricin (**1**) together with known isoflavones, isoirisolidone (**2**) and irisolidone (**3**). Compound **2**, a known synthetic compound [17], is now reported for the first time from a natural source, while compound **3** isolated previously from other *Iris* species [18] is reported for the first time from the species.

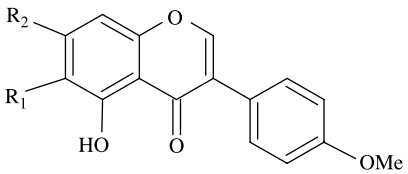
Compound **1** (isonigricin) was obtained as white shiny crystals, m.p. 249°C. Its molecular formula was determined as $C_{17}H_{12}O_6$ by an ion peak at *m/z* 312 $[M]^+$ and elemental analysis. The ^{13}C NMR spectrum showed signals for all the 17 carbons in **1** (Table 1). The DEPT experiment indicated the presence of one methyl, one methylene, and six methines, as well as nine quaternary carbons in the molecule. The UV spectrum of **1** exhibited the absorption maxima at 213, 264, and 322 nm, which are characteristic for an isoflavone structure. In the IR spectrum, prominent absorption bands were found at 3267 (free phenolic OH), 1632 (conjugated C═O), and 946 cm^{-1}(O—C—O). The ^1H NMR spectrum revealed the presence of one OMe group at δ 3.92, one D_2O replaceable singlet at δ 9.60 for a hydroxyl group and a singlet for H-2 at δ 8.20, characteristic of an isoflavone. The correlation of H-2 with C-2 (δ 152.5) in the HSQC spectrum and with C-3 (δ 122.4), C-4 (δ 174.1), C-9 (δ 151.0), and C-1′ (δ 124.2) in the HMBC spectrum further confirmed its isoflavone nature (Figure 1) [2]. A typical four-peak pattern of two doublets (J = 8.4 Hz each) was observed at δ 6.81 and 7.36 and was assigned to H-3′,5′ and H-2′,6′, respectively [5]. In addition to these, a two-proton singlet at δ 6.12 in the ^1H NMR spectrum revealed the presence of the methylenedioxy group at position C-6,7 (ring A) in **1** [19]. On acetylation, **1** gave a monoacetate (**1-OAc**). In the ^1H NMR spectrum (DMSO-d_6), the chemical shift of the proton in ring A of **1-OAc** remained virtually unchanged with respect to **1**, whereas the signals for the protons of the ring B in **1-OAc** were observed at δ 7.17 and

Table 1. ^{13}C NMR spectral data (125 MHz) of compounds **1** (DMSO-d_6) and **2** ($CDCl_3$).

	δ (ppm)	
Carbon no.	**1**	**2**
2	152.5	155.2
3	122.4	122.9
4	174.1	181.3
5	103.5	152.6
6	135.9	130.4
7	153.9	153.5
8	140.4	93.21
9	151.0	152.8
10	113.2	106.5
O—CH_2—O	102.6	–
1′	124.2	123.1
2′	130.2	130.2
3′	114.8	114.1
4′	157.1	159.8
5′	114.8	114.1
6′	130.2	130.2
OMe	60.8	55.4, 56.9



1



2: R_1 = OH, R_2 = OMe

3: R_1 = OMe, R_2 = OH

Figure 1. Structures of compounds **1**–**3** and key HMBC correlations of isonigricin (**1**).

7.54, respectively, for 3′, 5′- and 2′, 6′-aryl protons. The signal for H-2 in **1-OAc** was observed at δ 8.33, establishing thereby the presence of the OH group in ring B at the 4′ position. A one-proton singlet at δ 7.02 in the ^{1}H NMR spectrum of **1** was assigned to C-5, instead of at C-8, on the basis of the fact that H-8 of 6,7-dioxygenated ring A is found at δ 6.30–6.70 [20]. This is also supported by the HSQC and HMBC experiment of **1** (Figure 1). In the HSQC spectrum, a proton singlet at δ 7.02 couples with C-5 (δ 103.5), while, as in the HMBC spectrum, its coupling with C-4 (δ 174.1) confirms its placement at C-5. Therefore, the signal at δ 3.92 (singlet) was assigned to the OMe group at the C-8 position. Hence, the structure of **1** was determined as 4′-hydroxy-8-methoxy-6,7-methylenedioxyisoflavone, a new natural product.

Compound **2** was identified as 5,6-dihydroxy-4′,7-dimethoxyisoflavone (isoirisolidone) by comparing its spectral data with those available in the literature [17]. This isoflavone is reported for the first time from a natural source. Compounds **3**, **4**, and **5** were identified as irisolidone [18], β-sitosterol, and acetovanillone [16], respectively, on the basis of their physical and spectral data.

3. Experimental

3.1 General experimental procedures

Melting points were measured with a Buchi 570 apparatus and are uncorrected. The UV spectra were recorded on a Shimadzu UV-1650PC spectrophotometer. The IR spectra were taken on a Perkin–Elmer Paragon-1000 spectrometer. The ^{1}H and ^{13}C NMR, along with the 2D NMR, spectra were obtained on a Bruker 200 and 500 MHz spectrometer, using TMS as an internal standard. EIMS were measured with JOEL-MSD300 and Bruker Esquire 3000 mass spectrometers. Separation and purification was performed by column chromatography on silica gel (60–120 mesh size; E. Merck) and TLC on silica gel 60 F_{254} plates (0.25 mm). Detection of spots was done by using UV light or ceric ammonium sulfate solution.

3.2 Plant material

The rhizomes of the plant *I. kashmiriana* were purchased from Nehru Botanical Garden, Srinagar (J&K, India), and identified by Dr Ghulam Hassan Dar at Institute of Plant Taxonomy, University of Kashmir. A voucher specimen (accession no. 13595 and collection no. 1207 GH DAR) is deposited in the herbarium of the same institute.

3.3 Extraction and isolation

Powdered rhizomes of the air-dried plant material (1.5 kg) were extracted with petroleum ether (60–80°C; Soxhlet, 48 h), and the extract was concentrated and reduced to 250 ml volume and left overnight at room temperature (20–22°C) when a pale yellow solid precipitated out and was filtered. The crude material was dissolved in a mixture of chloroform:hexane (7:3) and left at 0°C to give a crystalline solid, which by recrystallization in the same solvent system afforded fine needles of compound **2** (138 mg). The filtrates obtained above were pooled and concentrated, and the residue (28.3 g) was obtained. Out of this, 20 g were charged on a silica gel column (400 g, 60–120 mesh), eluted with an increasing gradient of *n*-hexane:chloroform mixture to afford a mixture of fatty acids (not identified), compound **2** (40 mg), compound **3** (9 mg), compound **4** (91 mg), and compound **5** (73 mg).

The defatted rhizomes were extracted with methanol (Soxhlet, 36 h) and the extract desolventized and lyophilized (using Christ Alpha 1-4 lyophilizer) to give a dried material (68 g). Chromatography of the crude extract (60 g) was carried out on silica gel (60–120 mesh) using an increasing gradient of *n*-hexane:chloroform followed by an increasing gradient of chloroform:methanol mixture. Fractions of 200 ml volume each were collected, analyzed by TLC, and pooled accordingly. Fractions 25–30 eluted with *n*-hexane:chloroform (1:3), pooled, concentrated,

and rechromatographed in *n*-hexane:chloroform (1:3) yielded 93 mg of **2** and 11 mg of **3**. Fractions 57–61 (95:5 $CHCl_3$:MeOH) were combined and concentrated to yield a pale yellow amorphous powder (370 mg). This powder was again dissolved in methanol (50 ml, reflux conditions), and then cooled to afford colorless shiny crystals of **1** (355 mg).

Compound **1**. A colorless crystalline solid, 0.025% of dried rhizomes; m.p. 249°C; UV (MeOH) λ_{max} (nm): 213, 264, 322; IR ν_{max} (KBr) (cm^{-1}): 3267, 2952, 2914, 1632, 1591, 946; ^{1}H NMR δ (ppm) (200 MHz, DMSO-d_6): 3.92 (3H, s, —OMe), 6.12 (2H, s, —OCH_2O—), 6.81 (2H, d, $J = 8.43$ Hz, 3′,5′-H), 7.02 (1H, s, 5-H), 7.36 (2H, d, $J = 8.4$ Hz, 2′, 6′-H), 8.20 (1H, s, —OCH═C); ^{13}C NMR (125 MHz, DMSO-d_6): see Table 1; EIMS *m/z* (%): 312(100), 297(100), 244(50), 219(23), 194(36), 152(37), 146(12), 118(7), and 93(9); Elemental analysis: Found: C, 65.98%; H, 3.941%; calcd for $C_{17}H_{12}O_6$: C, 65.38%; H, 3.873%.

Compound **2**. A pale yellow crystalline solid, 0.011% of dried weight; m.p. 220°C; UV (MeOH) λ_{max} (nm): 213, 265, and 340 (+ $AlCl_3$: 237, 269, 312, 364; + $AlCl_3$ + HCl: 233, 274, 312, 362; + NaOAc: 222, 273, 341, + NaOAc + H_3BO_3: 221, 267); IR ν_{max} (KBr) (cm^{-1}): 3438, 3068, 2989, 2959, 1660, 1624; ^{1}H NMR δ (ppm) (500 MHz, $CDCl_3$): 3.85, 4.04 (3H each, s, 2 × —OMe), 6.53 (1H, s, 8-H), 6.99 (2H, dd, $J = 2.0$, 8.7 Hz, 3′,5′-H), 7.46 (2H, dd, $J = 2.0$, 8.7 Hz, 2′,6′-H), 7.88 (1H, s, —OCH═C); ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1; EIMS *m/z* (%): 314(100), 299(50), 252(7), 207(52), 203(11), 192(6), and 164(12); Elemental analysis: Found: C, 65.71%; H, 4.52%; calcd for $C_{17}H_{14}O_6$: C, 64.97%; H, 4.89%.

3.4 Acetylation of compound 1 (1-OAc)

Compound **1** (30 mg, 0.096 mmol) was allowed to react with acetic anhydride (0.15 mmol) in the presence of dimethylamino pyridine (2 mg), and the contents were heated at 60°C for 5 min. The reaction mixture was worked up by adding crushed ice and extracted with ethyl acetate (3 × 30 ml). The organic layer was washed with water (2 × 10 ml) and concentrated after drying over anhydrous sodium sulfate to give a crude product which on crystallization in CH_2Cl_2–hexane afforded **1-OAc**: a colorless solid (21 mg, 0.059 mmol, 61.5% yield).

Compound **1-OAc**. A white solid, m.p. 157–58°C; IR ν_{max} (KBr) (cm^{-1}): 3060, 2961, 1646, 1616, 945; ^{1}H NMR δ (ppm) (200 MHz, DMSO-d_6): 2.29 (3H, s, $OCOCH_3$), 3.92 (3H, s, —OMe), 6.19 (2H, s, —OCH_2O), 7.03 (1H, s, 5-H), 7.17 (2H, d, $J = 8.6$ Hz, 3′,5′-H), 7.54 (2H, d, $J = 8.6$ Hz, 2′,6′-H), 8.33 (1H, s, 2-H); ^{13}C NMR δ (ppm) (50 MHz, DMSO-d_6): 20.8, 60.8, 103.6, 102.6, 113.1, 121.4, 123.5, 129.4, 130.1, 135.9, 140.4, 151.0, 152.0, 152.7, 153.9, 169.2, 173.6; EIMS *m/z* (%): 354(90), 323(24), 311(100), 265(9), 250(15), 224(5), 179(3), 150(3), 148(2), 146(3).

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