Phytochemistry 69 (2008) 1880–1885

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

A benzil and isoflavone from *Iris tenuifolia*

Muhammad Iqbal Choudhary [a,*], Sumaira Hareem [a], Hina Siddiqui [a], Shazia Anjum [a], Shamsher Ali [a], Atta-ur-Rahman [a], Mudassir Israr Zaidi [b]

[a] H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan
[b] Department of Botany, University of Balochistan, Quetta 87300, Pakistan

ARTICLE INFO

Article history:
Received 27 November 2007
Received in revised form 12 March 2008
Available online 9 May 2008

Keywords:
Iris tenuifolia
Iridaceae
Phytochemical investigation
Benzil
Isoflavone
DPPH radical scavenging activity
X-ray diffraction studies

ABSTRACT

Two compounds, tenuifodione (**1**) and tenuifone (**2**), and 12 known compounds, izalpinin (**3**), alpinone (**4**), arborinone (**5**), irilin B (**6**), irisone A (**7**), irisone B (**8**), betavulgarin (**9**), β-sitosterol (**10**), 5,7-dihydroxy-2′,6-dimethoxyisoflavone (**11**), 2′,5-dihdroxy-6,7-methylenedioxy flavanone (**12**), irisoid A (**13**) and ethyl-β-D-glucopyranoside (**14**) were isolated from the whole plant of *Iris tenuifolia* Pall. All compounds, except **12**, were isolated for the first time from this plant. Compounds **2**, **3** and **11** have shown a considerable DPPH radical scavenging activity. Structures of these compounds were identified on the basis of spectroscopic techniques, including 2D NMR. Compounds **3**, **5** and **7** were also subjected to single-crystal X-ray diffraction analysis and their structures were unambiguously deduced.

1. Introduction

Iridaceae is comprised of 92 genera and 1800 species, distributed throughout the world, but rare in tropical lowlands (Ali and Mathew, 2000; Hooker, 1982). *Iris* is the largest and most complicated genus of this family. This genus has over 300 species, some of which are ornamental (Krishnan, 2001). Among them, 16 species are found in Pakistan (Ali and Mathew, 2000). The rhizomes, stalks and leaves of *Iris* plants have special layers to protect them from dryness (Ali and Mathew, 2000). The cultivated Irises show a wide variety of colors in their large and usually perfumed blossoms. The juice of fresh roots of *Iris* species, bruised with wine, is used for the treatment of dropsy. This juice is also used in cosmetic for the removal of freckles from the skin. *Iris* plants have also been used in the treatment of cancer, inflammation, bacterial and viral infections (Nadkarni, 1976).

Iris tenuifolia is an important member of this genus, which occurs in Olzitt Sommon of the middle Gobi district of Mongolia (Central Asia) and West Asia, including Balochistan regions of Pakistan and Iran. Root powder of the plant, mixed in curds, is used to treat diarrhea in folk medicine. From the underground parts of *I. tenuifolia*, several flavonoids and isoflavonoids have been previously isolated (Baquar, 1989; Kojima et al., 1997).

The flavonoids are often biologically active secondary metabolites and about 2% of all the photosynthesized carbon is converted into flavonoids (Harborne, 1988). A number of flavonoids have been found to posses anti-protozoal, anti-inflammatory and antioxidant activities. Insecticidal, antifungal and antibacterial activities have also been reported for some isoflavonoids (Ingham, 1983).

Benzil derivatives form an important class of natural products, some of which are inhibitors of the acid corrosion of steel, as well as used in photocurable coatings. They are also used as intermediates in the synthesis of various heterocyclic compounds such as imidazoles or quinoxalines (Giraud et al., 2006). Recently benzils have been identified as inhibitors of carboxylesterase enzymes, proteins involved in the metabolism of esterified drugs and xenobiotics (Hyatt et al., 2006).

During the current study, we have isolated two new compounds, tenuifodione (**1**) and tenuifone (**2**), along with 12 known compounds **3**–**14**. Their structures were elucidated on the basis of NMR spectroscopic data. DPPH radical scavenging activity of some of these compounds was also investigated. Compounds **3**, **5** and **7** were also analyzed by single-crystal X-ray diffraction analysis.

2. Results and discussion

Fractions eluted with pet. ether–dichloromethane (3:7), from the column chromatography of ethanol soluble part of *I. tenuifolia*, yielded compound **1** as a yellow solid. The IR spectrum exhibited

* Corresponding author. Tel.: +92 21 4824924 5; fax: +92 21 4819018 9.
E-mail address: hej@cyber.net.pk (M.I. Choudhary).

0031-9422/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.phytochem.2008.03.011

absorptions for hydroxyl (3435 cm^{-1}), conjugated ketone (1665 cm^{-1}), aromatic C=C (1592 cm^{-1}) and O–CH_2–O (925 cm^{-1}) functionalities, while UV absorption bands at 351 (3.29), 286 (3.66), 207 (3.88), 204 (3.89) and 243 (3.64) nm indicated a benzil-type skeleton (Miyase et al., 1999). The high resolution electron impact mass spectrum (HR EIMS) showed the M^+ at m/z 316.0568 ($C_{16}H_{12}O_7$, calcd. 316.0583) with 11 degrees of unsaturation. In the EIMS, the base peak at m/z 195 corresponded to the fragmented ring A ($C_9H_7O_5$), while another peak at m/z 121 corresponded to the fragmented ring B ($C_7H_5O_2$). Another peak at m/z 180 (M^+–CH_3) indicated the loss of a methyl group from fragment ion containing ring A (Ferrari et al., 1984).

The ^{1}H NMR spectrum of compound **1** showed the signals for five aromatic protons (Table 1). The most downfield singlets at δ_H 12.42 and 11.14 were due to the C-2′ and C-2″ OH protons, involved in hydrogen bondings with C-1 and C-2 carbonyl oxygens, respectively. Sharp singlets at δ_H 3.60 (3H) and 5.93 (2H) indicated the presence of methoxy and methylenedioxy groups in the molecule. A singlet at δ_H 6.26 was due to the aromatic H-3′ of the ring A. Four mutually coupled aromatic signals, resonated at δ_H 7.05 (br *d*, *J* = 8.4 Hz), 7.50 (*dd*, *J* = 8.4, 1.4 Hz), 6.88 (br *t*, *J* = 7.6 Hz) and 7.37 (*dd*, *J* = 7.9, 1.4 Hz), were assigned to aromatic H-3″, H-4″, H-5″ and H-6″ of the ring B, respectively (Choudhary et al., 2001a). This revealed the presence of two independent aromatic rings in the molecule. The coupling pattern of the di-substituted ring B indicated C-1″ and C-2″ substitutions. On the other hand, ring A contains substituents such as OCH_3 (δ_H 3.60) at C-6′ and the methylenedioxy group (δ_H 5.93) at C-4′ and C-5′ (Li et al., 1998).

The ^{13}C NMR spectrum of **1** showed a total of 16 carbon signals, resolved as one methyl, five methine, one methylene and nine quaternary carbons (Table 1). Two carbonyl carbons signals was resonated at δ_C 193.6 (C-1) and 195.5 (C-2) involved in hydrogen bondings with the C-2′ and C-2″ OH functionalities, respectively. Methylenedioxy carbon was resonated at δ_C 102.0. *O*-Methyl carbon signal was resonated at δ_C 59.4 (Mahabusarakam et al., 2004).

The structure of compound **1** was deduced on the basis of HMBC spectrum which showed cross peaks between H-3′ (δ_H 6.26) and C-1′ (δ_C 105.2), C-2′ (δ_C 164.2), C-4′ (δ_C 158.2) and C-5′ (δ_C 128.8) of ring A. The HMBC between methylenedioxy protons (δ_H 5.93) and C-4′ (δ_C 158.2) and C-5′ (δ_C 128.8) was also observed. Similarly *O*-methyl protons (δ_H 3.60) showed cross peak with C-6′ (δ_C 142.2) in the HMBC spectrum. The aromatic protons of ring B also showed long-range heteronuclear couplings which are as follows: H-3″ (δ_H 7.05) with C-1″ (δ_C 116.5), C-2″ (δ_C 162.8), and C-5″ (δ_C 119.4); H-4″ (δ_H 7.50) with C-2″ (δ_C 162.8) and C-6″ (δ_C 131.4); H-5″ (δ_H 6.88) with C-1″ (δ_C 116.5), C-3″ (δ_C 118.3) and C-4″ (δ_C 136.7); and H-6″ (δ_H 7.37) with C-2 (δ_C 195.5), C-2″ (δ_C 162.8) and C-4″ (δ_C 136.7). The above data led to the structure **1** for tenuifodione.

Fractions eluted with pet. ether–dichloromethane (1:9), from the column chromatography of ethanol soluble part of *I. tenuifolia*, yielded compound **2** as an amorphous powder. The IR spectrum exhibited absorptions for hydroxyl (3426 cm^{-1}), conjugated ketone (1649 cm^{-1}), aromatic C=C (1547 cm^{-1}) and O–CH_2–O (923 cm^{-1}) functionalities, while UV absorptions at 268 (4.03), 219 (4.23), 200 (4.95), 210 (4.17) and 250 (3.94) nm indicated an isoflavone-type skeleton (Markham and Mabry, 1975). The M^+ at m/z 314.0415 in the HR EIMS supported the formula $C_{16}H_{10}O_7$ (calcd. 314.0426), with 12 degrees of unsaturation. The base peak at m/z 180 indicated the presence of a methylenedioxy group between C-6 and C-7. Two major fragments, m/z 180 ($C_8H_4O_5$) and 134 ($C_8H_6O_2$), were formed by the retro Diels Alder's cleavage of compound **2** (Markham and Mabry, 1975; Atta-ur-Rahman et al., 2003).

The ^{1}H NMR spectrum of compound **2** showed the presence of five aromatic protons of two phenyl rings (Table 1). One-proton singlets at δ_H 7.97 and 6.48 were assigned to H-2 and H-8, respectively, characteristic of an isoflavone skeleton (Hanawa et al., 1991; Sui-Ming et al., 1987). A 2H singlet at δ_H 6.01 was assigned to the protons of methylenedioxy group. The chemical shift of the H-8 (δ_H 6.48) indicated the presence of 5-hydroxy-6,7-methylenedioxy substituents in the ring A of the isoflavone (Hikino et al., 1982; Shawl et al., 1984; Konno et al., 1977). The remaining three aromatic protons appeared as an ABX system due to H-4′, H-5′ and H-6′ [δ_H 6.82 (*dd*, *J* = 7.9, 1.5 Hz), 6.72 (br *t*, *J* = 7.9 Hz) and 6.62 (*dd*, *J* = 7.7, 1.5 Hz), respectively].

Table 1
^{1}H and ^{13}C NMR chemical shift assignments for compounds **1** and **2** in $CDCl_3$ and CD_3OD

Position	**1**		**2**	
	δ_C	δ_H (*J* = Hz)	δ_C	δ_H (*J* = Hz)
2	–	–	155.2	7.97 (1H, *s*)
3	–	–	121.8	–
4	–	–	181.7	–
5	–	–	141.4	–
6	–	–	130.4	–
7	–	–	154.6	–
8	–	–	89.4	6.48 (1H, *s*)
9	–	–	153.5	–
10	–	–	107.8	–
1′	105.2	–	118.7	–
2′	164.2	–	142.7	–
3′	93.5	6.26 (1H, *s*)	146.2	–
4′	158.2	–	115.5	6.82 (1H, *dd*, *J* = 7.9, 1.5)
5′	128.8	–	120.9	6.72 (1H, br *t*, *J* = 7.9)
6′	142.2	–	121.0	6.62 (1H, *dd*, *J* = 7.7, 1.5)
1″	116.5	–	–	–
2″	162.8	–	–	–
3″	118.3	7.05 (1H, br *d*, *J* = 8.4)	–	–
4″	136.7	7.50 (1H, *dd*, *J* = 8.4, 1.4)	–	–
5″	119.4	6.88 (1H, br *t*, *J* = 7.6)	–	–
6″	131.4	7.37 (1H, *dd*, *J* = 7.9, 1.4)	–	–
OCH_2O	102.0	5.93 (2H, s)	102.8	6.01 (2H, *s*)
1-C=O	193.6	–	–	–
2-C=O	195.5	–	–	–
OH of C-2′	–	12.42 (1H, *s*)	–	–
OH of C-2″	–	11.14 (1H, *s*)	–	–
OCH_3 of C-6′	59.4	3.60 (3H, *s*)	–	–

The ^{13}C NMR spectrum showed 16 carbon signals, including five methine, one methylene and 10 quaternary carbons (Table 1). The most downfield signal at δ 181.7 was due to carbonyl C-4, involved in a hydrogen bonding with the C-5 hydroxyl functionality (Agrawal and Mahesh, 1987). Highly deshielded methine carbon at δ_C 155.2 was assigned to the C-2. This further indicated its isoflavone nature (Atta-ur-Rahman et al., 2002). Carbon signals at δ_C 141.4 (C-5), 130.4 (C-6), 154.6 (C-7), 89.4 (C-8), 153.5 (C-9) and 107.8 (C-10) were characteristic of a 5-hydroxy-6,7-methylenedioxy-benzopyran-4-one ring system (Kachroo et al., 1990). Signals at δ_C 118.7 (C-1′), 142.7 (C-2′), 146.2 (C-3′), 115.6 (C-4′), δ_C 120.9 (C-5′) and 121.0 (C-6′) were due to a 2′,3′-dihydroxy substituted ring B. ^1H and ^{13}C NMR data of compound **2** is presented in Table 1.

Important HMBC interactions were observed between H-2 (δ_H 7.97) with C-3 (δ_C 121.8), C-4 (δ_C 181.7) and C-1′ (δ_C 118.7). Similarly, H-8 (δ_H 6.48) showed HMBC interactions with C-9 (δ_C 153.5), C-6 (δ_C 130.4) and C-10 (δ_C 107.8), further indicating the presence of a 5-hydroxy-6,7-methylenedioxy substituted ring A in the isoflavone skeleton (Fig. 1). The above data led to the structure **2** for tenuifone.

Compounds **1**–**14**, isolated from *I. tenuifolia*, showed a concentration-dependent antiradical activity by reducing the stable DPPH radicals to the yellow colored diphenylpicrylhydrazine derivative. Izalpinin (**3**), 5,7-dihydroxy-2′,6-dimethoxyisoflavone (**11**) and tenuifone (**2**) showed IC_{50} values between 159.153 ± 4.492, 293.939 ± 17.984 and 418.727 ± 10.219 μM, respectively (Table 2).

Flavonoids are known to posses antiradical activity by acting as hydrogen donor or chelating metals. The activity of flavonoids with polyhydroxylated substitution is based on the location of the hydroxyl substitution on the ring B. Hydroxyl substitution on the *ortho* position in the ring B yielded a lower activity as in irisone B (**8**), however additional hydroxylation on *meta* position of ring B enhanced the activity many fold, e.g. tenuifone (**2**). In case of irilin B (**6**) and 5,7-dihydroxy-2′,6-dimethoxy isoflavone (**11**), it was observed that OCH_3 at *ortho* position of ring B yield a higher activity than hydroxylation at the same position as in irilin B (**6**). With

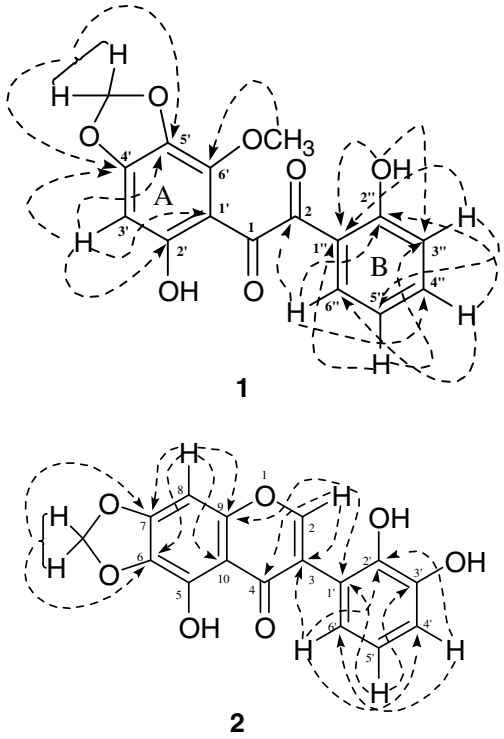

Fig. 1. Key HMBC interactions in compounds **1** and **2**.

Table 2
Antioxidant activities of compounds **1**–**14**

Compounds	% RSA	IC_{50} ± SEM (μM)
Tenuifodione (**1**)	–	–
Tenuifone (**2**)	83.741	418.727 ± 10.219
Izalpinin (**3**)	76.917	159.153 ± 4.492
Alpinone (**4**)	2.032	–
Arborinone (**5**)	–	–
Irilin B (**6**)	32.355	–
Irisone A (**7**)	–	–
Irisone B (**8**)	17.075	–
Betavulgarin (**9**)	18.969	–
β-Sitosterol (**10**)	–	–
5,7-Dihydroxy-2′,6-dimethoxy isoflavone (**11**)	84.843	293.939 ± 17.984
2′,5-Dihydroxy-6,7-methylenedioxy flavanone (**12**)	–	–
Irisoid A (**13**)	32.909	–
Ethyl-β-D-glucopyranoside (**14**)	–	–
Standrad BHT	92.30	44.2 ± 0.20

"–" Inactive.
BHT: butylated hydroxytoluene.
SEM: standard error of mean.
(Results are reported in ±standard error of mean of three experiments).
RSA: radical scavenging activity.
(IC_{50} values of test compounds were determined by using EZ-FIT program).

methylenedioxy group in ring A, irisone B (**6**) showed low activity. In addition, it was also observed that compound with conjugated double bond in ring C, as in izalpinin (**3**), has a higher antiradical activity than non-conjugated double bond at ring C as in alpinone (**4**).

3. Conclusions

Phytochemical investigation on *I. tenuifolia* resulted in the isolation of two new compounds, tenuifodione (**1**) and tenuifone (**2**), along with several known compounds. Compounds **3**, **4**, **5** and **14** were not reported earlier from genus *Iris*. Compounds **2**, **3** and **11** exhibited a good DPPH radical scavenging activity. Most of compounds **3**, **4**, **6**, **7**, **8**, **9**, **11** and **12** belong to phenolics class of compounds.

4. Experimental

4.1. General procedures

IR spectra were recorded on Jasco-320-A spectrometer. ^1H NMR spectra were recorded in $CDCl_3$ and $CDCl_3 + CD_3OD$ on a Bruker AM-300 and AM-400 spectrophotometers, while the ^{13}C NMR spectra were recorded on Bruker AM-400 and AM-600 spectrophotometers at MHz 100 and 150 in the same solvent. Chemical shifts are given relative to TMS (δ 0.00) as an internal standard (^1H), δ 77.0 (ppm) from $CDCl_3$ and δ 49.0 (ppm) from CD_3OD as a standard (^{13}C). Mass spectra (EIMS and HR EIMS) were measured in an electron impact mode on Finnigan MAT 12 or MAT 312 spectrometers and ions are given in *m*/*z* (%). TLC was performed with precoated silica gel G-25-UV_{254} plates and detection was carried out by spraying with ceric sulphate solution (1 g/100 ml of distilled water) in 10% H_2SO_4. Silica gel (E. Merck, 230–400 mesh) was used as a stationary phase for column chromatography. The diameter of VLC column was 9.5 cm. Melting points were determined on a Gallenkemp apparatus and are uncorrected. X-ray data were collected on a Bruker Smart Apex I, CCD 4-K area detector diffractometer.

4.2. Plant material

The whole plants of *Iris tenuofolia* Pall. were collected from Ziarat Valley, Quetta, Pakistan, and identified by Dr. Rasool Baksh Tareen, Department Botany, Balochistan University, Quetta, where

a specimen (voucher no. 1852) was also deposited in the herbarium.

4.3. Extraction and Isolation

The dried whole plant material (30 kg) was chopped and soaked in 90% EtOH + H_2O (120 l) for two weeks at room temperature and the resulting extract was concentrated to a gum (1.8 kg). This gum was subjected to a vacuum liquid chromatography (VLC). The VLC column was eluted with pure pet. ether, pet. ether–CH_2Cl_2, CH_2Cl_2, CH_2Cl_2–MeOH and finally with pure MeOH as a mobile phase. Each solvent passed from VLC column 1.5l and each fraction was collected in 1 l. On the evaporation of organic solvents, five fractions and 200.1 g, 250.6 g, 295.2 g, 100.4 g and 50.2 g were obtained.

Four compounds **3**–**6** were isolated from the pet. ether–CH_2Cl_2-soluble fraction. Seven compounds **1** and **7**–**12** were isolated from the CH_2Cl_2-soluble fraction. Three compounds **2** and **13**–**14** were isolated from CH_2Cl_2–MeOH-soluble fraction by using repeated column chromatography (silica gel) and preparative thin layer chromatography.

The pet. ether–CH_2Cl_2-soluble fraction was chromatographed on a silica gel column by using pet. ether–dichloromethane–methanol in increasing order of polarity to obtain four fractions (Fc-1 to Fc-4). The major fraction Fc-3, which was eluted with pet. ether–dichloromethane (5:5) was subjected to column chromatography over silica gel by using mixtures of pet. ether and dichloromethane as an eluent to obtain compounds **3** (8.70 mg), **4** (8.9 mg), **5** (7.54 mg) and **6** (8.43 mg).

The CH_2Cl_2-soluble fraction was chromatographed on a silica gel column by using pet. ether–dichloromethane–methanol in increasing order of polarity to obtain six fractions (Fc-1 to Fc-6). The major fractions Fc-4 and Fc-5, which were eluted with pet. ether–dichloromethane (2:8), subjected to column chromatography over silica gel and eluted with various mixtures of pet. ether and dichloromethane. This has yielded compounds **1** (5.10 mg), **7** (8.54 mg), **8** (10.9 mg), **9** (7.54 mg), **10** (4.93 mg), **11** (8.12 mg) and **12** (5.23 mg). The CH_2Cl_2–MeOH-soluble fraction was subjected to column chromatography on silica gel and column was eluted with increasing polarities of pet. ether–dichloromethane–methanol mixtures to obtain compounds **2** (5.80 mg), **13** (10.43 mg) and **14** (4.95 mg).

The known compounds **3**–**14** were characterized by comparison of physical and spectral data with the literature values (Jaipetch et al., 1983; Shawl and Kumar, 1992; Inoshiri et al., 1988; Hanawa et al., 1991; Elliger and Halloin, 1994; Jong and Hwang, 1995; Sadikum et al., 1996; Shawl et al., 1984; Kojima et al., 1997; Choudhary et al., 2001b; Prawat et al., 1995). Known compounds were first time isolated from this plant species.

4.4. Single-crystal X-ray diffraction data of compounds *3*, *5* and *7*

A plate-shaped colorless crystal of compound **3**[1] with dimension 0.40 × 0.15 × 0.05 mm was selected for X-ray diffraction studies. $C_{16}H_{12}O_5$: M_r 284.26; monoclinic; a = 15.2576(11) Å, b = 3.8832(3) Å, c = 22.5739(13) Å; $\alpha = \gamma = 90°$, β = 111.569(4)°; V = 1243.81 (16) Å^3, space group = $P2_1/c$, Z = 4, $D_{calc.}$ = 1.518 g/cm^3, $F(000)$ = 592.0, Mo Kα (λ 0.71073 Å). Intensity data of compound **3** was collected on a Bruker Smart Apex I, CCD 4-K area detector diffractometer attached with a KRYO-FLEX low temperature device. Data reductions were performed by using SAINT (Siemens, 1996). The structure was solved by direct methods (Sheldrick, 1997) and refined by full-matrix least squares on F^2 by using the SHELXTL-PC package. The intensity data within the θ range 2.81–28.21 were collected at 173 (2) K. A total of 11,949 reflections were recorded, of 4257 reflections were judged observed on the basis of I>2 s (1). The final R and Rw were 0.0444 and 0.1108, respectively. Fig. 2 was plotted with the aid of ORTEP (Johnson, 1976). H-atoms were placed in calculated positions, with C–H distances in the range 0.93–0.98 Å. The U_{iso} values were constrained to be 1.5U_{eq} of the carrier atom for methyl H atoms, and 1.2U_{eq} for the remaining H atoms. In the absence of significant anomalous dispersion effects, Friedel pairs were merged before the final refinement.

A block-shaped colorless crystal of compound **5**[2] with dimension 0.41 × 0.23 × 0.45 mm was selected for X-ray diffraction studies. $C_{30}H_{48}O$: M_r 424.68; orthorhombic; a = 7.6601(8) Å, b = 10.6690(12) Å, c = 31.839(4) Å; $\alpha = \gamma = \beta = 90°$; V = 2602.1(5) Å^3, space group = $P2_12_12_1$, Z = 4, $D_{calc.}$ = 1.084 g/cm^3, $F(000)$ = 944.0, Mo Kα (λ 0.71073 Å). Intensity data of compound **5** was collected on a Bruker Smart Apex I, CCD 4-K area detector diffractometer; data reductions were performed by using SAINT (Siemens, 1996). The structure was solved by direct methods (Sheldrick, 1997) and refined by full-matrix least squares on F^2 by using the SHELXTL-PC package. The intensity data within the θ range 1.28–25.0 were collected at 293 (2) K. A total of 15,591 reflections were recorded, of which 3448 reflections were judged observed on the basis of I>2 s (1). The final R and Rw were 0.0482 and 0.1090, respectively. Fig. 2 is plotted with the aid of ORTEP (Johnson, 1976). H-atoms were placed in calculated positions, with C–H distances in the range 0.93–0.98 Å. The U_{iso} values were constrained to be 1.5U_{eq} of the carrier atom for methyl H atoms, and 1.2U_{eq} for the remaining H atoms. In the absence of significant anomalous dispersion effects, Friedel pairs were merged before the final refinement.

A block-shaped colorless crystal of compound **7**[3] with dimension 0.33 × 0.25 × 0.17 mm was selected for X-ray diffraction studies. $C_{17}H_{12}O_6$: M_r 312.27; monoclinic, a = 7.0623(4) Å, b = 23.0586(12) Å, c = 8.3412(4) Å; $\alpha = \gamma = 90°$, β = 96.8090 (10)°; V = 1348.76 (12) Å^3, space group = $P2_1/c$, Z = 4, D_{calc} = 1.538 g/cm^3, $F(000)$ = 648.0, Mo Kα (λ 0.71073 Å). Intensity data of compound **7** was collected on a Bruker Smart Apex I, CCD 4-K area detector diffractometer attached with a KRYO-FLEX low temperature device. Data reductions were performed by using SAINT (Siemens, 1996). The structure was solved by direct methods (Sheldrick, 1997) and refined by full-matrix least squares on F^2 by using the SHELXTL-PC package. The intensity data within the θ range 2.61–28.29 were collected at 173 (2) K. A total of 9599 reflections were recorded, of which 3952 reflections were judged observed on the basis of I>2 s (1). The final R and Rw were 0.0445 and 0.1113, respectively. Fig. 2 is plotted with the aid of ORTEP (Johnson, 1976). H-atoms were placed in calculated positions, with C–H distances in the range 0.93–0.98 Å. The U_{iso} values were constrained to be 1.5U_{eq} of the carrier atom for methyl H atoms, and 1.2U_{eq} for the remaining H atoms. In the absence of significant anomalous dispersion effects, Friedel pairs were merged before the final refinement.

4.5. Tenuifodione (*1*)

M.P. 132–133 °C. IR ($CHCl_3$) ν_{max} cm^{-1}: 3435 (OH), 1665 (conjugated ketone C=O), 1592 (aromatic C=C), 925 (O–CH_2–O). UV: λ_{max} MeOH nm (log ε) 351 (3.29), 286 (3.66), 207 (3.88), 204 (3.89) and 243 (3.64). EIMS m/z (rel. int.): 316 [M]$^+$, 196 (48%), 195 (100%), 180 (92%) and 121 (30%). HR EIMS m/z: 316.0568 (Calcd. for $C_{16}H_{12}O_7$: 316.0583). ^{1}H and ^{13}C NMR: Table 1. HMBC: see Fig. 1.

[1] Crystallographic data for compound **3** has been deposited at the Cambridge Crystallographic Data Center (CCDC 655287) 12 Union Road, Cambridge, CB2 1EZ, UK (www.ccdc.cam.ac.uk/data_request/cif).

[2] Crystallographic data for compound **5** has been deposited at the Cambridge Crystallographic Data Center (CCDC 653388) 12 Union Road, Cambridge, CB2 1EZ, UK (www.ccdc.cam.ac.uk/data_request/cif).

[3] Crystallographic data for compound **7** has been deposited at the Cambridge Crystallographic Data Center (CCDC-655286) 12 Union Road, Cambridge, CB2 1EZ, UK (www.ccdc.cam.ac.uk/data_request/cif).

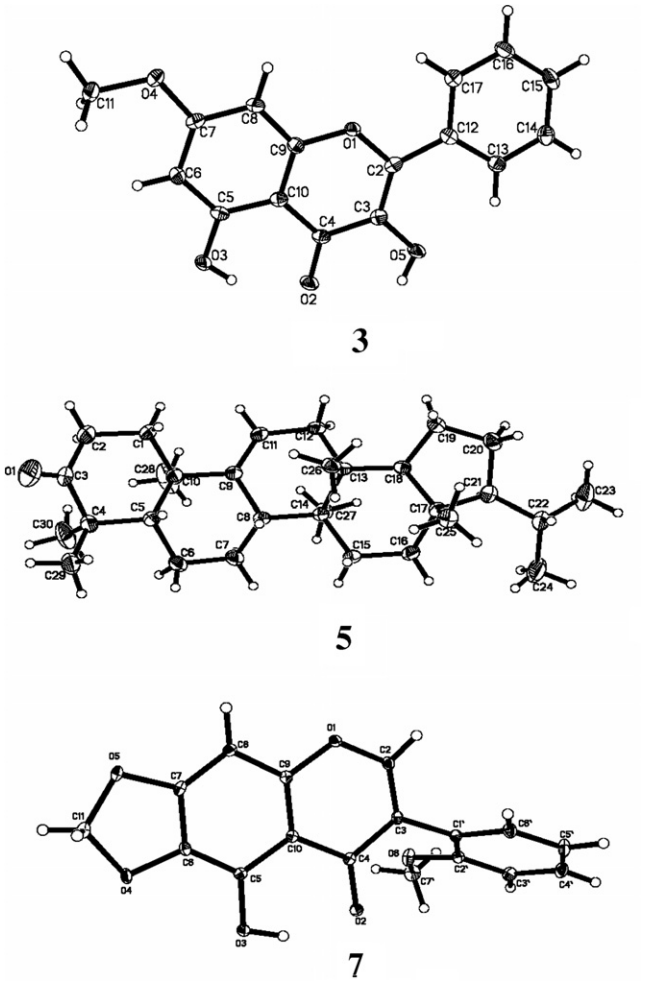

Fig. 2. The asymmetric units of compounds **3**, **5** and **7** showing 50% probability displacement ellipsoids and the atomic numbering.

4.6. Tenuifone (*2*)

M.P. 200–202 °C. IR (KBr) ν_{max} cm^{-1}: 3426 (OH), 1649 (conjugated ketone C=O), 1547 (aromatic C=C), 923 (O–CH_2–O). UV: λ_{max} MeOH nm (log ε) 268 (4.03), 219 (4.23), 200 (4.95) 210 (4.17) and 250 (3.94). EIMS *m*/*z* (rel. int.): 314 $[M]^+$, 268 (10%), 181 (32%), 180 (100%) and 134 (12%). HR EIMS *m*/*z*: 314.0415 (Calcd. for $C_{16}H_{10}O_7$: 314.0426), ^{1}H and ^{13}C NMR: Table 1. HMBC: see Fig. 2.

4.7. DPPH free radical scavenging assay

1,1-Diphenyl-2-picrylhydrazyl radical (DPPH), a stable free radical, shows a strong absorption at 515 nm (in ethanol), as a deep violet color. When its electron is paired off and corresponding hydrazine forms, the absorption vanishes. Resulting decolorization is stoichiometric with respect to the number of electrons taken up. This assay is basically a free radical colorimetry that relies on the reaction $DPPH^{\cdot} + AH \rightarrow DPPH\text{-}H + A^{\cdot}$ with specific antioxidant (AH) (Shaheen et al., 2005; Kumara and Karunakaran, 2007).

4.7.1. Protocol

Test samples were allowed to react with stable DPPH radicals for half an hour at 37 °C. The concentration of DPPH was kept as 300 μM. The test samples were dissolved in DMSO, while the DPPH solution was prepared in ethanol. After incubation, decrease in absorption was measured at 515 nm by using a multiplate reader (Spectra MAX-340, Molecular Devices). Percent radical scavenging activity of samples was determined in comparison with a DMSO treated control group (Lee et al., 1998), by using the following formula:

$$\%RSA = 100 - \{(OD\ test\ compound/OD\ control) \times 100\}$$

Acknowledgments

One of us (Sumaira Hareem) acknowledges the enabling role of the Higher Education Commission (Pakistan), and appreciates its financial support through the "Merit Scholarship Scheme for Ph.D. Studies in Science and Technology".

References

Agrawal, P.K., Mahesh, C.B., 1987. C-13 NMR of flavonoids. Elsevier Science Publishers, Amsterdam. p. 193.
Ali, S.I., Mathew, B., 2000. Flora of Pakistan, vol. 202. Karachi University Press, Karachi. pp. 4–29.
Atta-ur-Rahman, Nasim, S., Baig, I., Ara, J.I., Sener, B., Orhan, I., Choudhary, M.I., 2002. Isoflavonoids glycosides from the rhizomes of *Iris germanica*. Chem. Pharm. Bull. 50, 1100–1102.
Atta-ur-Rahman, Nasim, S., Baig, I., Orhan, I., Sener, B., Ayanoglu, F., Choudhary, M.I., 2003. Isoflavonoids glycosides from the rhizomes of *Iris germanica*. Helv. Chim. Acta 86, 3354–3362.
Baquar, S.R., 1989. Medicinal and poisonous plants of Pakistan. Printas, Karachi, 244–245.
Choudhary, M.I., Nur-e-Alam, M., Baig, I., Akhtar, F., Ahmed, S., Ndognii, P.Ö., Badarchiin, T., Purevsuren, G., Atta-ur-Rahman, 2001a. Five new peltogynoids from underground parts of *Iris bungei*. Chem. Pharm. Bull. 49, 1295–1298.
Choudhary, M.I., Nur-e-Alam, M., Baig, I., Akhtar, F., Ndognii, P.Ö., Babarchiin, T., Purevsuren, G., Nahar, N., Atta-ur-Rahman, 2001b. Four new flavones and a new isoflavones from *Iris bungei*. J. Nat. Prod. 64, 857–860.
Elliger, A.C., Halloin, M.J., 1994. Phenolics induced in *Beta vulgaris* by *Rhizoctonia solani* infection. Phytochemistry 37, 691–693.
Ferrari, F., Alves de Lima, R., Bettolo, G.B.M., 1984. 2,4,2′-Trihydoxy-4′-methoxy bezil from *Zollernia paraensis*. Phytochemistry 23, 2691–2692.
Giraud, A., Provot, O., Peyrat, J., Alami, M., Brion, J., 2006. Microwave-assisted efficient synthesis of 1,2-diaryldiketones: a novel oxidation reaction of diarylalkynes with DMSO promoted by $FeBr_3$. Tetrahedron 62, 7667–7673.
Hanawa, F., Tahara, S., Mizutani, J., 1991. Isoflavonoids produced by *Iris pseudacorus* leaves treated with cupric chloride. Phytochemistry 30, 157–163.
Harborne, J.B., 1988. Plant flavonoids in biology and medicine II: biochemical cellular, and medicinal properties. Alan R. Liss Inc., New York. pp. 17–27.
Hikino, H., Takahasi, M., Konno, C., 1982. Structure of ephedrannin A, a hypotensive principle of *Ephedra* roots. Tetrahedron Lett. 23, 673–676.
Hooker J.D., 1982. Flora of British India, Bishman S.M. P.S. 2, p. 665.
Hyatt, J.L., Tsurkan, L., Wierdl, M., Edwards, C.C., Danks, M.K., Potter, P.M., 2006. Mol. Cancer Ther. 5, 2281–2288.
Ingham, J.L., 1983. Progress in the Chemistry of Organic Natural Products, vol. 43. Springer-Verlag, New York. p. 88.
Inoshiri, S., Saiki, M., Kohda, H., Otsuka, H., Yamasaki, K., 1988. Monoterpene glucoside from *Berchemia racemosa*. Phytochemistry 27, 2869–2871.
Jaipetch, T., Reutrakul, V., Tuntiwachwuttikul, P., Santisuk, T., 1983. Flavonoids in the black rhizomes of *Boesenbergia pandurata*. Phytochemistry 22, 625–626.
Johnson, C.K., 1976. ORTEPII, Report ORNL-5138. Oak Ridge National Laboratory, Tennessee, USA.
Jong, T., Hwang, C., 1995. Two rare isoflavones from *Celosis argentia*. Planta Med. 61, 584–585.
Kachroo, K., Razdan, T.K., Qurishi, M.A., Khuroo, M.A., Koul, S., Dhar, K.L., 1990. Two isoflavones from *Iris kashmiriana*. Phytochemistry 29, 1014–1016.
Krishnan, M.S., 2001. Sastri, B.N. (Ed.), The Wealth of India: A Dictionary of Raw Material and Industrial Products, vol. 2. Council of Scientific and Industrial Research, New Delhi, pp. 254–256.
Kojima, K., Gombosurengyin, P., Ondogyni, P., Begzsurengyin, D., Zevgeegyin, O., Hatano, K., Ogihara, Y., 1997. Flavanones from *Iris tenuifolia*. Phytochemistry 44, 711–714.
Konno, C., Oshima, Y., Hikino, H., 1977. Mourusinol, isoprenoid flavone from *Morus* roots bark. Planta Med. 32, 118–124.
Kumara, A., Karunakaran, J.R., 2007. Activity-guided isolation and identification of free radical-scavenging components from an aqueous extract of *Coleus aromaticus*. Food Chem. 100, 356–361.
Lee, S.K., Zakaria, H., Chung, H., Luyengi, L., Gamez, E.J.C., Mehta, R.J., Kinghorn, D., Pezzuto, J.M., 1998. Evaluation of the antioxidant potential of natural products. Comb. Chem. High Throughput Screening 1, 35–46.
Li, W., Asada, Y., Yoshikawa, T., 1998. Antimicrobial flavonoids from *Glycyrrhiza glabra* hairy root cultures. Planta Med. 64, 746–747.
Mahabusarakam, W., Deachathai, S., Phongpaichit, S., Jansakul, C., Taylor, W.C., 2004. A benzil and isoflavones derivatives from *Derris scandens* Benth. Phytochemistry 65, 1185–1191.

Markham, K.R., Mabry, T.J., 1975. The Flavonoids Part 1. Academic Press, New York. pp. 50–126.
Miyase, T., Sano, M., Yoshino, K., Nonaka, K., 1999. Antioxidants from *Lespedeza homoloba* (II). Phytochemistry 52, 311–319.
Nadkarni, K.M., 1976. Indian Materia Medica, vol. 1. Popular Prakashan, Bombay. p. 541.
Prawat, H., Mahidol, C., Ruchirawat, S., Prawat, U., Tuntiwachwuttikul, P., Tooptakong, U., Taylor, W.C., White, A.H., 1995. Cyanogenic and non-cyanogenic glycosides from *Manihot esculenta*. Phytochemistry 40, 1167–1173.
Sadikum, A., Aminah, I., Ismail, N., Ibrahim, P., 1996. Sterols and sterol glycosides from the leaves of *Gynura procumbens*. Nat. Prod. Sci. 2, 19–23.
Shaheen, F., Ahmad, M., Khan, T.H., Jalil, S., Ejaz, A., Sultankhodjaev, M.N., Arfan, M., Choudhary, M.I., Atta-ur-Rahman, 2005. Alkaloids of *Aconitum* leaves and their anti-inflammatory, antioxidant and tyrosinase inhibition activities. Phytochemistry 66, 935–940.
Shawl, A.S., Kumar, T., 1992. Isoflavonoids from *Iris crocea*. Phytochemistry 31, 1399–1401.
Shawl, A.S., Vishwapaul, Zaman, A., Kalla, A.K., 1984. Isoflavonoids of *Iris spuria*. Phytochemistry 23, 2405–2406.
Sheldrick, G.M., 1997. SHELXTL-PC (Version 5.1). Siemens Analytical Instruments Inc., Madison, WI.
Siemens, 1996. SMART and SAINT. Siemens Analytical X-Ray Instruments Inc., Madison, WI, USA.
Sui-Ming, W., Chohachi, K., Yoshieru, O., John, M.P., Harry, H.S.F., Norman, R.F., 1987. Irisones A and B: two new isoflavones from *Iris missouriensis*. J. Nat. Prod. 50, 178–180.