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Alkylated benzoquinones from *Iris kumaonensis*☆

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Abstract

Six novel alkylated *p*-Benzoquinones irisoquin (A–F) (**1**–**2**, **4**–**7**) together with a known cytotoxic quinone, irisoquin (**3**) along with three known isoflavones, tectoregenin, iristectorin and irigenin were isolated from the rhizomes of *Iris kumaonensis* and characterized. The structures of compounds **1**–**2**, **4**–**7** were confirmed by extensive spectroscopic analysis, IR, MS, HRMS, GC, GC–MS, 1D (^{1}H, ^{13}C, NOE) and 2D (HMQC and HMBC) NMR and comparison with literature data of known compounds.

Keywords: *Iris kumaonensis*; Iridaceae; Rhizomes; Benzoquinones; Irisoquin A–F; Isoflavonoids

1. Introduction

Iris kumaonensis Wall ex Don. V. Lhathum (Iridaceae) is a perennial herb growing wild in western Himalaya from Kashmir to Uttranchal at an altitude of 2500–4000 m. Rhizomes of this plant are used for fever (Anon., 1959) and roots are used for kidney infections (Bhattacharjee, 1998). The genus *Iris* consists of about three hundred species and is distributed throughout the world. About twelve species are found in India (Bhattacharjee, 1998). The phytochemistry of this genus has been the subject of extensive investigation and found to be a rich source of flavones, isoflavones (Shawl and Kumar, 1992; Farag et al., 1999) and quinones (Wong et al., 1985). These classes of compounds have attracted considerable attention because of their antioxidant (Pietta, 2000) and cytotoxic (Hadfield et al., 2000) properties. Two isoflavones Iridin and Iriskumonin have previously been isolated from the rhizomes of *I. kumaonensis* (Rastogi and Mehrotra, 1991). During our ongoing research for characterization and identification of new secondary metabolites from western Himalayan flora the present investigation of *I. kumaonensis* rhizomes has led to the identification of six new alkylated Benzoquinones, **1** (irisoquin-A), **2** (irisoquin-B), **4** (irisoquin-C), **5** (irisoquin-D), **6** (irisoquin-E) and **7** (irisoquin-F) and a known benzoquinone (irisoquin) (**3**) which has been reported earlier from the hexane extract of *Iris missouriensis* and reported to possess cytotoxic activity (Wong et al., 1985). Three known isoflavones, tectorigenin (Shawl and Kumar, 1992), iristectorin-A (Shawl et al., 1984) and irigenin (Ali et al., 1983) were also identified from the $CHCl_3$ extract of the rhizomes of this plant. The identification of these compounds was carried out by spectral analysis (IR, GC–MS, HREIMS, ^{1}H and ^{13}C NMR) and also by comparing with the literature data of known compounds.

2. Results and discussion

Repeated column chromatography of the hexane extract of rhizomes led to the isolation of an orange coloured powder, which was almost homogenous on TLC. HPLC analysis gave a single broad peak. Its EIMS showed a base peak at *m*/*z* 168 while seven peaks of different intensities were observed at *m*/*z* 378, 392, 406, 420, 434, 448 and 462, all 14 amu apart in their molecular ion region, which did not seem to be fragment ions. To ascertain and confirm it, the sample was subjected to FDMS and confirmed our speculations that these peaks were molecular ion peaks of seven molecules which were observed as seven molecular ions

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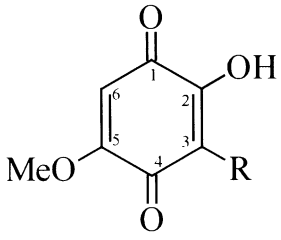
Table 1
GC, GCMS and HR EI–MS Data for compounds **1–7**

Compound	RT (GC)	Percentage (GC)	M^+ m/z	Base peak m/z	HREIMS m/z	Molecular formula
1	38.85	2.72	378	168	378.2773	$C_{23}H_{38}O_4$
2	41.08	71.46	392	168	392.2923	$C_{24}H_{40}O_4$
3	43.05	17.08	406	168	406.3083	$C_{25}H_{42}O_4$
4	45.76	2.96	420	168	420.3231	$C_{26}H_{44}O_4$
5	47.88	2.64	434	168	434.3387	$C_{27}H_{46}O_4$
6	49.94	1.64	448	168	448.3553	$C_{28}H_{48}O_4$
7	54.44	1.19	462	168	462.3697	$C_{29}H_{50}O_4$

on the above mentioned m/z values. The molecular formula of each M^+ ion was deduced by HREIMS (Table 1). The relative percentage of each compound and its MS was determined by GC, GC–MS and **2** was found to be a major compound. The data is given in Table 1.



1 R = $C_{16}H_{33}$
2 R = $C_{17}H_{35}$
3 R = $C_{18}H_{37}$
4 R = $C_{19}H_{39}$
5 R = $C_{20}H_{41}$
6 R = $C_{21}H_{43}$
7 R = $C_{22}H_{45}$

Compounds **1–7** contained 3-alkyl-2-hydroxy-5-methoxy-1,4-benzoquinone moiety as shown by following spectral data. IR spectra indicated the presence of a hydroxy and methoxy groups (3356 and 1222 cm^{-1}) and conjugated carbonyls of trisubstituted benzoquinone (1685, 1640 and 1605 cm^{-1}) (Kabo et al., 1983; Wong et al., 1985). In ^1H NMR spectrum a singlet at δ 3.86 (3H) and D_2O exchangeable broad singlet at δ 7.29 supported the attachment of a methoxy and a hydroxy group to benzoquinone ring. Further, a triplet observed at δ 2.43 (2H, $J = 6.5$ Hz) for methylene protons of alkyl side chain attached to quinone ring, a triplet at δ 0.87 for terminal methyl of long alkyl side chain and a signal for quinoid proton appeared at δ 5.86. ^1H and ^{13}C NMR data (Table 2) correlates well with the reported values (Wong et al., 1985) of compound **3**. The base peak at m/z 168.0431 ($C_8H_8O_4$) in the MS corresponds to hydroquinone fragment which further supports the common quinoid moiety (Kabo et al., 1987) in all the structures 1–7. The position of hydroxy, methoxy and alkyl side chain was finally confirmed by HMBC and NOE experiments (Fig. 1). The combined spectral data established that compounds **1–7** possessed common 3-alkyl-2-hydroxy-5-methoxy-1,4-benzoquinone moiety and difference exists only in the length of their side chain alkyl group. On the basis of MS it was clear that there was successive addition of one CH_2 unit to previous homologue. Thus alkyl side chains in **1**—$C_{16}H_{33}$, **2**—$C_{17}H_{35}$, **3**—$C_{18}H_{37}$, **4**—$C_{19}H_{39}$, **5**—$C_{20}H_{41}$, **6**—$C_{21}H_{43}$ and **7**—$C_{22}H_{45}$ respectively establish their structures as **1–7**. Compound **1** (irisoquin-A), **2** (irisoquin-B) **4** (irisoquin-C) **5** (irisoquin-D) **6** (irisoquin-E) and **7** (irisoquin-F) are new compounds while **3** (irisoquin) has been reported earlier from *Iris missouriensis* and possesses cytotoxic activity (Wong et al., 1985). On the basis of presence of **3** we

Table 2
^1H (300 MHz) and ^{13}C NMR (75 MHz) data of compounds **1–7** in $CDCl_3$

Position	δ^1H[a]	$\delta^{13}C$[b]	HMBC
1	–	182.85 (s)	
2	–	151.54 (s)	
3	–	119.29 (s)	
4	–	181.71 (s)	
5	–	161.10 (s)	
6	5.86 (s)	102.17 (d)	1,2,4,5
1′	2.43 (t, $J = 6.5$)	28.04 (t)	2, 3, 4, 2′
2′	1.44 (m)	28.04 (t)	
$(CH_2)_{n'}$[c]	1.24 (brs)	29.37–29.71 (t)	
$n'+1$	1.24 (brs)	31.91(t)	
$n'+2$	1.24 (brs)	22.70 (t)	
$n'+3$	0.87 (t, $J = 6.5$)	14.14 (q)	$n'+1$, $n'+2$
OCH_3	3.86 (s)	56.76 (q)	5
OH	7.29 (brs)	–	1, 2, 3

[a] Multiplicity and J values in Hz are given in parentheses.
[b] Multiplicity determined by DEPT and HMQC given in parentheses.
[c] **1** ($n' = 11$), **2** ($n' = 12$), **3** ($n' = 13$), **4** ($n' = 14$), **5** ($n' = 15$), **6** ($n' = 16$), **7** ($n' = 17$).

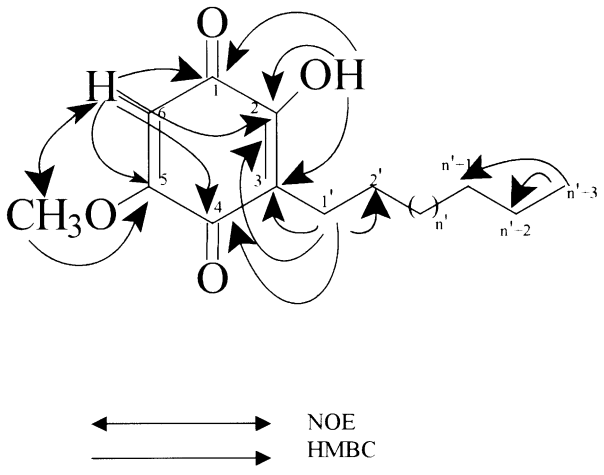


Fig. 1. HMBC and NOE correlation for 1–7.

conclude that the new quinones reported here may also possess cytotoxic activity as all are either lower or higher homologues of 3.

As one of the isolated benzoquinones (irisoquin, 3) has previously been reported from related *Iris* species, *I. missouriensis* we have characterized six new quinones of this series from *I. kumaonensis* which are homologues with different alkylated chain lengths. Apart from these two species, a potential antitumour benzoquinone, irisquinone having 3-(10-heptadecenyl)-5-methoxy-1,4-benzoquinone structure has been isolated from the seed oil of *Iris pallasi* (Wu, 1980) and has various effective anti-cancer properties (Hadfield et al., 2000). Irisquinone differs from Irisoquin-B (2) that, the former does not contain a 2-hydroxy group and has 10′–11′ double bond in its alkylated chain. A detailed investigation of benzoquinones on other *Iris* species is required to establish their biosynthetic significance, chemotoxanomic importance and their potential cytotoxic and anticancer activities. This will further provide insight in the distribution of benzoquinones in genus *Iris*. However, work in this direction is presently going on.

3. Experimental

3.1. General

IR spectra were measured on a JASCO FT/IR 5300, 1D and 2D NMR were recorded in $CDCl_3$/DMSO-d_6 on Bruker DRX 300 instrument using TMS as internal standard. EIMS and HREIMS were recorded on Finnigan MAT 8230, 600 mA, 3 KV mass spectrometer. FDMS was recorded on Finnigan MAT 900 S, 10 mA, 10KV mass spectrometer. GC and GC-MS was carried out at 70 ev using a Perkin Elmer GC-FID fitted with a Perkin Elmer Q-mass 910 mass spectrometer, SE-30 capillary column (length 30 m, ID = 0.25). Helium as carrier gas, operating conditions: injector 250 °C, split 1:20, temperature programme: 150–290 °C at the rate of 6 °C/min. HPLC was performed on waters 600, chromatogram on spherisorb (5μ, ODS-2, 4.6 × 250 mm column) with flow rate of 1 mL/min using solvent H_2O:MeOH (50:50) to MeOH in 45 min at ambient temperature.

3.2. Plant material

The rhizomes of *I. kumaonensis* were collected from Banjar area of Kullu district at an average height of 3500 m (msl) in Himachal Pradesh, India during Oct 1999. An authenticated voucher specimen (No. 452 VKK) has been deposited in the herbarium of the institute.

3.3. Extraction and isolation

1 kg of air dried powdered rhizomes of *I. kumaonensis* were extracted first with hexane (5 × 4 l) at room temperature (28–30 °C) yielding 12 g of dried extract followed by $CHCl_3$ extraction (5 × 4 l) yielding 22 g of dried extract.

10 g of hexane extract was subjected to normal phase column chromatography (5 × 100 cm) over silica gel-G (400 g) and eluted with hexane, hexane:benzene (1:1), benzene, benzene:$CHCl_3$ (3:1), $CHCl_3$ and $CHCl_3$:MeOH (9:1). Octacosane and octanoic acid were obtained by elution with hexane and hexane:benzene (1:1) respectively, whereas elution with benzene:$CHCl_3$ (3:1) afforded an orange gum (400 mg) which was rechromatographed over silica gel (50 g) and eluted with CH_2Cl_2:EtoAc (98:2). Fifteen equal fractions of 25 ml each were collected. On the basis of TLC pattern, fractions 7–10 were pooled which gave an orange powder (310 mg) after removal of solvent, containing compounds 1–7.

The $CHCl_3$ extract (10 g) was subjected to column chromatography over silica gel-G (400 g) using CH_2Cl_2, CH_2Cl_2:MeOH. From the CH_2Cl_2:MeOH (97:3) fraction, a yellow coloured amorphous powder (650 mg) was obtained which on repeated chromatography on silica gel-G using solvent system CH_2Cl_2:Me_2CO:-MeOH (96:2:2) afforded tectorigenin (120 mg), iristectorin (185 mg) and irigenin (212 mg).

3.4. 2-Hydroxy-3-alkyl-5-methoxy-1, 4-benzoquinones (1–7)

Orange coloured powder, mp 81.5 °C, IR (KBr) ν_{max} 3340, 2962, 2925, 1685, 1650, 1602, 1222 and 1210 cm^{-1}. For ^1H and ^{13}C NMR see Table 2, for MS, GC and GC–MS see Table 1. Other significant ions in GC–MS were observed at *m*/*z* 169, 167, 156, 139, 43 in all compounds (1–7). HREIMS: *Irisoquin-A* 1 (2-hydroxy-3-hexadecyl-5-methoxy-1,4-benzoquinone) *m*/*z* 378.2773 (calc. for

$C_{23}H_{38}O_4$, 378.2760) *Irisoquin-B*, **2** (2-hydroxy-3-heptadecyl-5-methoxy, 1,4-benzoquinone) *m/z* 392.2923 (calc. for $C_{24}H_{40}O_4$, 392.2956) *Irisoquin* **3** (2-hydroxy-3-octadecyl-5-methoxy-1,4-benzoquinone) *m/z* 406.3083 (calc. for $C_{25}H_{42}O_4$, 406.3072) *Irisoquin-C* **4** (2-hydroxy-3-nonadecyl-5-methoxy-1,4-benzoquinone) *m/z* 420.3231 (calc. for $C_{26}H_{44}O_4$, 420.3228) *Irisoquin-D* **5** (2-hydroxy-3-eicosanyl-5-methoxy-1,4-benzoquinone) *m/z* 434.3387 (calc. for $C_{27}H_{46}O_4$, 434.3384) *Irisoquin-E* **6** (2-hydroxy-3-docosanyl-5-methoxy-1,4-benzoquinone) *m/z* 448.3553 (calc. for $C_{28}H_{48}O_4$, 448.3540) *Irisoquin-F* **7** (2-hydroxy-3-docosanyl-5-methoxy-1,4-benzoquinone) *m/z* 462.3697 (calc. for $C_{29}H_{50}O_4$, 462.3696).

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