TWO ISOFLAVONES FROM *IRIS SONGARICA* SCHRENK.

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ABSTRACT

Two isoflavone, irilin A and irisone B were isolated from *Iris songarica* and their structures were determined by 1 and 2 D-NMR, IR, UV and MS. The effect of solvent on ^{1}H-NMR spectra of irisone B have been studied.

Key words: *Iris songarica*, isoflavone, irilinA, irisone B.

INTRODUCTION

Plants of genus *Iris* comprise over 3000 species in the world of which twenty species are present in Iran (1, 2). Medicinal plants belongs to this genus has been named Irsa in Iranian traditional medicine. These species have been introduced as diuretic and expectorant at low doses and as a strong purgative and emetic in high doses. It has been reported that Irsa is useful for pulmonary, asthma, liver and uterus diseases as well as hemorrhoid and gripe (3). Previous phytochemical studies have shown a variety of compounds including flavonoids, isoflavonoids and their glycosides, benzoquinone, triterpenoids especially iridals, xanthones and stilbene glycosides (4). This genus is rich in isoflavonoids which have a wide range of biological activity including anti-inflammatory, antioxidant and cancer chemopreventive properities (5,6).

Iris songarica is distributed in Iran, Pakistan, Afghanistan, Uzbekisatn, Kazakhstan, Soviet Union, Mongalia and Northern China (1,2,7). Powder of roots mixed with curd is used to cure diarrhea in Pakistan (7). To the best of our knowledge there has been no phytochemical study on this species. In this article isolation of two isoflavonids from this plant is described.

MATERIALS AND METHODS

The UV spectra were obtained using a Hitachi U-3200 spectrophotometer. The FT-IR spectra were recorded on a vector 22 instrument. The ^{1}H-NMR was recorded on a Bruker AM 400 and AMX 500 NMR (Avance) instruments using the UNIX data system at 400 and 500 MHz, respectively. The ^{13}C-NMR spectra were recorded at 100 and 125 MHz, respectively using $CDCl_3$ and C_5D_5N (deuterated pyridine) as solvents. ^{1}H-^{13}C HMBC and HMQC were recorded as mentioned above. EI MS spectra were recorded on a Finnigan MAT 312. FAB mass measurements were performed on Jeol JMS HX 110 mass spectrometer using glycerol as the matrix. CI MS and HREI MS were carried out on Jeol JMS 600 mass spectrometer. Column chromatography was carried out on silica gel (M&N), 70-230 and 230-400 meshes. Compounds on the TLC (M&N) were detected at 254 and 366 nm and by ceric sulfate as spraying reagent.

Experimental

The under ground parts of *I.songarica* were collected from Aliabad-e-Pishkoh in altitude 2545m, Yazd Province, Iran , in June 2002 and identified by Mr.GH.R. Barzegari (Natural resource institue of Yazd Province,Yazd, Iran). A voucher specimen (no.4077) was deposited at the the Herbarium of agriculture faculty of Karaj, Islamic Azad University. The fresh rhizomes and roots of *I.songarica* were cleaned under running tape water, cut into pieces and dried under shadow for two weeks. The powdered crude materials (1.6 Kg) were extracted by maceration with EtOH (80%) (3×8 lit.). The extract was evaporated and freez dried to give 60 g. of gummy extract. The extract was chromatographed on silica gel column (70-230 mesh, 700 g) and eluted with varying portion of *n*-Hexane, $CHCl_3$ and MeOH. Three fractions (I, II and III) were selected from eluted fraction of n-Hexane-$CHCl_3$ (6:4). The fraction I was loaded on silica gel (30 g) and the column was eluted with *n*-Hexane- acetone (95:5 (I_a), 90:10 (I_b) and 85:15 (I_c)). Fraction I_c were loaded on silica columns and the columns were eluted

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with *n*-Hexane-EtOAc (4:1) to purify irisone B (16.5 mg). Fraction II was pure irisone B. Fraction III was eluted with *n*-Hexane-EtOAc (4:1) to yield irilin A (10 mg).

Irilin A:

yellow amorphous solid. $[M]^+$ at m/z 314.07497 (calcd. for $C_{17}H_{14}O_6$, 314.07901); FAB MS $[M+1]^+$: 315, $[M-1]^+$:313. UV: λ_{max} (MeOH): 202.6, 263.2; +$AlCl_3$ 276.8, 312_{sh}, 390 nm, +($AlCl_3$ + HCl) without shift. IR ν_{max} (KBr) cm^{-1} : 3446(*br*-OH), 2950 (-C-H), 2835.9, 2273, 1736 (C=O) , 1649.6(C=C), 1560, 1541, 1453.8, 1324.5 (C-O). EI MS m/z (rel. int.): 313.9(100), 299 (45), 281(39), 196(35), 181(74.5), 153 (40.5), 145 (20). ^{1}H-NMR (table 1).

Irisone B:

white amorphous powder. $[M]^+$ at m/z 298.05149 (calc. for $C_{16}H_{10}O_6$, 298.04771); FAB MS $[M+1]^+$: 299, $[M-1]^+$: 297. CI MS $[M+1]^+$: 299. UV: λ_{max} (MeOH): 218, 243, 272; +$AlCl_3$: 221, 281, 321, 386 nm, +($AlCl_3$ + HCl) without shift. IR ν_{max} (KBr) cm^{-1} 3696, 3284(*br*-OH), 3085 (ar.=C-H), 2926(-C-H), 1677, 1622, 1560, 1471, 1332, 922. EI MS m/z (rel. int.): 298(64), 281(5.25), 180(100), 145(10.5). ^{1}H and ^{13}C-NMR (table 1).

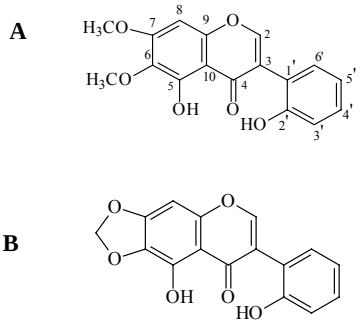

Figure 1. Structures of irilin A (A) and irisone B (B).

RESULTS AND DISCUSSION

The known isoflavonoids, irlin A and irisone B (figure 1) were isolated from the crude mixture by repetitive column chromatography. They were identified by interpretation of their MS, NMR, IR and UV spectra as well as by comparison of their spectral data with those reported in the literatures (8,9). The high resolution electron impact (HR EI) MS of irisone B showed the $[M]^+$ at m/z 298.05149 in agreement with the molecular formula $C_{16}H_{10}O_6$ (calcd. 298.04771). CI MS and FAB MS $[M+1]^+$ spectra confirmed the molecular weight at m/z 299. The EI MS showed fragments at m/z 180 and 118, belonging to retro Diles-Alders cleavage which showed OCH_2O residue in the ring A and a OH substitution in the ring B. UV absorption maxima at 264 and 336 nm (sh), suggested the presence of an isoflavone moiety.

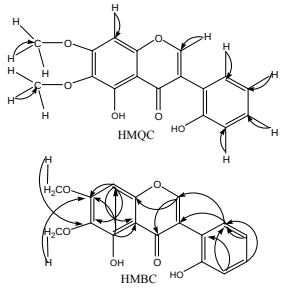

Figure 2. HMQC and HMBC correlation of irilin A (500 MHz in $CDCl_3$).

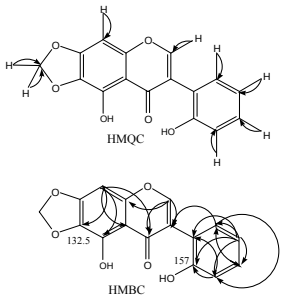

Figure 3. HMQC and HMBC correlation of Irisone B (400 MHz in C_5D_5N).

This data was further substantiated by a ^{1}H-NMR signal at 8.01, which is typical for the two hyrogens of an isoflavone (δ 7.8-8.1) (10). The ^{1}H-NMR aromatic signals (δ 7-7.6 in C_5D_5N and δ 7-7.4 in $CDCl_3$) showed a complex coupling patterns indicated a 2′ substituent in the phenyl moiety which was further confirmed by 2-D COSY. The position of the proton (δ 6.6 and 89.59) assigned on C-8. A chemical shift at δ 6.06 and 103 (2H) indicated a metylenedioxy (OCH_2O) group in C-6 and C-7. Two single peaks, δ 11.62 and 13.51, showed the position of hydroxyl group at C-2′ and C-5 respectively (8, 9). All the chemical shifts were confirmed by means of HMBC and HMQC (figure 2). The broad bond (BB) decoupled ^{13}C-NMR of B (C_5D_5N, 100 MHz) showed resonances for all sixteen carbons in the molecule. The Adept (BB and DEPT) spectra revealed the presence of one methylene, 6 methines, and nine quaternary carbons. A downfield quaternary signal at 181.87 indicated the presence of carbonyl. The ^{13}C-NMR spectra also showed the presences of twelve aromatic carbons (table 1), which comprise of 5 methines (δ 89.59, 116.89, 130.26, 119.52 and 132.55) and 7 quaternary carbons of which two were found to be oxygen-bearing centers (11).

Table 1. $^{1}H\&^{13}C$–NMR assignment for the isoflavoniods irilin A and irisone B (values in $CDCl_3$* and C_5D_5N**).

Pair proton or carbon	Irilin A ^{1}H-NMR* 500MHz	Irisone B ^{1}H-NMR** 500MHz	Irisone B ^{1}H-NMR** 500MHz	Irisone B ^{13}C-NMR** 100MHz
2	8.07 *s*	8.04 *s*	8.3 *s*	156.13 C-H
3				121.45 C
4				181.87 C
6				130.26 C-H
7				154.51 C
8	6.51 *s*	6.59 *s*	6.6 *s*	89.59 C-H
9				153.75 C
10				108.79 C
1′				118.96 C
3′	7.08 *d* (7.7)	7.09 *d* (7.76)	7.23 *dd* (8,0.8)	116.89 C-H
4′	7.35 *dt* (7.7,1.22)	7.35 *dt* (7.76,1.06)	7.32 *ddd* (8,7.2,1.6)	130.26 C-H
5′	6.98 *t* (7.7)	6.98 *t* (7.76)	7.03 *ddd* (7.5,7.2,0.8)	119.52 C-H
6′	7.15 *dd* (7.7,1.22)	7.15 *dd* (7.76,1.06)	7.59 *dd* (7.5,1.6)	132.55 C-H
5-OH	12.12 *s*	12.13 *s*	13.51 *s*	143.03 C
2′-OH	7.98 *s*	8.01 *s*	11.62 *s*	157.00 C
O-CH_2-O	-	6.13 *s*	6.07 *s*	103.24 H-C-H
OMe	3.89 *s*			61
OMe	3.96 *s*			56

Figures in parentheses refer to coupling constants in Hz.

The structural similarities between irisone B and irilin A was revealed by comparison of the ^{1}H-NMR spectra of these two compounds (table 1). In the ^{1}H-NMR spectrum of irilin A the signal belonging to 2′-OH (δ 8.07), a hydrogen bonded hydroxyl at C-5 (δ 12.13), a 2′- substituted B ring (δ 7.09, 3′-H; 7.35, 4′-H; 6.98, 5′-H; 7.15, 6′-H) and an 8-hydrogen (δ 6.59) were observed. Signal at δ 6.13 belonging to methylenedioxy group was not present while two signals were observed at δ 3.89 and 3.96 (3H). It could be concluded that the OCH_2O group has been replaced by two methoxy groups at C-6 and C-7.This was further supported by differences between high resolution electron impact (HR EI) MS spectra of irisone B, $[M]^+$ at m/z 298.05149 (calc. for $C_{16}H_{10}O_6$, 298.04771), as well as IR spectra (922 cm^{-1} OCH_2O in B, 1370 and 1454 cm^{-1} OCH_3 in A). The most interesting problem in comparative study of ^{1}H-NMR spectra of B in $CDCl_3$ and C_5D_5N was observation of clear meta coupling in C_5D_5N and shift of 2′-OH to downfield (table 1). Around 300 species isoflavone have been reported till 1994; of which 27 contained methylenedioxy moieties. Nine isoflavones with this residue belong to *Iris* species (6). Assessment of the biological activities of these two compounds is suggested in order to investigate the role of two OCH_3 substitutions or OCH_2O group on their biological activity.

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