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ISOCLADRASTIN AND KASHMIGENIN—TWO ISOFLAVONES FROM *IRIS KASHMIRIANA*

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Key Word Index—*Iris kashmiriana*; Iridaceae; rhizomes; isoflavones; isocladrastin; kashmigenin; junipegenin-B.

Abstract—From the rhizomes of the *Iris kashmiriana*, two new isoflavones, isocladrastin and kashmigenin, were characterized as 3′-hydroxy-6,7-4′-trimethoxy-isoflavone and 4′-hydroxy-3′,5′-dimethoxy-6,7-methylenedioxy-isoflavone, respectively. Also, junipegenin-B has been found present in this plant species.

INTRODUCTION

In our earlier work on *Iris kashmiriana* [1], we recorded the presence of two isomeric isoflavones, iriskashmirianin and isoiriskashmirianin [2], besides irilone [3], irisolone [4] and irisolone methyl ether [5], in the rhizomes of the plant. Herein, we report the isolation and characterization of two more new isoflavones, isocladrastin (**1**) and kashmigenin (**2**), in addition to the known Junipegenin-B [6].

RESULTS AND DISCUSSION

The continued chromatography of the ethyl acetate soluble fraction of the methanolic extract of the rhizomes of the *Iris kashmiriana* afforded compounds **1**–**3**, which responded to colour [7] tests for isoflavones. Their UV [8] and IR [2] spectra also conformed with the isoflavone skeleton.

The compound **1**, M^+ at *m/z* 328.2096, $C_{18}H_{16}O_6$, showed a low field resonance signal at δ7.91 (1H, *s*), due to H-2 of isoflavones. The resonance signals at δ3.92 (3H, *s*) and 3.96 (6H, *s*) revealed the presence of three methoxyls in the compound. A free phenolic group (ν_{max} 3250 cm^{-1}, *br*); δ5.70 (1H, *br s*, exch. D_2O) was confirmed by its conversion to the monoacetate **4**, M^+ at *m/z* 370.2338, $C_{20}H_{19}O_7$, (ν_{max} 1730 cm^{-1}), δ2.36 (3H, *s*, $OCOH_3$); and to the tetramethoxyisoflavone **5**, M^+ at *m/z* 342.2310, $C_{19}H_{18}O_6$, (ν_{max} 1025 cm^{-1}), δ3.96 (6H, *s*, $2\times OCH_3$), 3.98 (3H, *s*, OCH_3) and 4.02 (3H, *s*, OCH_3).

The MS of the compound contained the molecular ion peak as the base peak and two peaks of nearly equivalent abundance at *m/z* 152 (39.8%) and 148 (40.1%). The peak at *m/z* 152 was prominent even in the spectra of **4** and **5**. Its appearance could be rationalized by considering the loss of two methylenes from the *RDA*-fragment, involving ring-A, at *m/z* 180 (weak). This, together with the inertness of **1** towards the diagnostic UV shift reagents [8], placed the two methoxyls in the A-ring. The lone hydroxyl and a methoxyl was present in the B-ring.

Compound **1** gave positive Gibb's test [9], placing the hydroxyl in the ring-B at C-2′ or C-3′ with a free *para* position. Its ^1H NMR spectrum revealed an ABX pattern with two double doublets at δ7.02 (2H, J = 8.5, 2 Hz), attributable to H-5′ and H-2′, and 7.36 (1H, J = 8.5, 2 Hz), attributable to H-6′. These signals were split in the ^1H NMR spectra of **4** and **5**. The exact positions of the methoxyl and hydroxyls were decided by NOE experiments. The irradiation of the resonance signal at δ3.92 (3H, *s*), in the spectrum of **1**, increased the intensity of the signal at δ7.02, due to H-5′, by 35%, without affecting other resonance signals. On irradiation of the signal at δ3.92, in the spectrum of **4**, caused a 20% increase in the signal at δ7.61, due to H-2′. Further, the chemical shifts, multiplicities and coupling constants of the B-ring aromatic protons of **5** were in close agreement with that of 5,7,3′,4′-tetramethoxyisoflavone from *Corydalis africana* [10]. *Thus* **1**, *isocladrastrin*, has the structure: 3-hydroxy-6,7,4′-tri-*O*-methoxy-isoflavone.

Compound **2**, M^+ at *m/z* 342.1910, $C_{18}H_{14}O_7$, was also an isoflavone, δ7.70 (1H, *s*, H-2). The compound carried a hydroxyl, ν_{max} 3340 cm^{-1}, δ5.60 (1H, *br s*, exch. D_2O), which could be located at a position other than C-5 and C-2′. On acetylation, it gave a monoacetate **6**,

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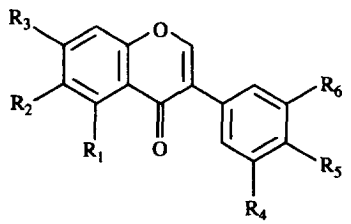

	R_1	R_2	R_3	R_4	R_5	R_6
1	H	OMe	OMe	OH	OCH	H
2	H	O-CH_2-O		OMe	OH	OMe
3	OH	OMe	OH	H	OMe	OMe
4	H	OMe	OMe	OAc	OMe	H
5	H	OMe	OMe	OMe	OMe	H
6	H	O-CH_2-O		OMe	OAc	OMe
7	H	O-CH_2-O		OMe	OMe	OMe
8	OAc	OMe	OAc	OMe	OMe	OMe

M^+ at *m/z* 384.2160 $C_{20}H_{16}O_8$ (ν_{max} 1730 cm^{-1}); δ2.40 (3H, *s*, $OCOCH_3$). The ^1H NMR spectrum of **2** also contained resonance signals of two magnetically non-equivalent methoxyl at δ3.89 and 4.05 (3H, each, *s*) which were shifted to δ3.96 and 4.35 (3H, each, *s*), respectively, in the spectrum of the acetate **6**. The two proton resonance signal at δ6.02 and 6.12 (2H, *s*), in the spectra of **2** and **6**, respectively, was due to the presence of a dioxymethylene function.

The MS of **2** and **6** contained two peaks of almost the same ionic abundance at *m/z* 164 (12.64 and 10.85%) and 178 (12.14 and 10.90%) arising from the RDA fragmentation of the γ-pyrone ring. The latter peak at *m/z* 178, in the MS of the compound **6**, originated from a less abundant (2.60%) peak *m/z* 220, by the loss of 42 amu. Further fragmentation of the ionic peak at *m/z* 164 was in agreement with the presence of the dioxymethylene group in the ring-A. The fragment ion at *m/z* 178 underwent loss of a methyl radical to give the fragment ions at *m/z* 163 and 162. The latter fragment underwent further successive loss of CO, CH_3 and CO to give the ion peaks at *m/z* 134, 119 and 91.

The ^1H NMR spectra of **2** and **6** contained signals for the *para* coupled protons at δ6.80 and 6.70 (1H, each, *s*), due to H-5 and H-8, respectively. The two singlets at δ7.03 and 7.23 (1H, each, *d*, J = 2.2 Hz), in the ^1H NMR spectrum of **2**, shifted to δ7.32 and 7.40 (1H each, *d*, J = 2.2 Hz, in the spectrum of **6**, showed that the B-ring protons were *meta* coupled. The irradiation of the methoxyl signal at δ3.69, in the spectrum of the compound **6**, there was an increase of 36% in the intensity of the signal at δ7.32, due to H-2′, and the irradiation of the signal at δ4.35, there was an increase of 27% in the intensity of the signal at δ7.40, due to H-6′, showing that the *ortho* positions to the methoxyls were free. Thus, the hydroxyl in the ring-B was flanked by two methoxyls. On comparing the chemical shifts of the *meta* coupled B-ring protons with those of the irigenin [6] (δ6.65 and 6.70), the chemical shifts were found to be different. On methylation, **2** gave the methyl ether **7**, M^+ at *m/z* 356.1923, $C_{19}H_{16}O_7$, whose B-ring protons showed identical chemical shifts and coupling constants as that of irigenin methyl ether [11]. The compound **2**, kashmigenin, was thus assigned the structure 4′-hydroxy-6,7-methylenedioxy-3′,5′-dimethoxyisoflavone.

The spectral data (UV, IR, ^1H NMR and MS) of the compound **3**, its diacetate **8** and dimethyl ether **9** was identical with junipegenin-B, previously isolated from *Juniperus macropoda* [6].

EXPERIMENTAL

Mps are uncorr. ^1H NMR at 90 MHz and 60 MHz in $CDCl_3$ and HRMS was carried out at 70 eV.

Extraction and isolation. The fresh bulbs of *Iris kashmiriana* procured from Tangmarg area (Kashmir, India) vouch No. UD/1986-413 were chopped into small pieces, ground mechanically and extracted with hot MeOH. The extract was filtered, freed from the solvent under reduced pressure and re-extracted with hot EtOAc. The EtOAc soluble portion was chromatographed on silica gel using gradient C_6H_6–EtOAc systems. The fractions of CC were monitored by TLC on silica gel G. The C_6H_6–EtOAc (9:1) afforded a mixture of compounds which on rechromatography over silica gel columns followed by disc chromatography over same absorbent, using C_6H_6–$CHCl_3$ (9:1) afforded three mixtures from which the compounds **1**–**3** were recovered by chromatography over silica gel using C_6H_6–EtOAc (9:1). The compounds were finally purified by crystallization from C_6H_6–MeOH.

General procedure for acetylation. 20–25 mg of the compound dissolved in C_5H_5N (5 ml) was treated with AC_2O (2 ml) and left for 24–48 hr. The reaction was monitored by TLC. The acetates were treated with acidulated water, the ppt. was filtered, dried and dissolved in chloroform. The mixture was separated by chromatography using petrol–benzene (3:7) and the acetates were crystallized from $CHCl_3$–MeOH.

General procedure for methylation. 20–30 mg of the compound was dissolved in dry methanol (25–40 ml) and anhydrous K_2CO_3 (0.5–1 g) was added to the solution. The MeI (5 ml) was now added, dropwise with constant shaking to the solution. The mixture was refluxed on water bath for 2 hr, filtered and freed from solvent and crystallized from C_6H_6–MeOH.

Isocladrastin **1**. mp 230–231°; M^+ at *m/z* 328.2096 (calc. for $C_{18}H_{16}O_8$, 328.326); UV: λ_{max}^{MeOH} 261, 324 (infl) nm; no shifts with diagnostic reagents. IR: ν_{max}^{KBr} cm^{-1} 3250 (*br*–OH), 1640, 1620, 1580, 1510, 1450, 1320, 920. ^1H NMR (90 MHz), $CDCl_3$: δ3.92 (3H, *s*; 4′-OCH_3) 3.96 (6H, *s*; 6-OCH_3, 7-OCH_3), 5.70 (1H, *br s*, exch. D_2O 3′-OH), 6.80 (1H, *s*, H-8), 7.02 (2H, *dd*, J = 8.5 Hz 2.5 Hz H-2′, H-5′), 7.36 (1H, *dd*, J = 8.5, 2Hz H-6′), 7.58 (1H, *s*, H-5), 7.91 (1H, *s*, H-2). MS: *m/z* at 328 (M^+) 327 (M^+-H), 313 (M^+-CH_3), 300 (M^+-CO), 298, 285, 283, 282, 270,

243, 152 (RDA, ring-A, 39%), 148 (RDA, ring-B, 40.5%), 147, 137, 134, 133, 124, 122, 109, 105, 76.

Isocladrastin monoacetate **4**. mp 176°, 177° M^+ at *m/z* 370.2338 (calc. for $C_{20}H_{18}O_7$, 370.364). UV (MeOH): λ_{max}^{MeOH} 256, 320 296 nm IR: ν_{max}^{KBr} cm^{-1} 1730, 1620, 1585, 1430, 1380, 1245, 920. ^1H NMR (90 MHz) $CDCl_3$; δ2.36 (3H-*s*-3'-$OCOCH_3$), 3.92 (3H, *s*, 4'-OCH_3), 3.96 (6H, *s*-*b*'-OCH_3), 6.80 (1H, *s*, H-8), 7.12 (1H-*d*-overlapped, J = 8.5 Hz, H-5'), 7.29 (1H, *d*, J = 1.2 Hz, H-2'), 7.57 (1H, *s*, H-5), 7.61 (1H, *dd*, J = 8.5, 1.2 Hz, H-6'), 7.97 (1H, *s*, H-2) MS: *m/z* at 370 (M^+), 328 (M^+-CH_2CO, 100%), 190 (RDA, ring-B $COCH_2$), 134, 124.

Isocladrastin methyl ether, **5**. mp 198–199°, M^+ at *m/z* 342.2310 (calc. for $C_{19}H_{18}O_6$, 342.353) UV λ_{max}^{MeOH} 273, 333 nm. IR: ν_{max}^{KBr} cm^{-1} 1615, 1590, 1490, 1320, 1245, 920. ^1H NMR (90 MHz) $CDCl_3$; δ3.96 (6H, *s*, 6,7-OCH_3) 3.98 (3H, *s*,-3'-OCH_3) 4.02 (3H, *s* 4'-OCH_3), 6.86 (1H, *s*, H-8) 6.90 (1H, *d*, J = 8.0 Hz H-5'), 7.05 (1H, *d*, J = 2.0 Hz, H-2'), 7.23 (1H, *dd*, J = 8, 2 Hz, H-6') 7.60 (1H, *s* H-5) 7.94 (1H, *s* H-2).

Kashmigenin, **2**. mp 199–200°, M^+ at *m/z* 342.1910 (calc. for $C_{18}H_{14}O_7$, 342.31). Light green colour with $FeCl_3$, OH, UV: λ_{max}^{MeOH} 285, 328 (infl). IR: ν_{max}^{KBr} cm^{-1} 3340 (br, *s*, OH) 1660, 1610, 1580, 1490, 1310, 1220, 940. ^1H NMR ($CDCl_3$, 60 MHz); δ3.98 (3H, *s*-3'-OCH_3), 4.05 (3H, 5'-OCH_3), 5.60 (1H, *s*, *br* exch. D_2O, 4'-OH), 6.88 (2H, *s*, H-5, H-8), 7.23 (H-2', H-6'), 7.70 (1H, *s*, H-2), 6.02 (2H, *s*,–OCH_2–O), MS: *m/z* at 342 (M^+), 314 (M^+) (–OCH_2, 100%), 286, 178 (RDA, ring-B 2.14%, 164 (RDA, ring-A, 2.86%, 163, 258, 244, 136, 149, 121, 106.

Kashmigenin monoacetate **6**. mp 176–177°, M^+ at *m/z* 384.2160 (calc. for $C_{20}H_{16}O_8$, 384.348) UV: λ_{max}^{MeOH} 270, 320 nm. IR: ν_{max}^{KBr} cm^{-1} 1730, 1620, 1590, 1450, 1320, 1245, 1120, 1050, 940. ^1H NMR ($CDCl_3$) 60 MHz: δ2.40 (3H, *s*, 4'-OAc), 3.69 (3H, *s*, 3'–OCH_3), 4.25 (3H, *s*, 5'–OCH_3), 6.12 (2H, *s*, –OCH_2O–), 6.89 (2H, *s*, H-5, H-8), 7.32 (^1H, *d*, J = 2.2 Hz), H-6', 7.40 (^1H, *d*, J = 2.2 Hz, H-2'), 7.70 (1H, *s*, H-2).

Kashmigenin methyl ether **7**. mp 182–185°, M^+ at *m/z* 356.2051 (calc. for $C_{19}H_{16}O_7$, 356.337). UV λ_{max}^{MeOH} nm 258, 310 nm. IR: ν_{max}^{KBr} cm^{-1} 1630, 1620, 1585, 1490, 1330, 1245, 920. ^1H NMR ($CDCl_3$, 60 MHz): δ3.69 (6H, *s*, 3',4'–OCH_3), 3.72 (3H, *s*,-5'–OCH_3), 6.12 (2H, *s*, OCH_2O), 7.32 (2H, integ. together, H-2', H-6'), 6.89 (1H, *s*, H-8), 6.93 (1H, *s*, H-5), 7.70 (1H, *s*, H-2). MS: *m/z* 356 (M^+, 100%), 341 (M^+–CH_3), 326 (M^+–CH_2); 183 (RDA, ring-B, 16.5%), 164 (RDA, ring-A, 15.2%), 169, 164, 141, 134, 127, 106.

Janipegenin **B-3**. mp 187–188°, M^+ at *m/z* 344.2068 (calc. for $C_{18}H_{16}O_7$, 344.326); green colour with $FeCl_3$; pink colour with Na–Hg HCl, UV: λ_{max}^{MeOH} 268, 340 nm. IR: ν_{max}^{KBr} cm^{-1} 3370 (OH), 1650, 1575, 1450, 1365, 1150, 1065, 1015 ^1H NMR (DMSO-d_6, 60 MHz): 3.98 (6H, *s*, 3',4'–OCH_3) 4.12 (3H, *s*, 6-OCH_3) 6.68 (1H, *s*, H-8), 7.10 (2H, *d*, J = 8.5 Hz, H-5', H-6'), 7.38 (1H, *d* submerged, J = 2.0 Hz, H-2') 8.02 (1H, *s*, H-2) 13.0 (1H, *br*-*s*, exch. D_2O,–5–OH) MS: *m/z* at 344 (100%), 329, 326, 315, 301, 172, 162, 157.

Diacetate of janipegenin-**B**, **8**. mp 160–161°; M^+ at 428, 2450 (calc. for $C_{22}H_{20}O_9$, 428.402) UV: λ_{max}^{MeOH} 240, 325 (infl); I.R.; ν_{max}^{KBr} cm^{-1} 1770, 1640, 1610, 1580, 1490, 1380, 1230, 1190, 1080, ^1H NMR ($CDCl_3$, 60 MHz). δ2.40 (3H, *s*, 7-OAc), 2.46 (3H, *s*, 5-OAc), 3.90 (3H, *s*, 6-OCH_3), 3.96 (6H, *s*, 3', 4'-OCH_3), 6.97 (2H, *d*, J = 8.5 Hz, H-5'-6'), 7.06 (1H, *s*, H, 2'), 7.20 (1H, *s*, H-8), 7.83 (1H, *s*, H-2) M:S *m/z* at 428 (M^+), 386, 344, 266, 224, 182, 168, 162, 148, 140, 130.

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