



ISOCLADRASTIN AND KASHMIGENIN—TWO ISOFLAVONES FROM *IRIS KASHMIRIANA*

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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, junipegeenin-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 Junipegeenin-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.

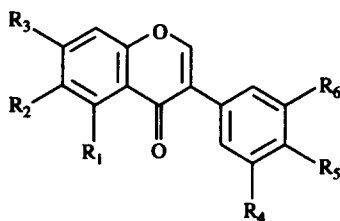
The compound 1, M^+ at m/z 328.2096, $C_{18}H_{16}O_6$, showed a low field resonance signal at δ 7.91 (1H, s), due to H-2 of isoflavones. The resonance signals at δ 3.92 (3H, s) and 3.96 (6H, s) revealed the presence of three methoxyls in the compound. A free phenolic group (ν_{\max} 3250 cm^{-1} , br); δ 5.70 (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}), δ 2.36 (3H, s, $OCOCH_3$); and to the tetramethoxyisoflavone 5, M^+ at m/z 342.2310, $C_{19}H_{18}O_6$, (ν_{\max} 1025 cm^{-1}), δ 3.96 (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 δ 7.02 (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 δ 3.92 (3H, s), in the spectrum of 1, increased the intensity of the signal at δ 7.02, due to H-5', by 35%, without affecting other resonance signals. On irradiation of the signal at δ 3.92, in the spectrum of 4, caused a 20% increase in the signal at δ 7.61, 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, isocladrastin, 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, δ 7.70 (1H, s, H-2). The compound carried a hydroxyl, ν_{\max} 3340 cm^{-1} , δ 5.60 (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 C₂₀H₁₆O₈ (ν_{\max} 1730 cm⁻¹); δ 2.40 (3H, s, OCOCH₃). The ¹H NMR spectrum of **2** also contained resonance signals of two magnetically non-equivalent methoxyl at δ 3.89 and 4.05 (3H, each, s) which were shifted to δ 3.96 and 4.35 (3H, each, s), respectively, in the spectrum of the acetate **6**. The two proton resonance signal at δ 6.02 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, CH₃ 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 δ 6.80 and 6.70 (1H, each, s), due to H-5 and H-8, respectively. The two singlets at δ 7.03 and 7.23 (1H, each, d, *J* = 2.2 Hz), in the ¹H NMR spectrum of **2**, shifted to δ 7.32 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 δ 3.69, in the spectrum of the compound **6**, there was an increase of 36% in the intensity of the signal at δ 7.32, due to H-2', and the irradiation of the signal at δ 4.35, there was an increase of 27% in the intensity of the signal at δ 7.40, 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] (δ 6.65 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, C₁₉H₁₆O₇, 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 CDCl₃ 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 C₆H₆-EtOAc systems. The fractions of CC were monitored by TLC on silica gel G. The C₆H₆-EtOAc (9:1) afforded a mixture of compounds which on rechromatography over silica gel columns followed by disc chromatography over same absorbent, using C₆H₆-CHCl₃ (9:1) afforded three mixtures from which the compounds **1-3** were recovered by chromatography over silica gel using C₆H₆-EtOAc (9:1). The compounds were finally purified by crystallization from C₆H₆-MeOH.

General procedure for acetylation. 20–25 mg of the compound dissolved in C₅H₅N (5 ml) was treated with Ac₂O (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 CHCl₃-MeOH.

General procedure for methylation. 20–30 mg of the compound was dissolved in dry methanol (25–40 ml) and anhydrous K₂CO₃ (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 C₆H₆-MeOH.

Isocladrastin 1. mp 230–231°; M⁺ at *m/z* 328.2096 (calc. for C₁₈H₁₆O₈, 328.326); UV: $\lambda_{\max}^{\text{MeOH}}$ 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), CDCl₃: δ 3.92 (3H, s, 4'-OCH₃) 3.96 (6H, s, 6-OCH₃, 7-OCH₃), 5.70 (1H, *br* s, exch. D₂O 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, 2 Hz 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⁺-CH₃), 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 C₂₀H₁₈O₇, 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) CDCl₃; δ 2.36 (3H, s-3'-OCOCH₃), 3.92 (3H, s, 4'-OCH₃), 3.96 (6H, s, b'-OCH₃), 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⁺-CH₂CO, 100%), 190 (RDA, ring-B COCH₂), 134, 124.

Isocladrastin methyl ether, 5. mp 198–199°, M⁺ at *m/z* 342.2310 (calc. for C₁₉H₁₈O₆, 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) CDCl₃; δ 3.96 (6H, s, 6,7-OCH₃) 3.98 (3H, s, 3'-OCH₃) 4.02 (3H, s 4'-OCH₃), 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 C₁₈H₁₄O₇, 342.31). Light green colour with FeCl₃, 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 (CDCl₃, 60 MHz); δ 3.98 (3H, s-3'-OCH₃), 4.05 (3H, s, 5'-OCH₃), 5.60 (1H, s, br exch. D₂O, 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, -OCH₂-O), MS: *m/z* at 342 (M⁺), 314 (M⁺) (-OCH₂, 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 C₂₀H₁₆O₈, 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 (CDCl₃) 60 MHz: δ 2.40 (3H, s, 4'-OAc), 3.69 (3H, s, 3'-OCH₃), 4.25 (3H, s, 5'-OCH₃), 6.12 (2H, s, -OCH₂O-), 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 C₁₉H₁₆O₇, 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 (CDCl₃, 60 MHz): δ 3.69 (6H, s, 3',4'-OCH₃), 3.72 (3H, s, 5'-OCH₃), 6.12 (2H, s, OCH₂O), 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⁺-CH₃), 326 (M⁺-CH₂); 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 C₁₈H₁₆O₇, 344.326); green colour with FeCl₃; 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'-OCH₃) 4.12 (3H, s, 6-OCH₃) 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. D₂O, -5-OH) MS: *m/z* at 344 (100%), 329, 326, 315, 301, 172, 162, 157.

Diacetate of junipegenin-B, 8. mp 160–161°; M⁺ at 428, 2450 (calc. for C₂₂H₂₀O₉, 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 (CDCl₃, 60 MHz). δ 2.40 (3H, s, 7-OAc), 2.46 (3H, s, 5-OAc), 3.90 (3H, s, 6-OCH₃), 3.96 (6H, s, 3', 4'-OCH₃), 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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