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PHENOLIC CONSTITUENTS OF *IRIS MILESII* RHIZOMES

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Key Word Index—*Iris milesii*; Iridaceae; rhizomes; isoflavones; flavanone; flavonol; ferulic acid derivatives; benzoquinone derivatives.

Abstract—Analysis of a methanol extract of the rhizomes of *Iris milesii* resulted in the isolation of a new isoflavone, 5,6,7,4′-tetrahydroxy-8-methoxyisoflavone along with prunetin, sakuranetin, 2:6-dimethoxy-1,4-benzoquinone, tectorigenin, irigenin,4-β(D-glucosyloxy)-ferulic acid methyl ester, quercetin-3-methyl ether, tectoridin, iridin and iristectorin B.

INTRODUCTION

In continuation of our work on *Iris* species [1–4], we came across *I. milesii* growing wild in abundance around Budhal near Poonch, on which no chemical work has been reported in the literature. Here, we wish to report the isolation and characterisation of a new isoflavone viz. 5,6,7,4′-tetrahydroxy-8-methoxyisoflavone along with some other compounds [5] isolated from extracts of the rhizomes of this *Iris* species.

RESULTS AND DISCUSSION

Compound **1**, mp 280°, analysed for $C_{16}H_{12}O_7$ and gave a strong ferric chloride test. Its IR displayed bands at 3600–3200 and 1660 cm^{-1} suggesting the presence of a chelated –C=O group. It formed tetraacetate, mp 125°, analysing for $C_{24}H_{20}O_{11}$, indicating the presence of four hydroxyl groups. The UV spectrum of **1** gave band II and band I at 272 and 320 nm, respectively, confirming it to be an isoflavone. Band II shifted to 303 nm upon addition of aluminium chloride and further shifted to 285 nm with aluminium chloride–hydrochloric acid, indicating the presence of a hydroxyl at C-5. Band II further exhibited a bathochromic shift of 12 nm with NaOAc, showing the presence of another hydroxyl at C-7. ^{1}H NMR (DMSO-d_6) displayed a sharp singlet integrating for one proton at δ8.4 assigned to the C-2 proton and a pair of *ortho* doublets (J = 8.5 Hz) each integrating for two protons centred at 7.40 and 6.83 were assigned to C-2′, C-6′ and C-3′, C-5′ protons, respectively. The doublet centered at 6.83 exhibited a downfield shift of 0.25 ppm on acetylation, indicating the presence of a 4′-hydroxyl group. Mass spectrometry also supported the presence of a hydroxyl group in ring B by giving retro-Diels–Alder ions at m/z 118 and 155 $[M-118-43]^+$. Methoxyl protons resonated at 3.8. A sharp singlet integrating for one proton at 12.0 and a multiplet integrating for three protons at 9.0 disappeared on exchange with D_2O.

On the basis of the above data, the three hydroxyl groups were thus placed at C-4′, C-5 and C-7. While the position of the fourth hydroxyl group was established by acetonide formation when **1** resulted in the formation of two acetonides with a marked difference in their R_f values, which could arise only if the hydroxyl group was placed at C-6. In this case the methoxyl group could be at C-8. Had the fourth hydroxyl group been at C-8, it would have formed only one acetonide, thereby confirming the structure **1** as 4H-1-benzopyran-4-one-3-(4 hydroxyphenyl)-5,6,7-trihydroxy, 8-methoxy.

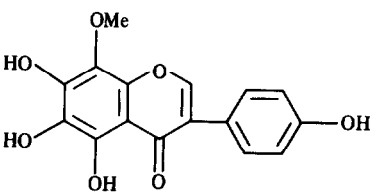
Further investigations resulted in the isolation of 10 more compounds, identified as prunetin; sakuranetin; 2,6-dimethoxy-1,4-benzoquinone; tectorigenin; irigenin; 4-β(D-glucosyloxy)-ferulic acid methyl ester; quercetin 3-methylether; tectoridin; iridin and iristectorin B. All the compounds were identified from their UV, IR, ^{1}H NMR and mass spectra.

EXPERIMENTAL

The plant under investigation was collected from Budhal near Poonch (J&K State, India). The plant was identified as *Iris milesii* M. Foster by the botanists of the Herbarium of Central Council of Research in Ayurveda and Siddha, Jammu Branch, (Voucher specimen No 6925, 6924, 7700, 7183).

Dry rhizomes (1.5 kg) were air dried, finely powdered and the defatted material was extracted with MeOH (3 × 3 l.). MeOH extracts were concd in vacuum to afford a residue (150 g). The residue was slurried with silica gel and extracted with $CHCl_3$, EtOAc and MeOH successively in a Soxhlet.

The MeOH extract (20 g) was subjected to CC on silica gel (1.5 kg) and eluted successively with $CHCl_3$–MeOH mixtures of increasing polarity. Compound **1** was obtained from $CHCl_3$–MeOH (4.1) eluates, needles (200 mg), [R_f = 0.5



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($CHCl_3$–MeOH, 17:3); 0.45 (C_6H_6–EtOAc, 2:3)], mp 280°. (Found: C, 60.60; H, 3.79 required: C, 60.63; H, 3.80%), analysed for $C_{16}H_{12}O_7$; UV λ_{max}^{MeOH} nm: 272, 320, + $AlCl_3$ 303, 350 (sh); + $AlCl_3$ + HCl 285, 355; + NaOAc 285, 245, + NaOMe 285, 235. IR ν_{max}^{KBr} cm^{-1}: 3600–3200, 1660 (–C=O), 1600, 1500, 1430, 1320, 1200, 1080, 1020 and 800. EIMS (probe) 70 eV, *m/z* (rel. int.): $[M]^+$ 316 (100), 301 (80), 273 (50), 183 (20), 155 (50), 118 (25) etc Acetylation of compound **1** gave a tetraacetate, colorless needles, mp 125; analysed for $C_{24}H_{20}O_{11}$. IR ν_{max}^{KBr} cm^{-1}: 3000, 1760 (OAc) 1635, (–C=O), 1595, 1480, 1400, 1350, 1150, 1000 and 840. ^1H NMR (60 MHz, $CDCl_3$): δ7.8 (1H, *s*, H-2), 7.42 (2H, *d*, *J* = 8 5 Hz, H-2′ and H-6′), 7.1 (2H, *d*, *J* = 8.5 Hz, H-3′ and H-5′), 3 8 (3H, *s*, OMe), 2 42, 2.35, 2.30 (12H, 3s, 4 × OAc).

Formation of acetonide. Compound **1** (20 mg) was refluxed with dry Me_2CO (15 ml) and *p*-toluenesulfonic acid (2 mg) at 100° for 3–4 hr The formation of two acetonides was checked by TLC, they showed a marked difference in their R_f values (C_6H_6–EtOAc, 2:3), R_f = 0.8 and 0.3.

Tectoridin was obtained from the $CHCl_3$–MeOH (3:1) eluates, white amorphous powder (2 g), mp 258°, analysed for $C_{22}H_{22}O_{11}$, hexaacetate, mp 80°, analysed for $C_{34}H_{34}O_{17}$, acidic hydrolysis afforded tectorigenin. Iridin from $CHCl_3$–MeOH (7.3) eluates, white amorphous powder (800 mg), mp 208°, analysed for $C_{24}H_{26}O_{13}$, acidic hydrolysis yielded irigenin. Iristectorin B from $CHCl_3$–MeOH (7:3) eluates, white amorphous powder (200 mg), mp 280°, analysed for $C_{23}H_{24}O_{12}$, hexaacetate, mp 90°, analysed for $C_{35}H_{36}O_{18}$, acidic hydrolysis afforded yellow crystalline substance, mp 240°, analysed for $C_{17}H_{14}O_7$, identified as iristectorigenin.

The $CHCl_3$ residue (10 g) was chromatographed over silica gel (900 g), prunetin was obtained from the C_6H_6–$CHCl_3$ (1:9) eluates, yellow needles (30 mg), mp 240°, analysed for $C_{16}H_{12}O_5$, diacetate, mp 222°; analysed for $C_{20}H_{16}O_7$. Sakuranetin from $CHCl_3$ eluates, as yellow needles (20 mg), mp 152°, analysed for $C_{16}H_{14}O_5$, diacetate, mp 97°, analysed for $C_{20}H_{18}O_7$. 2·6-Dimethoxy-1,4-benzoquinone from $CHCl_3$–MeOH (19:1) eluates, yellow needles (20 mg), mp 252°, analysed for $C_8H_8O_4$. Tectorigenin from $CHCl_3$–MeOH (9:1) eluates, yellow needles (2 g), [R_f 0.5 ($CHCl_3$–MeOH, 25:2), 0.6 (C_6H_6–EtOAc, 2:3)], mp 230°, analysed for $C_{16}H_{12}O_6$, triacetate, mp 190°, analysed for $C_{22}H_{18}O_9$. Irigenin from $CHCl_3$–MeOH (17:3) eluates, white needles (500 mg), [R_f 0.6 ($CHCl_3$–MeOH, 25:2), 0.7 (C_6H_6–EtOAc, 2:3)], mp 185°, analysed for $C_{18}H_{16}O_8$.

The EtOAc residue (10 g) was chromatographed over silica gel (1 kg) and eluted successively with $CHCl_3$–MeOH mixtures of differing proportions.

4-β(D-Glucosyloxy)ferulic acid Me ester was obtained from $CHCl_3$–MeOH (41:9) eluates, a gum (200 mg), analysed for $C_{17}H_{22}O_9$, tetraacetate, gum, analysed for $C_{25}H_{30}O_{13}$, acidic hydrolysis yielded white needles, mp 63°, analysed for $C_{11}H_{12}O_4$, identified as ferulic acid Me ester; monoacetate, mp 82°, analysed for $C_{13}H_{14}O_5$. Quercetin-3-methyl ether from $CHCl_3$–MeOH (4:1), eluates yellow solid, mp 259°, analysed for $C_{16}H_{12}O_7$, tetraacetate, mp 220°, analysed for $C_{24}H_{20}O_{11}$.

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