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ISOFLAVONES OF TWO *IRIS* SPECIES

V K AGARWAL, R K THAPPA, S G AGARWAL, M S MEHRA* and K L DHAR

Regional Research Laboratory, Jammu Tawi 180001, India, *Bareilly College, Bareilly, India

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Key Word Index—*Iris milesii*, *Iris kumaonensis*, Iridaceae, rhizomes, isoflavones

Abstract—Analysis of the methanol extracts of the rhizomes of *Iris milesii* resulted in the isolation of a new isoflavone, 5,6,7,4′-tetrahydroxy-3′-methoxyisoflavone and that of *Iris kumaonensis*, iriskumaonin methyl ether, iriskumaonin, irisflorentin, junipegenin-A, irigenin and iridin

INTRODUCTION

Recently we reported a new isoflavone from *Iris milesii* [1] Further work on the same species has now afforded another new isoflavone characterized as 5,6,7,4′-tetrahydroxy-3′-methoxyisoflavone *Iris kumaonensis* on chemical investigation resulted in the isolation of iriskumaonin methyl ether [2], irisflorentin [3, 4], junipegenin-A [5], being reported here for the first time from *Iris kumaonensis*, along with iriskumaonin [6], irigenin and iridin Junipegenin-A has been reported recently only from *Juniperus macropoda* (Cupressaceae)

RESULTS AND DISCUSSION

Compound **1**, mp 257°, analysed for $C_{16}H_{12}O_7$ (M^+ 316) and gave a strong $FeCl_3$ test Its IR spectrum displayed bands at 3600–3200 and 1650 cm^{-1} suggesting the presence of a chelated C=O group It formed a tetraacetate, mp 88°, analysing for $C_{24}H_{20}O_{11}$ and tetramethyl ether, mp 188°, analysing for $C_{20}H_{20}O_7$, indicating the presence of four hydroxyl groups The UV spectrum had maxima at 320 and 278 nm respectively, indicating it to be an isoflavone The presence of hydroxyl groups at C-5 and C-7 was indicated by the application of diagnostic shift reagents

The ^1H NMR spectrum (60 MHz, $CDCl_3$ + TFA) established its nature as isoflavone A sharp singlet at δ 7 99 was assigned to a C-2 proton, a *meta* coupled singlet (1H) at 7 2 due to a C-2′ proton and a broad singlet (2H) at 6 97 assigned to C-5′ and C-6′ protons The C-8 proton was located at 6 66 which moved downfield to 7 20 on acetylation and methoxyl protons resonated at 4 00 Retro-Diels–Alder ions at *m/z* 148 and 168 established the presence of one hydroxyl and one methoxyl in ring B and three hydroxyls in ring A

The positions of the methoxyl group at C-3′ and hydroxyl group at C-4′ in the ring B were ascertained by the oxidative degradation of **1** with permanganate to give a compound, mp 207°, identified as vanillic acid (lit mp 210°) [7] (co-TLC, mmp)

Compound **1** on methylation afforded a tetramethyl ether, mp 188°, analysing for $C_{20}H_{20}O_7$, and was found to be identical in all respects (co-TLC, mmp, ^1H NMR) with the dimethyl ether of junipegenin-B, mp 186° [8], thereby confirming the structure of **1**

Iris kumaonensis on chemical investigation gave six isoflavones, identified as iriskumaonin, iriskumaonin methyl ether, irisflorentin, junipegenin-A, irigenin and iridin All the compounds were identified from their UV, IR, ^1H NMR and mass spectra

EXPERIMENTAL

The plant *Iris milesii* was collected from Budhal near Poonch (J & K State, India) and *Iris kumaonensis* from Mari (Lahaul), (Voucher specimen no 12603, Herbarium, RRL, Jammu) Dry rhizomes (1 5 kg) of each species were separately dried, finely powdered and the defatted material was extracted with MeOH (3 × 3 l) The MeOH extracts were concd *in vacuo* to afford *ca* 150 g of each residue

The MeOH-residue (20 g) of *Iris milesii* was subjected to CC over silica gel (1 5 kg) and eluted successively with $CHCl_3$–MeOH mixtures of increasing polarity Compound **1** was obtained from $CHCl_3$–MeOH (17 3) eluates as yellow needles (40 mg), [R_f 0 65 ($CHCl_3$–MeOH, 17 3), 0 50 (C_6H_6–EtOAc, 2 3)], mp 257°, (Found C, 60 60, H, 3 79, required C, 60 62, H, 3 81 %), analysed for $C_{16}H_{12}O_7$ UV λ_{max}^{MeOH} nm 278, 320 (sh), + $AlCl_3$ 312, 340 (sh), + $AlCl_3$ + HCl 300, 345 (sh), + NaOAc 292, 325 (sh), + NaOMe 318, 340 (sh) IR ν_{max}^{KBr} cm^{-1} 3600–3200 and 1650 (C=O) EIMS (probe) 70 eV, *m/z* (rel int) $[M]^+$ 316 (100), 301 (80), 168 (40), 148 (20), 140 (18) and 133 (10) Acetylation of **1** (Ac_2O–C_5H_5N) gave a tetraacetate, crystallized from MeOH, needles, mp 88°, analysed for $C_{24}H_{20}O_{11}$ ^1H NMR (60 MHz, $CDCl_3$) δ 7 8 (1H, *s*, H-2), 7 23–7 17 (3H, *m*, H-8, H-2′ and H-5′), 7 03 (1H, *br s*, H-6′), 3 8 (3H, *s*, OMe), 2 33, 2 18, 2 17, 2 16 (12H, 4*s*, 4 × OAc) Methylation of **1** with DMS–K_2CO_3–Me_2CO gave a tetramethyl ether, plates from MeOH, mp 188°, analysed for $C_{20}H_{20}O_7$ ^1H NMR ($CDCl_3$, 90 MHz) δ 7 76 (1H, *s*, H-2), 7 12 (1H, *br s*, H-2′), 6 95 (1H, *d*, J = 8 Hz, H-6′), 6 80 (1H, *d*, J = 8 Hz, H-5′), 6 63 (1H, *s*, H-8), 3 88 (15H, *s*, 5 × OMe)

The MeOH residue (20 g) of *Iris kumaonensis* on silica gel (1 5 kg) CC with eluents $CHCl_3$–*n*-hexane and $CHCl_3$–MeOH mixtures in increasing polarity afforded the compounds, iriskumaonin methyl ether from $CHCl_3$–*n*-hexane (4 1) eluates, needles (50 mg), mp 183°, analysed for $C_{19}H_{16}O_7$, irisflorentin from $CHCl_3$–*n*-hexane (9 1) eluates, needles (200 mg), mp 180°, analysed for $C_{20}H_{18}O_8$, iriskumaonin from $CHCl_3$–MeOH (49 1) eluates, needles (500 mg), mp 207–208°, analysed for $C_{18}H_{14}O_7$ irigenin from $CHCl_3$–MeOH (19 1) eluates, yellow needles (2 g), [R_f = 0 6 ($CHCl_3$–MeOH, 25 2), 0 7

(C_6H_6–EtOAc, 2 3)], mp 185°, analysed for $C_{18}H_{16}O_8$, junipegenin-A from $CHCl_3$–MeOH (17 3) eluates, analysed for $C_{16}H_{12}O_7$, iridin from $CHCl_3$–MeOH (4 1) eluates, amorphous powder (2 g), mp 208°, analysed for $C_{24}H_{26}O_{13}$

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ISOSOJAGOL, A COUMESTAN FROM *PHASEOLUS COCCINEUS*

MELANIE J O'NEILL, S A ADESANYA and MARGARET F ROBERTS

Department of Pharmacognosy, The School of Pharmacy, University of London, 29–39 Brunswick Square, London, WC1, U K

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Key Word Index—*Phaseolus coccineus*, Leguminosae, coumestan, isosojagol

Abstract—A novel coumestan isolated from *Phaseolus coccineus* has been characterized as 3,9-dihydroxy-10-(γ,γ-dimethylallyl)-coumestan and named isosojagol

INTRODUCTION

Previous research has resulted in the isolation of five coumestans from *Phaseolus* species following either infection with fungi or treatment with $CuCl_2$ Coumestrol **1** has been found to occur in *P vulgaris*, *P lunatus*, *P aureus* and *P calcaratus* [1], psoralidin **2** has been detected in *P lunatus* [1], sojagol **3** has been isolated from *P aureus* [1], phaseol **4** occurs in *P aureus* [2] and aureol **5** has been obtained from *P aureus* [2] and *P mungo* [Adesanya, O'Neill and Roberts, unpublished] The present report describes the isolation of three coumestans from the runner bean *P coccineus* which in addition to coumestrol and aureol produces a novel coumestan, isosojagol **6** after treatment with $CuCl_2$

RESULTS AND DISCUSSION

The ethyl acetate extract from $CuCl_2$-treated *P coccineus* seedlings was fractionated on a polyamide column using a chloroform–methanol gradient Purification of fractions by TLC revealed three fluorescent substances which gave purple colours with Fast Blue Salt B reagent [3] UV spectroscopy suggested that the three compounds may be coumestans and two of the substances were subsequently identified as coumestrol **1** and aureol **5** by a comparison of their TLC, UV, mass spectral and ^1H NMR characters with authentic standards and literature values [2, 4]

The UV spectrum of the third coumestan contains principal maxima at 206, 246 and 347 nm with the mid-wavelength maximum having a lower intensity than that at 347 nm Addition of sodium acetate produced a bathochromic shift indicating a free hydroxyl at C-3 The presence of one or more other phenolic functions in the molecule was revealed by further UV spectral shifts upon addition of sodium methoxide The mass spectrum gave a plausible $[M]^+$ peak at m/z 336 and a fragmentation pattern similar to those observed for the prenylated coumestans psoralidin [5], sojagol [6] and phaseol [2] Intense signals at m/z 281 and 280 in the spectrum of the new compound could be attributed to loss of C_4H_7 and C_4H_8 radicals from a prenylated $[M]^+$ at m/z 336 A minor peak at m/z 253 could result from loss of CO from the ion at m/z 281 Such a transition is typical of coumestans in which removal of the lactonic carbonyl is an important fragmentation

The ^1H NMR spectrum indicated that the compound possessed a γ,γ-dimethylallyl side chain rather than a 2,2-dimethylchromene ring Signals were also observed for five aromatic protons, three of which show *ortho* coupling, one is *meta* coupled and one shows both *ortho* and *meta* coupling The two possible structures which can account