(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 $C_{24}H_{26}O_{13}$

Acknowledgements—The authors are thankful to Mr B K Kapai for the plant material (*I kumaonensis*) and the Instrumentation Section for providing the spectral data

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Phytochemistry, Vol 23, No 11, pp 2704–2705, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00

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

(*Received* 27 *April* 1984)

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

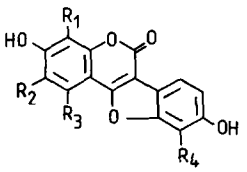
Previous research has resulted in the isolation of five coumestans from *Phaseolus* species following either infection with fungi or treatment with $CuCl_2$ Coumestrol **1** has been found to occur in *P vulgaris*, *P lunatus*, *P aureus* and *P calcaratus* [1], psoralidin **2** has been detected in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in *P aureus* [2] and aureol **5** has been obtained from *P aureus* [2] and *P mungo* [Adesanya, O'Neill and Roberts, unpublished] The present report describes the isolation of three coumestans from the runner bean *P coccineus* which in addition to coumestrol and aureol produces a novel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



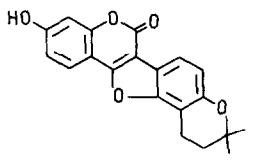
1 $R_1 = R_2 = R_3 = R_4 = H$

2 $R_1 = R_3 = R_4 = H$, $R_2 = CH_2CH{=}C(CH_3)_2$

4 $R_2 = R_3 = R_4 = H$, $R_1 = CH_2CH{=}C(CH_3)_2$

5 $R_1 = R_2 = R_4 = H$, $R_3 = OH$

6 $R_1 = R_2 = R_3 = H$, $R_4 = CH_2CH{=}C(CH_3)_2$



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for these features are 3,9-dihydroxy-4(γ,γ-dimethylallyl)-coumestan (phaseol **4**), which has been isolated previously from *P aureus* [2] and 3,9-dihydroxy-10(γ,γ-dimethylallyl)-coumestan **6** Since the ^{1}H NMR spectrum resonance frequencies of the new compound differ from those observed for phaseol, the new compound was provisionally characterized as 3,9-dihydroxy-10(γ,γ-dimethylallyl)-coumestan Confirmation of this identity was obtained by acid cyclization of the prenyl side chain which produced a compound whose UV and mass spectra closely resembled literature values for sojagol [6] The UV spectral maximum of the cyclized compound at 346 nm in ethanol underwent a shift to 362 nm in sodium acetate and a further shift to 374 nm in sodium methoxide Apparently, therefore, cyclization of the prenyl moiety involves the C-9 hydroxyl rather than the C-3 hydroxyl The new coumestan was subsequently assigned the trivial name isosojagol

EXPERIMENTAL

Extraction and purification P coccineus var Scarlet Emperor seeds, obtained from Mr Fothergill's seeds, England, were germinated, grown and the seedlings treated with aq $CuCl_2$ as previously described [7] Seedlings (3 5 kg) were exhausted with EtOH and the EtOH extracts were concd *in vacuo* The residue was partitioned between H_2O and EtOAc and the organic fraction was concd to a viscous liquid (6 68 g) which was chromatographed on a column of polyamide (Polyclar Gaf) Elution was achieved using a $CHCl_3$–MeOH gradient, starting at 10% and increasing the MeOH proportion to 100% over 22 l at which time elution ceased, 75 ml fractions were collected Isosojagol eluted between 2025 and 3150 ml, coumestrol between 3225 and 4875 ml and aureol between 4957 and 5850 ml Further purification was achieved by TLC on silica gel GF_{254} developed in hexane–EtOAc–MeOH (6 4 1) (solvent A) and $CHCl_3$–*iso*-PrOH (9 1) (solvent B)

Coumestrol **1** Yield 9 2 mg Detected as a blue fluorescent band on TLC at R_fs = 0 23 in solvent A and 0 63 in solvent B UV, MS and ^{1}H NMR characteristics as in refs [4, 6]

Aureol **5** Yield 10 7 mg Detected as a yellow fluorescent band on TLC at R_fs 0 60 in solvent A and 0 65 in solvent B UV, MS and ^{1}H NMR characteristics as given in ref [2]

Isosojagol **6** Yield 6 2 mg Detected as a blue fluorescent band on TLC at R_fs 0 44 in solvent A and 0 50 in solvent B UV λ_{max}^{EtOH} nm 206, 246, 266 (sh), 295 (sh) 307, 347, EtOH + NaOAc 265, 315, 362, EtOH + NaOMe 205, 275, 320, 380 MS *m/z* (rel int) 336 (52) $[M]^+$, 281 (44) $[M-C_4H_7]^+$, 280 (100) $[M-C_4H_8]^+$, 253 (9) $[M-C_4H_7-CO]^+$, ^{1}H NMR 400 MHz (Me_2CO-d_6) δ7 81 (1H, *d*, J = 8 6 Hz, C-1 or C-7), 7 75 (1H, *d*, J = 8 5 Hz, C-7 or C-1), 7 19 (1H, *d*, J = 2 2 Hz, C-4), 7 07 (1H, *d*, J = 8 7 Hz, C-8), 7 04 (1H, *dd*, J = 8 5, 2 2 Hz, C-2), 5 33 (1H, *br t*, $J \approx$ 7 4 Hz, C-2'), 3 62 (2H, *br d*, $J \approx$ 7 4 Hz, C-1'), 1 89 (3H, *s*, Me), 1 67 (3H, *s*, Me)

Acid cyclization of **6** Isosojagol (3 mg), HOAc (1 ml) and conc H_2SO_4 (2 drops) were kept at room temp in the dark for 4 hr TLC in solvent A gave a single fluorescent product (2 1 mg) at R_f 0 75 UV λ_{max}^{EtOH} nm 207, 244, 266 (sh), 295 (sh), 307, 346, 360 sh, EtOH + NaOAc 210, 307, 362, 386 (sh), EtOH + NaOMe 206, 255 (sh), 275, 315 (sh), 374, MS *m/z* (rel int) 336 (75) $[M]^+$, 281 (50) $[M-C_4H_7]^+$, 280 (100) $[M-C_4H_8]^+$

Acknowledgements—The authors are grateful to The Federal Government of Nigeria for financial support (to S A A), to Dr G Hawkes (Queen Mary College, University of London) for ^{1}H NMR spectra and to Mr D Carter and Mr K Welham (S O P) for MS

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