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# GENISTEIN 7-(2″-*p*-COUMAROYLGLUCOSIDE) FROM *TRIFOLIUM REPENS*

V. K. SAXENA and A. K. JAIN

Natural Products Laboratory, Department of Chemistry, Dr. H. S. Gour Vishwavidyalaya, Sagar (M.P.) 470003, India

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**Key Word Index**—*Trifolium repens*; Leguminosae; genistein 7-*O*-(2″-*p*-coumaroyl-β-D-glucopyranoside); isoflavone.

**Abstract**—A new acetylated isoflavone, genistein 7-(2″-*p*-coumaroylglucoside), has been characterized from *Trifolium repens*.

Earlier reports of the occurrence of genistein and biochanin-A [1] from *Trifolium repens* [2] encouraged us to investigate this plant further.

A new acylated isoflavone (**1**) was isolated from a 95% ethanolic extract of the dried plant by extraction with ethyl acetate and column chromatography of the concentrated ethyl acetate extract to give a single spot on TLC. It gave positive tests for an isoflavone glycoside [3], $C_{30}H_{26}O_{12}$, $[M]^+$ at *m/z* 578, mp 250–252°, and analysed for C = 62.28%, H = 4.49%, calculated; found: C = 62.24%, H = 4.53%.

UV spectral analysis indicated the presence of free hydroxyl groups at C-5 and C-4′, and acid hydrolysis yielded genistein, *p*-coumaric acid and D-glucose (by co-PC and co-TLC). On treatment with methanolic NaOMe it gave genistein 7-*O*-β-D-glucopyranoside, mp 263–265° and methyl coumarate ($R_f$ 0.73), which were identified by UV, IR, $^1$H NMR and MS analysis.

Permethylation [4] of **1** gave a hexa-*O*-methyl ether, **2**. $C_{36}H_{38}O_{12}$, $[M]^+$ at *m/z* 662, which on acid hydrolysis yielded genistein 5,4′-dimethyl ether indicating that the acyl residue was attached to the sugar moiety. On acetylation with acetic anhydride–pyridine it gave a hexaacetate, **3**, $C_{42}H_{38}O_{18}$, $[M]^+$ at *m/z* 830. $^1$H NMR analysis of **3** showed the presence of one *p*-coumaroyl residue, and the chemical shifts for the protons H-1″ (5.52 ppm) and H-2″ (5.06 ppm) indicated acylation at position C-2″ [5], thus confirming **1** to be genistein 7-*O*-(2″-*p*-coumaroyl-β-D-glucopyranoside), which was further confirmed by its mass spectral analysis.

## EXPERIMENTAL

Plants of *Trifolium repens* (L.) were supplied by M/s Himalaya range drug field, Simla, and authenticated by the Botany Department of this university.

*Spectral data for* **1**. UV $\lambda_{max}^{MeOH}$ nm: 265, 332; + NaOMe 275, 284, 360; + $AlCl_3$ 274, 304, 378; + $AlCl_3$–HCl 243, 380; + NaOAc 278, 323; + $H_3BO_3$ 264, 330. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3348, 1774, 1705, 1560–1610, 1282, 1216, 1150, 825.

*Acid hydrolysis of* **1**. A methanolic soln of **1** (350 mg) was refluxed with 10% HCl (50 ml) and run on TLC ($C_6H_6$–$Me_2CO$–$H_2O$, 4:3:1) giving genistein ($R_f$ 0.36), *p*-coumaric acid ($R_f$ 0.47) and D-glucose. Genistein, $C_{15}H_{10}O_5$, mp 296–297°; UV $\lambda_{max}^{MeOH}$ nm: 260, 331; + NaOMe 271, 326; + $AlCl_3$ 274, 304, 370; + $AlCl_3$–HCl 280, 312, 380; + NaOAc 280, 324; + $H_3BO_3$ 268, 330.

*Alkaline methylation of* **1**. **1** in absolute MeOH was treated with 2% NaOMe overnight, neutralized, evaporated to a syrup and extracted with dry $Et_2O$. The $Et_2O$-soluble fraction on TLC (EtOAc–HOAc–$H_2O$, 3:2:3) gave methyl *p*-coumarate ($R_f$ 0.72) and genistein 7-glucoside ($R_f$ 0.38). *Methyl* p-*coumarate*: $^1$H NMR ($CDCl_3$, TMS as internal standard) δ7.70 (*d*, *J* = 8 Hz, H-β), 7.56 (*d*, *J* = 4.6 Hz, H-2, H-6), 6.88 (*d*, *J* = 4.5 Hz, H-3, H-5), 6.28 (*d*, *J* = 7.8 Hz, H-α), 2.46 (*s*, OAc), 3.83 (*s*, OMe). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 2970, 1720, 1680, 1610, 1560, 1330, 820. MS *m/z*: 178 $[M]^+$ 147, 119, 91, 69, 55. *Genistein* 7-*glucoside*: UV $\lambda_{max}^{MeOH}$ nm: 265, 328; + NaOMe 275, 320; + $AlCl_3$ 270, 308, 372; + $AlCl_3$–HCl 282, 308, 382; + NaOAc 278, 320; + $H_3BO_3$ 272, 334.

*Genistein* 7-O-β-D-*glucopyranoside permethyl ether* was prepared as described by Brimacombe [6] and worked up in the usual manner.

*Genistein* 5,4′-*di*-O-*methyl ether*. The permethyl ether of genistein 7-glucoside on acid hydrolysis gave genistein 5,4′-dimethyl ether.

**1** *Hexa*-O-*acetate* (**3**). **1** was heated with $Ac_2O$–pyridine for 4 hr and the acetate worked up in the usual manner. **3** crystallized from MeOH, $CHCl_3$, mp 240–242°. $^1$H NMR ($CDCl_3$, TMS as internal standard): δ8.04 (*s*, H-2), 6.75 (*d*, *J* = 2.5 Hz, $C_6$-H) 2.51 (*s*, 3H of 5-OAc), 7.42 (*d*, *J* = 2.4 Hz, $C_8$-H), 2.28 (*s*, $C_4$′-OAc), 7.46 (*d*, *J* = 9 Hz, $C_2$′-H, $C_6$′-H) 7.12 (*d*, *J* = 9 Hz, $C_3$′–$C_5$′-H), 4.43 (*d*, *J* = 7.0 Hz, 1″ anomeric proton) 2.12 (*s*, C-3″ OAc), 2.02 (*s*, C-4″ OAc), 3.93 (*s*, C-6″ OAc) 5.46 (m-6 proton of glucose unit). MS *m/z*: 578 $[M]^+$, 459, 431, 270, 269, 165, 153, 152, 135, 124, 118.

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REFERENCES

1. Shutt, D. A. (1976) *Endeavour* **35**, 110.
2. Chopra, R. N., Nayar, S. L. and Chopra, I. C. (1974) *Supplement of Glossary of Indian Medicinal Plants*, p. 97. CSIR, New Delhi.
3. Narayana, V. and Seshadri, T. R. (1971) *Indian J. Chem.* **9**, 14.
4. Wagner, H. and Seligman, O. (1973) *Tetrahedron* **29**, 3029.
5. Ansari, F. R., Ansari, W. H., Rahman, W., Seligman, O., Chari, V. M., Wagner, H. and Osterdahl, B. (1979) *Planta Med.* **36**, 196.
6. Brimacombe, J. S., Jones, B. P., Stacey, M. and Willard, J. J. (1966) *Carbohydr. Res.* **2**, 167.

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# TWO 12a-HYDROXYROTENOIDS FROM *BOERHAAVIA COCCINEA*

I. MESSANA*, F. FERRARI and A. E. GOULART SANT'ANA†

Centro Chimica dei Recettori e delle Molecole Biologicamente Attive del C.N.R., Istituto di Chimica, Università Cattolica del Sacro Cuore, Largo F. Vito, 1-Roma, Italy; †Depto de Quimica, U.F.A.L., 57.000 Maceiò, Brazil

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**Key Word Index**—*Boerhaavia coccinea*; Nyctaginaceae; roots; 12a-hydroxyrotenoids; $^{13}$C NMR.

**Abstract**—Two new 12a-hydroxyrotenoids, (−)-4,11,12a-trihydroxy-9-methoxyrotenoid and (−)-4,9,11,12a-tetrahydroxyrotenoid, were isolated from the roots of *Boerhaavia coccinea*. Their structures were established by spectroscopic methods.

## INTRODUCTION

*Boerhaavia coccinea* is a Brazilian plant known by the trivial name of pega-pinto. The roots are used in traditional medicine for the treatment of liver, loins and urinary diseases [1]. Only two species of *Boerhaavia* have been extensively studied, *B. repens* [2, 3] and *B. diffusa* [4], from which alkaloids and polysaccharides have been isolated. In this paper we report the isolation of (−)-4,11,12a-trihydroxy-9-methoxyrotenoid (**1**) and (−)-4,9,11,12a-tetrahydroxyrotenoid (**2**) from a methanolic extract of roots of *B. coccinea*.

## RESULTS AND DISCUSSION

Compound **1**, $C_{17}H_{14}O_7$, $[M]^+$ at *m/z* 330, $[\alpha]_D^{20}$ −339°, was obtained as an amorphous powder. It showed UV maxima (MeOH) at 340 (sh), 293 and 216 nm, and IR bands ($CHCl_3$) at 3550 (br, OH) and 1630 cm$^{-1}$ (chelated C=O). The $^1$H NMR spectrum of **1** exhibited the signals of five aromatic protons, two of which were *meta*-coupled (δ6.08 and 6.10, 2H, 2*d*, $J$ = 2 Hz), a methoxyl group (δ3.86, 3H, *s*), a chelated hydroxyl (δ11.85, 1H, *s*, exchangeable with $D_2O$) and an ABC system (δ4.44, 1H, *dd*, $J$ = 5.5 and 10 Hz; δ4.48, 1H, *t*, $J$ = 10 Hz; δ4.77, 1H, *dd*, $J$ = 5.5 and 10 Hz), attributed to a O–$CH_2$–CH–O sequence. In agreement with the latter assignment, the $^{13}$C NMR spectrum of **1** (see Experimental) showed resonances at 77.1 and 62.4 ppm (doublet and triplet, respectively, in the off-resonance decoupled $^{13}$C NMR spectrum), together with a signal at 67.0 ppm (singlet).

The aforementioned data suggested for **1** the structure of a 12a-hydroxyrotenoid. After acetylation, **1** was transformed into the corresponding derivative **3**, $C_{23}H_{20}O_{10}$, which showed in its $^1$H NMR spectrum the signals of an aliphatic acetyl group (δ1.88), assigned to the 12a position, and two aromatic acetyl groups (2.29 and 2.37).

In the mass spectrum of **1** the base peak at *m/z* 167 ($C_8H_7O_4$) originated from the typical retro-Diels–Alder fragmentation of 6a,12a-saturated rotenoids [5], thus confirming the suggested structure and the assignment of the methoxyl and the chelated hydroxyl group to the D-ring (Scheme 1). On account of the presence in the $^1$H NMR spectrum of **1** of a low-field shifted signal (δ7.78, 1H, *dd*, $J$ = 3 and 7 Hz), the third hydroxyl group could be located on C-4 and, therefore, the structure of 4,11,12a-trihydroxy-9-methoxyrotenoid was unambiguously assigned to **1**. The *trans*-B/C ring junction was assigned to **1** on the basis of the H-1 chemical shift value [6], whereas it was impossible to correlate the Cotton effect curve to the absolute configuration on account of the absence of proper models. Compound **2**, $C_{16}H_{12}O_7$, $[M]^+$ at *m/z* 316, crystals from $CHCl_3$–MeOH, mp 237–240°, $[\alpha]_D^{20}$ −440°, exhibited UV maxima (MeOH) at 335 (sh), 293 and 231 nm. The structure of a 9-demethyl derivative of **1** could be assigned to **2** by comparison of the $^1$H NMR and mass spectra of the two compounds (see Experimental). Moreover, **1** and **2** when treated with diazomethane gave the same methyl derivative by TLC comparison.

* To whom correspondence should be addressed.