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AN ISOFLAVONE GLUCOSIDE FROM *RETAMA SPHAEROCARPA* BOISSIER

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Key Word Index—*Retama sphaerocarpa*; Fabaceae; isoflavones glucosides; genistin; daidzin; 6′-methoxypseudobaptigenin 7-*O*-β-glucoside; 7-hydroxy-6′-methoxy-3′,4′-methylenedioxy-isoflavone 7-*O*-β-glucoside; ^{1}H and ^{13}C NMR spectra.

Abstract—The new isoflavone, 7-hydroxy-6′-methoxy-3′,4′-methylenedioxyisoflavone 7-*O*-β-glucoside (6′-methoxypseudobaptigenin 7-*O*-β-glucoside) and two known isoflavones, genistein 7-*O*-β-glucoside (genistin) and daidzein 7-*O*-β-glucoside (daidzin) have been isolated from the aerial parts of *Retama sphaerocarpa* and characterized by mp, UV, IR, FAB-MS and NMR data.

INTRODUCTION

As part of our continuing study of *Retama sphaerocarpa* (Fabaceae) [1—4] for novel compounds which might possess biological activity, we have undertaken a reexamination of the chemical constituents of the aerial parts of *R. sphaerocarpa*. We report here the isolation of a new isoflavone, 7-hydroxy-6′-methoxy-3′,4′-methylenedioxyisoflavone 7-*O*-β-glucoside (**1**), and two known isoflavones, genistin (**2**) [5] and daidzin (**3**) [6]. Their structures have been established on the basis of spectroscopic and chemical evidence.

RESULTS AND DISCUSSION

Chromatography of the ethyl acetate extract of the aerial parts of *Retama sphaerocarpa* gave 7-hydroxy-6′-methoxy-3′,4′-methylenedioxyisoflavone 7-*O*-β-glucoside (6′-methoxypseudobaptigenin 7-*O*-β-glucoside) (**1**) and genistein 7-*O*-β-glucoside (genistin), while chromatography of the chloroform extract of the aerial parts gave daidzein 7-*O*-β-glucoside (daidzin). The structural elucidation of the new compound (**1**) is described in the present study. The compounds (**2**) and (**3**) are known and exhibited mp, UV spectral, FABS-MS, ^{1}H and ^{13}C NMR analytical data identical with those of genistin [5] daidzin [6].

7-hydroxy-6′-methoxy-3′,4′-methylenedioxyisoflavone 7-*O*-β-glucoside (**1**) was isolated as fine needles (133–135°) and gave positive Labat test for methylenedioxy-groups [7]. The UV spectrum [λ_{max}/MeOH: 215, 245, 260, 296] [8] indicated that **1** is an isoflavone glycoside. IR showed α-β unsaturated carbonyl group and methylenedioxy group absorption at 1640 and 926 cm^{-1} respectively. The ^{1}H NMR spectrum displayed it to be an 6′-methoxy-3′,4′-methylenedioxy-isoflavone glycoside; the spectrum of the compound showed a characteristic singlet at δ 8.08 (H-2) of an isoflavone, the sharp singlets at δ 3.70 (3H) and δ 6.02 (2H) indicated the presence of one methoxy and one methylenedioxy group, two doublets at δ 7.86 (J = 8.7 Hz) and δ 7.11 (J = 2.3) could be assigned to H-5 and H-8 respectively, a double doublet at the δ 7.16 (J = 8.7, 2.3 Hz) corresponding to H-6 and the singlets at δ 6.83 and δ 6.75 are attributed to H-2′ and H-5′ respectively. The sugar showed anomeric one proton doublet at δ 5.20 with diaxial coupling (J = 7.4 Hz). ^{13}C NMR spectrum exhibited two signals at δ 102.2 and δ 57.0 that were assigned to carbons corresponding to methylenedioxy and methoxy group, besides, the ^{13}C-NMR spectrum confirmed the presence of the sugar residue by one anomeric carbon signal at δ 101.4,^{13}C. Multiplicities were determinated by DEPT pulse sequence. The FAB-mass spectrum displayed $[M+Na]^+$ at m/z 497 (30%).

Acid hydrolysis (6% HCl) of a small amount of **1** afforded a substance identified by spectroscopic methods as 6′-methoxypseudobaptigenin [9]. Chromatographic analysis of the sugar (glucose) was carried out by GC/MS and direct comparison with an authentic sample. Thus, this compound is 7-hydroxy-6′-methoxy-3′,4′-methylenedioxyisoflavone 7-*O*-β-glucoside and this is the first report of this compound in the literature.

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EXPERIMENTAL

General

mp: uncorr. ^1H and ^{13}C in DMSO-d_6. FAB-MS (thioglycerol + NaI matrix) CC: Sephadex LH 20 and silica gel. Acid hydrolysis and UV, IR spectral analyses with shift reagents were carried out according to standard procedures [8]. Flavonoids detection: UV, 366 nm and $AlCl_3$ reagent. Sugar analysis by CG/MS.

Plant material

The aerial parts of *R. sphaerocarpa* were collected, in May 1992, during the flowering period from Zahara de la Sierra (Cádiz, Spain). The identity was kindly verified by Dr A. Aparicio (Department of Botany of the Faculty of Pharmacy, University of Sevilla) and a voucher specimen is deposited, labelled SEVF.

Extraction and isolation

Air-dried, powdered aerial parts (500 g) of *R. sphaerocarpa* were extracted by Soxhlet successively for 24 hr with Et_2O and for 48 hr with MeOH. The MeOH extract was evaporated to dryness and suspended in 50 ml H_2O, then it was extracted successively with $CHCl_3$, EtOAc and BuOH. The concentrated EtOAc extract (1g) was dissolved in CH_2Cl_2-MeOH (1:1) and roughly chromatographed on Sephadex LH20 (15 × 300 mm), using mixtures of CH_2Cl_2-MeOH (1:1). The isoflavonoids glucosides **1** (15 mg) and **2** (9 mg) was isolated from the intermediate fractions by crystallization. The chloroform extract was fractionated on a silica column and the fraction eluted with EtOAc-MeOH-H_2O (80:3:3) and followed on Sephadex LH20 both give **3** (5 mg).

7-hydroxy-6′-methoxy-3′,4′-methylenedioxyisoflavone 7-O-β-glucoside (**1**). Fine needles, mp 133–135. UV λ_{max}^{MeOH} nm: 215, 245, 260, 308, unaffected on addition of NaOMe, NaOAc or $AlCl_3$. IR ν_{max}^{BrK} cm^{-1}: 1640, 926. ^1H NMR (200 MHz, DMSO-d_6): δ 8.02 (1H, *s*, H-2), 7.98 (1H, *d*, J = 8.9 Hz, H-5), 7.17 (1H, *dd*, J = 8.5, 2.6 Hz, H-6), 7.10 (1H, *d*, J = 2.3 Hz, H-8), 6.87 (1H, *s*, H-2′), 6.82 (1H, *s*, H-5′), 6.00 (2H, *s*, OCH_2O), 3.70 (3H, *s*, OCH_3), 5.5 (1H, *d*, J = 7.5 Hz, H-1″). ^{13}C NMR (50 MHz, DMSO-d_6); δ 56.5 (*q*, OCH_3), 60.6 (*t*, C-6″), 69.6 (*d*, C-4″), 73.1 (*d*, C-2″), 76.4 (*d*, C-5″), 77.2 (*d*, C-3″), 95.5 (*d*, C-5′), 99.9 (*s*, C-1″), 101.1 (*t*, OCH_2O), 103.4 (*d*, C-8), 110.9 (*d*, C-2′), 122.6 (*s*, C1′), 118.3 (*s*, C-10), 115.3 (*d*, C-6), 121.7 (*s*, C-3), 126.8 (*d*, C-5), 140.3 (*s*, C-3′), 147.9 (*s*, C-4′), 152.8 (*s*, C-6′), 154.7 (*d*, C-2), 157.0 (*s*, C-9), 161.3 (*s*, C-7), 174.2 (*s*, C-4). FAB-MS: *m/z* 497 (30%) $[M+Na]^+$.

REFERENCES

1. Martín-Cordero, C., Gil Serrano, A. M. and Ayuso González, M. J., *Plantes méd. et Phytothèr*, 1991, **25**, 157.
2. Martín-Cordero, C., Gil, A. M. and Ayuso, M. J., *J. Chem. Ecol.*, 1993, **19**, 2389.
3. Chacón, J. A., Martín-Cordero, C. and Ayuso, M. J., *Cytobios*, 1994, **80**, 155.
4. Martín-Cordero, C., Saenz, M. T., Ayuso, M. J. and Caviedes, M. A., *Fitoterapia*, 1995, **66**, 495.
5. Yuldashev, M. P., Batirov, E. Kh. and Malikov, V. M., *Chem. Nat. Compd.*, 1990, **26**, 465.
6. Kobayashi, M. and Ohta, Y., *Phytochemistry*, 1983, **22**, 1257.
7. Harper, S. H., Kemp, A. D., Umderwood, W. G. E. and Campbell, R. V. M., *J. Chem. Soc.*, 1969, (C), 1787.
8. Mabry, T. J., Markham, K. R. and Thomas, M. P., *The Systematic Identification of Flavonoids.*, 1970, Springer, Berlin.
9. Rao, E. V., Murthy, M. S. R. and Ward, R. S., *Phytochemistry*, 1984, **23**, 1493.