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A NEW ISOFLAVONE GLYCOSIDE FROM THE AERIAL PARTS OF *Retama sphaerocarpa*

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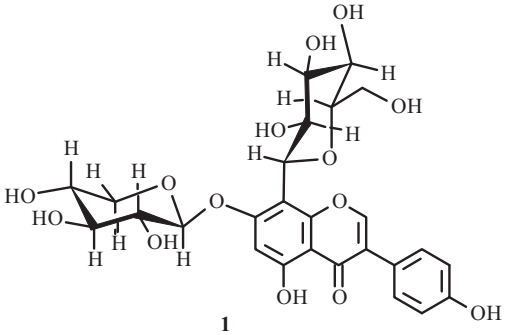
*A new isoflavone glycoside, genistein 7-*O*-xylosyl 8-C-glucoside (**1**), was isolated from the leaves of* Retama sphaerocarpa. *Its structure was elucidated by spectroscopic methods.*

Keywords*: Retama sphaerocarpa,* isoflavone glycoside, spectroscopic methods.

The genus *Retama* is located in the Atlas regions and the Sahara [1] (Arabic common name R′tem) and represented by three species in the flora of Algeria: *Retama monosperma*, *Retama retam*, and *Retama sphaerocarpa.* The last one is used to cure rabies in folk medicinal traditions in the east of Algeria. Investigations of *Retama sphaerocarpa* have led to the isolation of alkaloids [2–4], isoflavonoids [5–7], and flavonoids [8–11]. Previous studies showed that isoflavonoid glucosides are common in this genus. We now report the results of chemical examination of the methanolic extract of the flowering stems of *R. sphaerocarpa* Boissier (Fabaceae).

The powdered aerial parts (950 g) of *R. sphaerocarpa* were extracted with 70% MeOH. The MeOH extract was evaporated to dryness. The residue was dissolved in boiling water and extracted with ethyl acetate and *n*-BuOH successively. Solvents were evaporated and the residue of the ethyl acetate and *n*-BuOH extracts was dissolved in small volumes of MeOH. Two-dimensional paper chromatography using 15% AcOH and BAW (*n*-BuOH–AcOH–H_2O, 4:1:5 upper phase) as solvents had shown that the ethyl acetate and *n*-BuOH extracts contain almost the same compounds representing flavonoids. The *n*-BuOH extract was applied to a column of polyamide MN SC6 and eluted with a gradient of toluene–MeOH with increasing polarity. Compound **1** was isolated by preparative PC on Whatman 3MM paper using 15% AcOH, then by preparative TLC on polyamid DC6 eluting with H_2O–MeOH–methyl ethyl acetone–acetylacetone 13:3:3:1).



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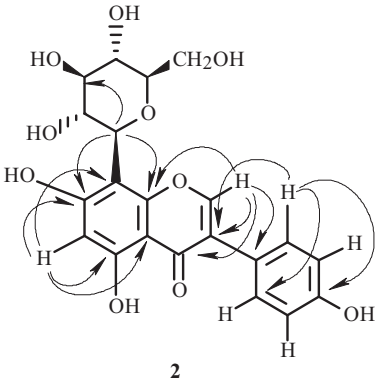

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Fig. 1. Important HMBC correlations for the structure elucidation of compound **2.**

Absorption bands at 257, 263 sh, and 324 sh nm in the UV spectrum of compound **1** in methanol and the singlet at 8.2 ppm in the ^{1}H NMR spectrum suggested that this compound was an isoflavone. Upon addition of NaOAc, the spectrum was unaffected (relative to the spectrum in methanol), suggesting that the C-7 hydroxyl group was substituted, and the presence of the 5-hydroxyl group is highlighted by the band II bathocromic shift δ λII = 10 nm after addition of $AlCl_3$ + HCl [12]. The ^{1}H NMR spectrum of compound **1** was obtained in CD_3OD, and the AA'BB' system consists of two proton doublets (J = 8.6 Hz) at δ 7.40 and 6.85 typical of a *para*-substituted B ring of the flavonoid.

We observed the presence of two anomeric sugar protons at 4.9 (1H, d, J = 9.9 Hz, anomeric proton of glucose H-1″) and 4.2 (1H, d, J = 6.6 Hz, anomeric proton of xylose H-1‴). Acid hydrolysis of **1** produced genistein 8-C-glucoside (**2**) and *D*-xylose. The identification of *D*-xylose in the water layer was carried out by comparison with an authentic sample (R_f 0.66) on TLC plates of silica gel eluted with acetone–H_2O (9:1) and was confirmed by the ^{1}H NMR coupling pattern. On the other hand, the resistance of the second sugar to acid hydrolysis confirmed the *C*-glycosyl structure. The ^{1}H and ^{13}C NMR spectral data of compound **2** were in agreement with published data [13, 14]. The positive electrospray MS exhibited a quasi-molecular ion $[M + H]^+$ at *m/z* 565, suggestive of the empirical formula $C_{26}H_{28}O_{14}$. Other important peaks appeared at *m/z* 433 $[M – 133(xylose) + H]^+$ corresponding to the loss of the xylosyl moiety from the protonated molecule. Then compound **1** was identified as genistein 7-*O*-xylosyl 8-C-glucoside.

EXPERIMENTAL

Compound **1**, $C_{26}H_{28}O_{14}$. UV (MeOH, λ_{max}, nm): 257, 263, 324; NaOAc: 263, 327; $AlCl_3$: 273, 307; $AlCl_3$/HCl: 274, 307, 364 nm. ES-MS positive ion mode *m/z*: 565 $[M + H]^+$, 587 $[M + Na]^+$, 433 $[M – 133(xylose) + H]^+$. ^{1}H NMR (CD_3OD, δ, ppm, J/Hz): 8.6 (1H, s, H-2), 7.4 (2H, d, J = 8.64, H-2′, H-6′), 6.85 (2H, d, J = 8.64, H-3′, H-5′), 6.28 (1H, s, H-6), 4.9 (1H, d, J = 9.9, anomeric proton of glucose H-1″), 4.2 (1H, d, J = 6.6, anomeric proton of xylose H-1‴).

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