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Novel flavonol glycoside from *Retama sphaerocarpa* Boissier

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Abstract

A novel flavonol glycoside, rhamnazin-3-*O*-β-glucopyranosyl-(1→5)-α-arabinofuranoside, was isolated from the aerial parts of *Retama sphaerocarpa* (Fabaceae) and its structure determined by mp, UV, IR, FAB-MS, GC–MS and NMR data.

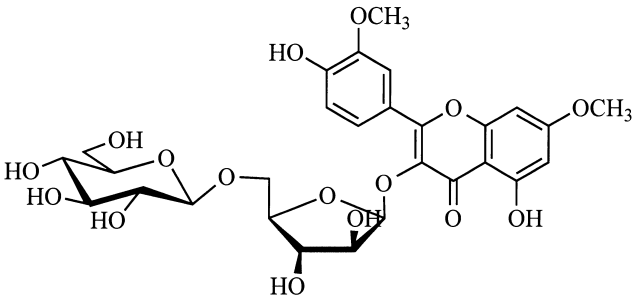
Keywords: *Retama sphaerocarpa*; Fabaceae; Flavonol glycoside; Rhamnazin-3-*O*-β-glucopyranosyl-(1→5)-α-arabinofuranoside; ^{1}H and ^{13}C NMR spectra

1. Introduction

Retama sphaerocarpa (Fabaceae) has been the subject of several chemical and pharmacological investigations (Martín-Cordero, Gil Serrano, & Ayuso González, 1991; Martín-Cordero, Gil, & Ayuso, 1993; Chacón, Martín-Cordero, & Ayuso, 1994; Martín-Cordero, Saenz, Ayuso, & Caviedes, 1995; López Lázaro, Martín-Cordero, Iglesias-Guerra, & Ayuso González, 1998). We have previously shown that an aqueous extract of aerial parts of *R.sphaerocarpa*, which is rich in flavonoids, possesses cytotoxic activity (Martín-Cordero et al., 1995). Therefore, continuing with our studies on *R.sphaerocarpa*, we have looked for novel compounds which might possess biological activity. For that, we have undertaken a reexamination of the chemical constituents of *R.sphaerocarpa*. We now report the isolation of a new flavonol glycoside (**1**), the rhamnazin-3-*O*-β-glucopyranosyl-(1→5)-α-arabinofuranoside. Its structure has been established on the basis of spectroscopic and chemical evidence.

2. Results and discussion

The butanol-soluble fraction of the methanolic extract of the aerial parts after separation by column chromatography gave rhamnazin-3-*O*-β-glucopyranosyl-(1→5)-α-arabinofuranoside (**1**). The structural elucidation of the new compound is described in the present study.



The compound (**1**) was isolated as yellow powder (166–168°C) which gave characteristic flavonoid colour reactions. The UV spectrum gave maxima at (λ_{max}/MeOH: 254, 265sh, 291sh, 353) and confirmation of the substitution of the 3-hydroxyl group was shown by the hypsochromic shift of band I compared with that of its aglycone ($\Delta\lambda_{max}$ 18 nm). The bathochromic shift induced by NaOMe (+59 nm without decrease in intensity relative to band I in MeOH) indicating the presence of a free OH-4′ group. The UV absorption spectrum, on addition of $AlCl_3$+HCl suggested the presence of 5-hydroxyl group. Its IR spectrum showed OH and α, β unsaturated C═O absorption at 3500–3400 and 1668 cm^{-1}, respectively and a broad C–O stretching band in the region 1100–1000 cm^{-1} suggesting its glycosidic nature (Mabry, Markham, & Thomas, 1970).

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The ^{1}H NMR spectrum in DMSO-d_6 exhibited two singlets at δ 3.86 (3H) and δ 3.87 (3H), indicating the presence of two methoxy groups, two doublets at δ 6.38 ($J=2.2$ Hz) and δ 6.77 ($J=2.2$ Hz) could be assigned to H-6 and H-8, respectively, a doublet at the δ 6.92 ($J=9$ Hz) corresponding to H-5′, a double doublet at the δ 7.67 ($J=2$, 9 Hz) and the doublet at δ 7.68 ($J=2$ Hz) are attributed to H-6′ and H-2′, respectively. In this spectrum the arabinose anomeric proton resonance was at δ 5.55, proving the attachment of this moiety to C-3 of the aglycone rhamnazin. The half-line width of this resonance (ca. 0.8 Hz) confirmed the α-configuration at the arabinose anomeric carbon. The anomeric β-glucoside proton was found resonating as a doublet ($J=7.6$ Hz) at δ 4.00 ppm. The phenolic proton (OH-5) signal is presented at δ 12.6. ^{13}C NMR spectrum showed 15 signals attributable to the aglycone and hence 11 signals that must be attributed to the sugar moiety. This presented two signals at δ 55.6 and δ 56.1 that were assigned to carbons corresponding to two methoxyl groups. ^{13}C multiplicities were determined by DEPT pulse sequence. The FAB-mass spectrum displayed $[M+H]^+$ at m/z 625, $[M+Na]^+$ at m/z 647. These results were according to the molecular weight calculated for: aglycon + hexose + pentose. In addition, a high resolution FAB-mass spectrum was performed and the molecular weight of the $[M+Na]^+$ pseudomolecular ion was determined. The observed m/z was 647.160163 and the one calculated for $C_{28}H_{32}O_{16}+Na$ is 647.158805.

Acid hydrolysis (6% HCl) of a small amount of **1** afforded a substance identified by spectroscopy methods as rhamnazin (Mabry et al., 1970; Agrawal, 1989).

Chromatographic analysis of the sugars was carried out by GLC/MS of the TMS ethers of methyl glycosides showed the presence of glucose and arabinose in a 1:1 molar ratio.

Methylation analysis using $NaBH_4$ showed the presence of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol arising from terminal glucopyranose and 1,2,5-tri-*O*-acetyl-3-4-di-*O*-methylarabinitol which could arise from 2-linked arabinopyranose, 4-linked arabinopyranose, or 5-linked arabinofuranose. In order to elucidate the arabinose linkage, a methylation analysis using $NaBD_4$ was performed. The identification of 1,2,5-tri-*O*-acetyl-1-deuterio-3-4-di-*O*-methylarabinitol showed that the arabinose residue could be 5-linked arabinofuranose or 4-linked arabinofuranose. The chemical shifts for the ^{13}C resonances were assigned by comparing with literature data (Bock, Pedersen, & Pedersen, 1984; Agrawal, 1989). Signals at 82.2 and 84.6 ppm were assigned, respectively, to C-2 and C-4 of an arabinofuranose residue. The signal shifted downfield at 68.3 ppm was assigned to C-5 of a 5-linked arabinofuranoside residue. Signals at 103.0 ppm and 108.3 ppm were assigned to C-1 of β-glucopyranose and α-arabinofuranose residues, respectively.

The chemical and spectroscopic data just described indicate that the flavonol glycoside isolated from *R. sphaerocarpa* has the rhamnazin-3-*O*-β-glucopyranosyl-(1→5)-α-arabinofuranoside structure. This is the first report of this compound in the literature.

At present, we have initiated bioassays with this compound to evaluate its possible antitumoral activity. Moreover, the common flavonols are of special systematic interest because they occur in combined form as glycosides, and the nature of the sugar or sugars present and the positions of their attachment to the flavonoid nucleus is often specific to a particular plant group. Rhamnazin-3-*O*-β-glucopyranosyl-(1→5)-α-arabinofuranoside isolated from *R.sphaerocarpa* has an unusual glycosilation because of the nature and sequence of sugars present. On the other hand, methylated derivates of flavonols are less known in the Leguminosae (Harbone, Boulter, & Turner, 1971).

3. Experimental

3.1. General

mp: uncorr. ^{1}H 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 (Mabry et al., 1970). Flavonoids detection: UV, 366 nm and $AlCl_3$ reagent. Sugar analysis by CG–MS, monosaccharides were determined as their trimethylsilylated methyl glycosides (Chaplin, 1982). Methylation analysis. The sample was methylated twice by the method of Ciucanu and Kerek (1984) and them reduced and acetylated by the method of Blakeney, Harris, Henry and Stone (1983).

3.2. 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.

3.3. Extraction and isolation

Air-dried, powdered aerial parts (500 g) of *R. sphaerocarpa* were extracted by Soxhlet successively for 24 h with Et_2O and for 48 h with MeOH. The MeOH extract was evaporated to dryness and suspended in 5O ml H_2O, then it was extracted successively with $CHCl_3$, EtOAc and BuOH. The butanol extract was fractionated on a silica column and the fraction eluted with EtOAc–

MeOH–H_2O (80:3:3) and followed on Sephadex LH20 give **1** (14 mg).

3.4. *Rhamnazin-3-O-β-glucopyranosyl*-(1→5)-α-*arabinofuranoside* (**1**)

Yellow powder, mp 166–168. UV λ_{max}^{MeOH} nm:254, 265sh, 291sh, 353; NaOMe, 260, 314, 412; $AlCl_3$, 270, 292sh, 365sh, 404; $AlCl_3$/HCl, 270, 292sh, 364, 401; NaOAc, 261, 292sh, 354, 374, 412; NaOAc/H_3BO_3, 253, 263sh, 291sh, 354. IR ν_{max}^{BrK} cm^{-1}: 3500–3400, 1668, 1100–1000. ^1H NMR (200 MHz, DMSO-d_6): δ 12.6 (OH, s, H-5), 7.68 (1H, d, $J=2$ Hz, H-2′), 7.67 (1H, dd, $J=9$, 2 Hz, H-6′), 6.92 (1H, d, $J=9$ Hz, H-5′), 6.77 (1H, d, $J=2.2$ Hz, H-8), 6.38 (1H, d, $J=2.2$ Hz, H-6), 5.55 (arabinose H-1, d, $J=0.8$ Hz), 4.11 (arabinose H-2, d, $J=3.9$ Hz), 4.00 (glucose H-1, d, $J=7.6$ Hz), 3.86 (3H, s, OCH_3), 3.87 (3H, s, OCH_3). ^{13}C NMR (50 MHz, DMSO-d_6); δ 55.6 (q, OCH_3), 56.1 (q, OCH_3), 61.0 (t, C-6‴), 68.3 (t, C-5″), 70.0(d, C-4‴), 72.5 (d, C-2‴), 73.3 (d, C-5‴), 76.7 (d, C-3‴), 77.6 (d, C-3″), 82.2 (d, C-2″), 84.6 (d, C-4″), 92.4 (d, C-8), 97.9 (d, C-6), 103.0 (d, C-1‴), 105.0 (s, C-10), 108.3 (d, C-1″), 112.6 (d, C-2′), 115.4 (d, C-5′), 120.7 (s, C1′), 122.9 (d, C-6′), 133.6 (s, C-3), 147.2 (s, C-4′), 149.6 (s, C-3′), 156.3 (s, C-2), 157.0 (s, C-9), 160.8 (s, C-5), 165.2 (s, C-7), 177.7 (s, C-4). FAB-MS: m/z 625 (5%)$[M+]^+$ 647(15%) $[M+Na]^+$.

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