Retamatrioside, a New Flavonol Triglycoside from *Retama sphaerocarpa*

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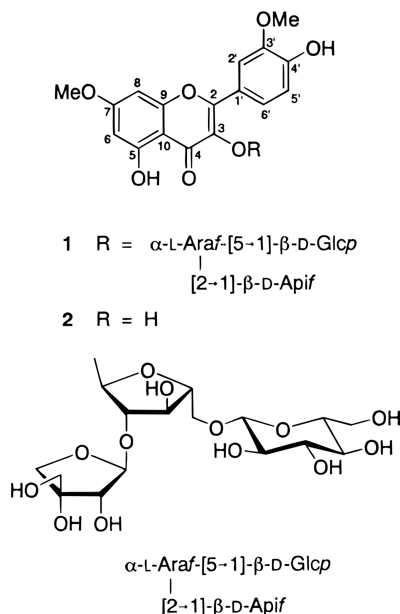
A new flavonol triglycoside, retamatrioside (**1**), has been isolated from the aerial parts of *Retama sphaerocarpa*. The structure of **1** has been determined as rhamnazin 3-*O*-*β*-D-glucopyranosyl-(1→5)-[*β*-D-apiofuranosyl(1→2)]-α-L-arabinofuranoside, using spectroscopic methods.

The genus *Retama* (syn. *Lygos*) (Fabaceae) includes four species with a distribution in the Mediterranean area, North Africa, and the Canary Islands. *Retama sphaerocarpa* Boiss. is a perennial shrub, common in the Iberian Peninsula and northwest Africa.[1] We began a phytochemical investigation on plants in this genus that has resulted so far in the isolation of quinolizidine alkaloids[2,3] and isoflavones.[4] In this paper we report the isolation of retamatrioside (**1**) as a new flavonol triglycoside from the aerial parts of *R. sphaerocarpa* collected in Spain. The structure of this natural product was established by spectral analysis, including homonuclear $^{1}H-^{1}H$ (COSY) and heteronuclear $^{1}H-^{13}C$ one-bond (HSQC) and multiple-bond (HMBC) correlation spectroscopy.

Retamatrioside (**1**) was obtained as yellow needles (166−168 °C), from the *n*-BuOH-soluble portion of the methanolic extract from the aerial parts of *R. sphaerocarpa,* after chromatography on Si gel and Sephadex LH-20. Acid hydrolysis (6% HCl) of a small amount of **1** afforded a substance, identified by spectroscopic methods[5,6] as rhamnazin (**2**).



The glycosidic fraction of **1** was analyzed by GC−MS of its TMS ether and, as a result, the presence of three different monosaccharides, in an 1:1:1 molar ratio, was shown. Two residues were identified as glucose and arabinose. As for the third residue, it was identified as apiose by comparing its ^{1}H and ^{13}C NMR resonance data (Table 1) with previously reported data.[7]

Table 1. ^{1}H and ^{13}C NMR Data of Compound **1**[a]

position	δ_H	δ_C	HMBC[b]
aglycon			
2		156.3	
3		133.6	
4		177.8	
5		160.8	
6	6.37 (d, 2.2)	97.9	(5),[c] (7), 8, 10
7		165.2	
8	6.75 (d, 2.2)	92.4	6, (7), (9), 10
9		157.0	
10		105.0	
1′		120.7	
2′	7.64 (d, 2.1)	112.6	(1′), (3′), 4′, 6′
3′		147.2	
4′		149.6	
5′	6.92 (d, 9.0)	115.4	1′, 3′, (4′)
6′	7.61 (dd, 2.1, 8.4)	122.9	2′, 4′, *2*[d]
$OH_{4'}$	9.55 (s)		
OH_5	12.50 (s)		
OMe_7	3.86 (s)	56.1	7
$OMe_{3'}$	3.86 (s)	55.7	3′
α-Ara			
1″	5.78 (s)	106.2	4″
2″	4.21 (d, 3.5)	87.8	(3″), *1‴*
3″	3.90 (dd, 3.5, 6.2)	76.2	
4″	3.69 (m)	83.4	
5″	3.67 (m)	67.8	3″, *1⁗*
	3.42 (m)		
β-Api			
1‴	5.02 (d, 3.1)	108.0	4‴, *2″*
2‴	3.76 (d)	76.0	(1‴)
3‴		78.6	
4‴	3.84 (d, 8.5)	73.5	1‴, 2‴, (3‴)
	3.55 (d)		
5‴	3.30	63.0	2‴, (3‴), 4‴
β-Glc			
1⁗	4.02 (d, 7.8)	102.7	*5″*
2⁗	2.90 (t, 8.7)	73.2	(1⁗), (3⁗)
3⁗	3.09 (t, 8.5)	76.6	(2⁗), (4⁗)
4⁗	2.99 (t, 9.6)	70.0	(3⁗), 6⁗
5⁗	3.03 (td, 2.0, 5.8)	76.7	(4‴)
6⁗	3.38 (dd, 11.7)	61.0	
	3.62 (dd)		

[a] Recorded in DMSO-d_6; chemical shifts are reported as δ values (ppm) from TMS at 500 MHz for ^{1}H and 125 MHz for ^{13}C; signal multiplicity and coupling constants (Hz) are shown in parentheses. [b] Carbons showing long-range couplings to proton, $^{n}J_{CH}$ ($n \geq 2$). [c] $^{n}J_{CH}$ correlations are shown in two-bond parentheses. [d] Inter-residue coupling are shown in italics.

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The UV spectrum of **1** was practically identical to that of its aglycon **2**, except in regard to a hypsochromic shift of band I ($\Delta\lambda_{max}$ 20 nm) due to substitution of the OH-3 group in **1**. The FABMS of **1** displayed a $[M + Na]^+$ ion at *m/z* 780 (75%). These results were in accord with the molecular weight calculated for the aglycon + 1 hexose + 2 pentoses.

The chemical shifts in the ^1H and ^{13}C NMR spectra of **1** were assigned from DQF−COSY and HSQC experiments (Table 1). In the lowfield region, besides the correlation cross-peaks arising from the aglycon (6, 8, 2′, 5′, and 6′), three other correlation signals were observed, corresponding to each anomeric position of the three glycosidic residues present in **1**. For the arabinofuranose unit (Ara), the C-1 resonance was located at δ 106.2 and the H-1 resonance at δ 5.78, suggesting the attachment of this moiety to C-3 of the aglycon, rhamnazin (**2**).[6,8] These values and the small $J_{1,2}$ value (0.8 Hz) suggested that this residue was in the α-anomeric form. Furthermore, the downfield shifts[6,8] of C-2 (δ 87.8) and C-5 (δ 67.8) indicated that these positions were substituted (this was further confirmed by the HMBC data, as described below). The glucose (Glc) residue was identified as the β anomer by the chemical shifts (4.03 and 102.7 for H-1 and C-1, respectively) and $J_{1,2}$ (7.8 Hz) values. Moreover, the *J* coupling pattern found for all the protons of this residue (see Table 1) was characteristic of a β-glucopyranose. The anomeric carbon resonance of the apiose (Api) was located at δ 108.0, which is characteristic of the β anomer.[9] The methylenic nature of C-5 (Ara), C-6 (Glc), and C-4 and C-5 (Api) was demonstrated unequivocally by the negative sign of their one-bond proton−carbon correlation cross-peaks in the HSQC NMR spectrum.

The substitution pattern and the confirmation of the assignments made were obtained from a $^1H-^{13}C$ long-range correlation NMR experiment (HMBC) (Table 1). Of significance was the appearance of cross-peaks corresponding to the long-range coupling of H_2 (Ara) with C_1 (Api), along with the coupling of H_1 (Api) with C_2 (Ara), which showed that apiose was linked to position 2 of the arabinose unit. In addition, the coupling of H_5 and C_5 (Ara) with C_1 and H_1 (Glc), respectively, revealed a →5→1 bond between these two residues. On the basis of the chemical and spectroscopic data obtained and the assumption that Glc and Api are members of the commonly found D series and Ara of the L series, the new flavonol glycoside isolated from *R. sphaerocarpa* was assigned the structure rhamnazin 3-*O*-β-D-glucopyranosyl-(1→5)-[β-D-apiofuranosyl(1→2)]-α-L-arabinofuranoside.

The common flavonols are of systematic interest in the Leguminosae 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.[11] Rhamnazin 3-*O*-β-D-glucopyranosyl-(1→5)-[β-D-apiofuranosyl(1→2)]-α-L-arabinofuranoside (**1**) has an unusual glycosylation pattern because of the nature and sequence of sugars present. Flavones with apiose as a sugar moiety are known,[10] but very few flavonol glycosides containing this sugar have been reported.

Experimental Section

General Experimental Procedures. The melting point was determined on a Thermovar HT 1 B11 instrument and is uncorrected. UV spectra in MeOH were recorded on a Perkin−Elmer Lambda 3 spectrometer. The IR spectrum with KBr plates was obtained on a Perkin−Elmer 1310 spectrometer. Samples were examined by NMR spectroscopy as solutions (10 mg/mL) in 99.6% DMSO-d_6. Spectra were recorded at 303 K on a Bruker AMX 500 spectrometer operating at 500.13 MHz (^1H) and 125.75 MHz (^{13}C). Chemical shifts are given in parts per million (ppm), using the DMSO-d_6 signals (2.49 ppm, and 39.5 ppm for ^1H and ^{13}C, respectively) as references. The 2D homonuclear proton double-quantum filtered correlation experiment (DQF−COSY)[12] was performed in the phase-sensitive mode using the Bruker standard pulse sequence. The 2D heteronuclear one-bond proton−carbon correlation experiment[13] was registered in the ^1H detection mode (inverse detection) via single-quantum coherence (HSQC). ^{13}C decoupling was achieved by the GARP scheme. This experiment was slightly modified by the implementation of an editing block in the sequence.[14] The long-range proton−carbon correlation experiment (HMBC)[15] was collected in the ^1H detection mode. A delay time of 80 ms between the first and second pulses and 96 scans per increment were used. The FABMS (thioglycerol + NaI matrix) was obtained on a Kratos MS80RFA mass spectrometer.

Plant Material. The aerial parts of *R. sphaerocarpa* were collected at Zahara de la Sierra (Cádiz, Spain), in May 1996, during the flowering period. The identity was verified by Dr. A. Aparicio, Department of Botany, Faculty of Pharmacy, University of Seville, where a voucher specimen is deposited (SEVF).

Extraction and Isolation. Air-dried, powdered aerial parts (500 g) of *R. sphaerocarpa* were extracted by a Soxhlet apparatus, successively for 24 h with Et_2O and for 48 h with MeOH. The MeOH extract (107 g) was evaporated to dryness and suspended in 50 mL H_2O, then it was extracted successively with $CHCl_3$, EtOAc, and *n*-BuOH to yield three fractions $CHCl_3$ (18 g), EtOAc (9 g), and *n*-BuOH (54 g). A portion (3.2 g) of the *n*-BuOH extract was fractionated on a Si gel column and eluted with EtOAc−MeOH−H_2O (80:3:3). The fractions C66−C94 were then passed over Sephadex LH-20, eluting with Cl_2CH_2−MeOH. Fractions C25−C30, obtained in the last column, yielded **1** (12 mg) as yellow needless.

Rhamnazin 3-*O*-β-D-glucopyranosyl-(1→5)-[β-D-apiofuranosyl(1→2)]-α-L-arabinofuranoside (1): yellow needles (MeOH), mp 166−168 °C; UV (MeOH) λ_{max} (log ϵ) 250 (4.38), 265 sh, 291 sh, 351 (4.28) nm; NaOMe, 264, 314, 410; $AlCl_3$, 271, 292 sh, 365 sh, 406; $AlCl_3$−HCl, 271, 292 sh, 365 sh, 406; NaOAc, 262, 292 sh, 409; NaOAc−H_3BO_3, 254, 352; IR (BrK) ν_{max} 3500−3400, 1654, 1618, 1581, 1509 cm^{-1}; ^1H NMR (DMSO-d_6 , 500 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 1; FABMS *m/z* 780 (75) $[M + Na]^+$.

Acid Hydrolysis of Compound 1. Compound **1** (5 mg) was diluted in 1 mL MeOH and refluxed for 1 h with 6 N HCl (5 mL). The solution was extracted with EtOAc, and the residue from the organic phase was identified by spectroscopic methods[5,6] as rhamnazin (**2**). The aqueous phase was concentrated and the sugars were identified by comparing the retention time of their TMS ethers with standard samples by GC−MS.[16]

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References and Notes

(1) Heywood, V. H. In *Flora Europaea*, Heywood, V. H., Burgess, N. A., Moore, D. M., Valentine, D. H., Walters, S. H., Webb, D. A., Eds. Cambridge University Press: London, 1968; p 101.
(2) Martín-Cordero, C.; Gil Serrano, A. M.; Ayuso González, M. J. *Plantes Méd. Phytothèr.* **1991**, *25*, 157−160.
(3) Martín-Cordero, C.; Gil, A. M.; Ayuso, M. J. *J. Chem. Ecol.* **1993**, *19*, 2389−2393.
(4) López Lazaro, M.; Martín-Cordero, C.; Iglesias-Guerra, F.; Ayuso González, M. J. *Phytochemistry* **1998**, *48*, 401−402.
(5) Mabry, T. J.; Markham, K. R.; Thomas, M. P. *The Systematic Identification of Flavonoids*; Springer: New York, 1970; pp 41−57.
(6) Agrawal, P. K.; Bansal, M. C. In *Carbon-13 NMR of Flavonoids*; Agrawal, P. K., Ed. Elsevier: Amsterdam, 1989; pp 283−364.
(7) Mathias, L.; Vieira, I. J. C.; Braz-Filho, R.; Rodrigues- Filho, E. *J. Nat. Prod.* **1998**, *61*, 1158−1161.
(8) Agrawal, P. K. *Phytochemistry* **1992**, *31*, 3307−3330.
(9) Puvanesarajah, V.; Darvill, A. G.; Albersheim, P. A. *Carbohydr. Res.* **1991**, *218*, 211−222.

(10) Harborne, J. B.; Williams, C. A. In *The Flavonoids. Advances in Research*; Harborne, J. B., Mabry, T. J., Eds. Chapman & Hall: London, 1982; p 261.
(11) Harborne, J. B.; Boulter, D.; Turner, B. L. In *Chemotaxonomy of the Leguminosae*; Academic Press: New York, 1971; pp 37−42.
(12) States, D. J.; Haber Korn, R. A.; Ruben, D. J. *J. Magn. Reson.* **1982**, *48*, 286−292.
(13) Bodenhausen, G.; Ruben, D. J. *Chem. Phys. Lett.* **1980**, *69*, 185−189.
(14) Parella, T.; Sánchez-Ferrando, F.; Virgili A. *J. Magn. Reson.* **1997**, *126,* 274−277.
(15) Frey, M. H.; Leupin, W.; Sorensen, O. W.; Denny, W. A.; Ernst, R. R.; Wüthrich, K. *Biopolymers* **1985***, 24*, 2371−2380.
(16) Markham, K. R. *Techniques of Flavonoids Identifications*; Academic Press: London, 1982; p 52.

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