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A new flavonol glycoside from leaves of *Zizyphus lotus*

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Zizyphus lotus (L.) Lam. (Rhamnaceae) (Syn.: *Z. rotundifolia*, *Z. lotos*, *Z. lotoidea*, *Rhamnus lotus*) is a mediterranean tree or shrub. Its edible fruit is a common food in Maghreb, and the root bark is used as an antidiabetic in Tunisia. We previously reported the isolation and structure of seven cyclopeptide alkaloids [1–3] and four saponins [4] from the root bark. The present work is the first analysis of the leaves of this species. We report here the isolation and structural elucidation of a new flavonol glycoside (**1**), as well as rutin (**2**) and 3′,5′-diglucosylphloretin (**3**) isolated for the first time from this species [5].

Compound **1** was obtained as a yellow amorphous powder. The UV spectrum in methanol showed characteristic site bands for flavonols in the range of 255 to 355 nm. The bathochromic shift on addition of NaOMe indicated the occurrence of free phenols. The permanent bathochromic effects on addition of $AlCl_3$, $AlCl_3$/HCl, NaOAc and NaOAc/H_3BO_3 suggested the absence of *ortho*-diphenols on the B-ring [6]. The IR spectrum showed a wide OH band at 3406 cm^{-1}, an alkene band at 1610 cm^{-1} and a carbonyl band at 1660 cm^{-1}. ESI-MS spectrum (positive mode) showed one peak at *m/z* 779, identified as $[M + Na]^+$ and suggesting a molecular formula determined as $C_{33}H_{40}O_{20}$.

In the ^1H NMR spectrum, the presence of two *meta* doublets at δ ppm 6.38 (d, J = 1.8 Hz,) and 6.20 (d, J = 1.8 Hz,) suggested a 5,7-disubstituted A-ring. Three aromatic protons with *meta* and *ortho* coupled signals at 7.51 (d, J = 2.1 Hz), 7.66 (dd, J_1 = 8.7 Hz, J_2 = 2.1 Hz) and 7.27 (d, J = 8.7 Hz), suggested a 3′,4′-disubstituted B-ring, corresponding to a quercetol skeleton. The ^1H and ^{13}C NMR spectra of **1** showed that this flavonol glycoside contained three sugars with signals of anomeric carbons at 99.0, 100.9 and 103.2, and anomeric protons at 5.58, 4.51 and 5.13 respectively (Table). Analysis of the COSY spectrum assigned the osidic protons of each unit. Two α-rhamnoses with anomeric protons at 5.58 (d, J = 1.2 Hz) and 4.51 (d, J < 1 Hz) were deduced from the values of the coupling constants and the presence of two methyl proton doublets at 1.28 (d, J = 6.2 Hz) and 1.14 (d, J = 6.2 Hz). The anomeric proton at 5.13 (d, J = 7.7 Hz) with exclusive axial-axial coupling constants suggested the presence of a β-glucose. The common D-configuration for glucose and L-configuration for rhamnose were assumed, being the most frequently encountered among the plant glycosides.

Table: ^{13}C NMR spectral data (125 MHz, CD_3OD) and ^1H NMR spectral data (500 MHz, CD_3OD) for compound 1

Aglycone	^{13}C δ ppm	Sugar	^{13}C δ ppm	^1H δ ppm, J (Hz))
2	156.9	β-D-Glucose		
3	134.4	1‴	103.2	5.13 (d, 7.7)
4	*	2‴	74.2	3.50 (t, 9)
5	157.4	3‴	76.7	3.44 (t, 8.9)
6	99.2	4‴	70.0	3.27 (t, 9.2)
7	167.0	5‴	75.8	3.35 (m)
8	94.0	6‴	67.2	3.37 (dd, 9.1, 5.9)
9	*			3.85 (br d, 9.4)
10	103.7	α-L-Rhamnose		
1′	124.7	1⁗	100.9	4.51 (br s, <1)
2′	117.2	2⁗	70.6	3.63 (dd, 3.2, 1.5)
3′	146.2	3⁗	70.7	3.54 (dd, 9.5, 3.4)
4′	146.6	4⁗	72.4	3.29 (t, 9.3)
5′	115.5	5⁗	68.2	3.46 (dq, 9.5, 6.1)
6′	121.1	6⁗	16.4	1.14 (d, 6.2)
		α-L-Rhamnose (4′)		
		1″	99.0	5.58 (d, 1.2)
		2″	70.3	4.17 (dd, 3.3, 1.7)
		3″	70.5	4.00 (dd, 9.6, 3.4)
		4″	72.3	3.50 (t, 9.7)
		5″	69.5	3.75 (dq, 9.4, 6.2)
		6″	16.6	1.28 (d, 6.2)

* not detected

The identification of the aglycone as quercetol was ascertained from combined analysis of ^{13}C spectral data, 2D-HSQC and 2D-HMBC experiments. Most of the carbons could be assigned, except the carbonyl C-4 and the C-9, which were not detected [7]. The ambiguous assignment of C-3′ and C-4′ was resolved by analysis of the HMBC spectrum, which showed correlations between C-4′ at 146.6 and protons H-2′ at 7.81, H-6′ at 7.66 and H-5′ at 7.27, whereas C-3′ at 146.2 did not exhibit any correlation with H-6′. A supplementary ^3J HMBC correlation was observed between C-4′ and the anomeric proton of rhamnose H-1′ at 5.58 (Fig.). In the same experiment, the correlation between the anomeric proton of the second rhamnose H-1⁗ at 4.51 and carbon C-6‴ of glucose at 67.2 indicated the presence of a rutinose moiety. The occurrence of the heteronuclear correlation between the anomeric proton of the glucose H-1‴ at 5.13 and the carbon signal at 134.4 indicated that the rutinose was linked at the C-3 of the aglycone (Fig.). Thus, the structure of compound **1** is the quercetol 4′-*O*-α-L-rhamnopyranosyl-3-*O*-α-L-rhamnopyranosyl-(1⁗→6‴)-β-D-glucopyranoside.

The structure of rutin was established by comparison of measured UV and NMR spectral data with spectroscopic data available from the literature [7, 8].

The 3′,5′-di-C-β-D-glucopyranosylphloretin showed ^1H and ^{13}C NMR data in full agreement with those given by Markham et al. [9] and Ogawa et al. [10].

Experimental

1. Equipment

Optical rotation was measured on a Perkin-Elmer 241 polarimeter. UV analysis were run on a Philips PU 720 UV/Vis scanning photometer with the sample solutions prepared in a 4 ml quartz cell (1 cm optical pathway). In the case of the flavonoids, all shift reagents were added separately to the methanolic solution of each substance investigated [6]. IR spectra were recorded by a Midac série M IRTF spectrometer in anhydrous KBr pellets. ESI-MS experiments were performed on a ZABSPEC-T Micromass spectrometer (compounds **1** and **2**) or a Bruker Esquire LC-MS instrument (compound **3**). NMR spectra were recorded in CD_3OD on an Avance DRX 500 Bruker spectrometer relative to CD_3OD (δ_H 3.33, δ_C 47.53). Centrifugal partition chromatography was performed on a HPCPC Sanki Series

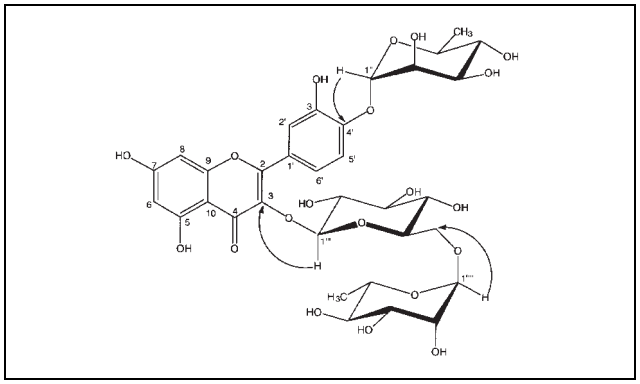

Fig.: Most significant correlations from HMBC experiment for compound **1**

1000 connected to a Techlab TIP50 gradient pump using analytical grade solvents. Detection was performed by an ELSD IIA. Analytical and preparative thin layer chromatographies were performed on Whatman K6F 250 µm silica plates using the elution system $CHCl_3-CH_3OH-H_2O$ (10.8 : 5.8 : 1). The chromatograms were sprayed with 50% aqueous H_2SO_4 and heated 3 min with hot-air gun.

2. Plant material

Zizyphus lotus (L) Lam. leaves were collected in Cherahil, Tunisia in September 1994 and identified by Pr M.A. Nabli, University of Tunis. A voucher specimen (No. KG/ZL/F-010) has been kept in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy of Monastir, Tunisia.

3. Extraction and isolation

The air-dried leaf powder of *Z. lotus* (300 g) was successively defatted with hexane and chloroform, then extracted with CH_3OH in a Soxhlet apparatus. The methanolic extract was evaporated under reduced pressure and low temperature, then dissolved in 130 ml CH_3OH and poured into 650 ml Et_2O. The dried precipitate (21.3 g) was dissolved in H_2O and submitted to dialysis in a 6–8000 Da-porosity cellulose tubing for 24 h. The retained part was freeze-dried and gave 7.17 g of a brown amorphous powder (2.4% w/w yield). Centrifugal partition chromatography was used to fractionate this extract with the EtOAc/n-BuOH/H_2O biphasic solvent system. Three batches of precipitate (1.35, 1.04 and 0.96 g) were submitted to linear gradient elution (95 : 1:4 to 46 : 40 : 14) at 1200 or 1300 rpm, with 2 ml/min flow and during 420 or 480 min. Detection was performed by an on-line evaporative light-scattering detector using N_2 as vector gas. Fractions size was 4 ml. The fractions were analysed by TLC eluted with $CHCl_3-CH_3OH-H_2O$ (10.8 : 5.8 : 1). TLC-similar fractions from each batch have been pooled together and submitted to preparative TLC eluted with $CHCl_3-CH_3OH-H_2O$ (10.8 : 5.8 : 1), giving pure compounds **1** (9 mg), **2** (11 mg) and **3** (9 mg).

Quercetol 4′-*O*-α-L-rhamnopyranosyl-3-*O*-α-L-rhamnopyranosyl-(1‴→6‴)-β-D-glucopyranoside (**1**): R_f value: 0.27. $\alpha_D = -30{,}0°$ (C = 0.16 CH_3OH); UV λ_{max} (CH_3OH): 257, 268, 351; +NaOMe: 274, 303, 383; +$AlCl_3$: 269, 299 sh., 353, 399; +$AlCl_3$/HCl: 276, 299 sh., 353, 400; +NaOAc: 273, 360; +NaOAc/H_3BO_3: 255, 268, 300 sh., 350 nm. IR, υ^{KBr}_{max}, cm^{-1}: 3406, 1660, 1610; ESI-MS: *m/z* 779 $[M + Na]^+$, 301 $[aglycone + H]^+$; ^1H NMR (500 MHz, CD_3OD): δ 6.20 (H-6, d, J = 1.9 Hz), 6.38 (H-8, d, J = 1.8 Hz), 7.27 (H-5′, d, J = 8.7 Hz), 7.66 (H-6′, dd, J = 2.1, 8.7 Hz), 7.81 (H-2′, d, J = 2.1 Hz). Osidic part: see Table; ^{13}C NMR: see Table.

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