Two New Glycosides from *Astragalus caprinus*

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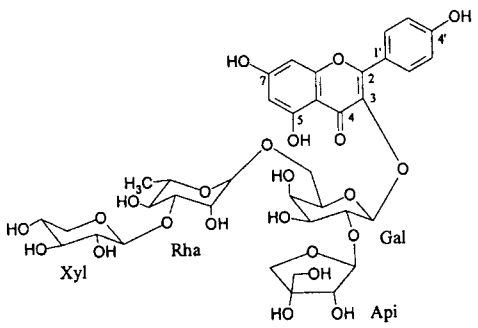
A new glycoside of flavonol (**1**) and a new glycoside of a cycloartane-type triterpene (**2**) were isolated from the leaves and the roots of *Astragalus caprinus*, respectively. Their structures were elucidated in turn by spectroscopic data interpretation as 3-*O*-{[*β*-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)]-[*β*-D-apiofuranosyl(1→2)]}-*β*-D-galactopyranosyl kaempferol (**1**) and 3-*O*-(*β*-D-xylopyranosyl)-24-*O*-(*β*-D-glucopyranosyl)-20,25-epoxycycloartane-3*β*,6α,16*β*,24α-tetrol (**2**).

Astragalus caprinus Maire (Fabaceae), endemic to North Africa, is widely distributed in Tunisia.[1] An infusion of the leaves of this perenial herb is used in Tunisian traditional medicine to treat hemorrhoids. The chemical studies on the genus *Astragalus* deal with many saponins and flavonoids,[2−11] but there have been no previous phytochemical reports on *A. caprinus*. This paper deals with the isolation and characterization of a new glycoside of the flavonol kaempferol (**1**) and a cycloartane-type triterpene saponin (**2**) from the leaves and the roots, respectively, of *A. caprinus*.



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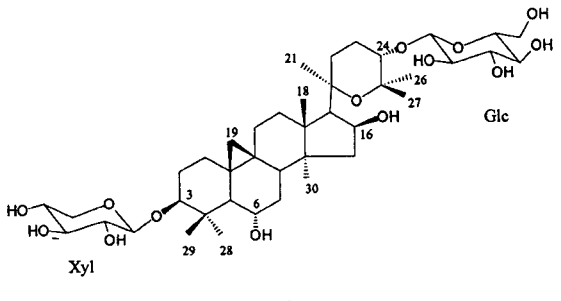

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Compound **1** was obtained as a major flavonoid from the *n*-BuOH-soluble part of the methanolic extract of the dried leaves of *A. caprinus*. Its fluorescence, chromatographic behavior, and UV spectral properties suggested **1** to be a kaempferol 3-polyglycoside.[12] The high-resolution FAB mass spectrometry (HRFABMS) (positive-ion mode) of **1** exhibited a pseudomolecular ion peak at *m/z* 881.2347 [M + Na]$^+$ (calcd 881.2327), consistent with a molecular formula of $C_{37}H_{46}O_{23}$.The electrospray ionization mass spectrometry (ESIMS) (positive-ion mode) displayed a quasimolecular ion peak [M + H]$^+$ at *m/z* 859. Other fragment ions peaks at *m/z* 727 [M + H − 132]$^+$, 581 [M + H − 132 − 146]$^+$, 449 [M + H − 132 − 146 − 132]$^+$, and 287 [M + H − 132 − 146 − 132 − 162]$^+$ corresponded to successive losses of four sugar moieties, namely, a pentosyl, a rhamnosyl, a pentosyl, and a hexosyl, respectively. Indeed, acid hydrolysis yielded kaempferol and four distinct sugars, which were identified as apiose, galactose, rhamnose, and xylose by co-TLC with authentic samples.[13] The structural elucidation of **1** was based on 2D NMR spectroscopy (see Table 1). A HSQC-TOCSY experiment was performed to identify the spin systems of sugar units, starting from anomeric protons at *δ* 5.37 (d, *J* = 7.8 Hz), 5.47 (d, *J* = 1.6 Hz), 4.52 (d, *J* = 1.4 Hz), and 4.34 (d, *J* = 7.2 Hz). While this approach led to a complete identification of α-rhamnopyranosyl (Rha) and *β*-xylopyranosyl (Xyl) units, only the cross-peaks corresponding to the *β*-apiofuranosyl (Api) C-1/H-1 (*δ* 109.8/5.47) to C-2/H-2 (*δ* 77.0/4.08) and *β*-galactopyranosyl (Gal) C-1/H-1 (*δ* 100.4/5.37) to C-4/H-4 (*δ* 69.6/3.76) units could be assigned. Therefore, a HMBC experiment was used to find other correlations between Gal C-4 at *δ* 69.6 and H_2-6 at *δ* 3.67 and 3.50 and between Api C-3 at *δ* 79.9 and H-1 at *δ* 5.47, H_2-4 at *δ* 4.12 and 3.74, and H_2-5 at *δ* 3.77 and 3.67, thus completing most signal assignments. The common D-configuration for Gal, Xyl, and Api and the L-configuration for Rha were assumed according to those most often encountered among the plant glycosides. Additionally, the HMBC experiment allowed the glycosidic linkages to be determined. Thus, the observation of long-range couplings between kaempferol C-3 (*δ* 133.7) and galactose H-1 (*δ* 5.37) proved the Gal unit to be attached at C-3 of kaempferol. Furthermore, long-range correlations between Gal C-2 (*δ* 75.8) and Api H-1 (*δ* 5.47), Gal C-6 (*δ* 66.6) and Rha H-1 (*δ* 4.52), and Rha C-3 (*δ* 81.3) and Xyl H-1 (*δ* 4.34) as well as reverse correlations proved the sequence of the tetrasaccharide moiety at C-3 to be [Xyl-(1→3)-Rha-(1→6)][Api-(1→2)]-Gal-

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Table 1. ^{13}C and ^{1}H NMR Data for Compound **1** (125 and 500 MHz, δ ppm)

	in CD_3OD		in DMSO-d_6	
position	δ_C	δ_H (J Hz)	δ_C	δ_H (J Hz)
kaempferol				
-2	157.7 s		156.7 s	
-3	133.7 s		133.8 s	
-4	178.5 s		178.1 s	
-5	162.1 s		161.8 s	
-6	99.0 d	6.21 d (2.1)	99.5 d	6.17[a]
-7	165.2 s		165.2 s	
-8	93.9 s	6.40 d (2.1)	94.6 s	6.39[a]
-9	157.5 s		157.3 s	
-10	104.7 s		104.6 s	
-1′	122.0 s		121.9 s	
-2′, -6′	131.4 d	8.12 d (9.1)	131.7 d	8.08 (8.8)
-3′, -5′	115.2 d	6.91 d (9.0)	115.9 d	6.86 d (8.7)
-4′	160.4 s		160.5 s	
Gal-1	100.4 d	5.37 d (7.8)	100.0 d	5.50 d (7.6)
-2	75.8 d	3.95 (7.8, 9.0)	75.5 d	3.75 dd (7.7, 9.7)
-3	74.3 d	3.69 dd (9.0, 3.6)	74.2 d	3.57[a]
-4	69.6 d	3.76 dd (3.6, 1.0)	69.1 d	3.59[a]
-5	74.4 d	3.63 m	74.2 d	3.57[a]
-6	66.6 t	3.67[a]	66.3 t	3.54[a]
		3.50[a]		3.27[a]
Api-1	109.8 d	5.47 d (1.6)	109.7 d	5.30[a]
-2	77.0 d	4.08[a]	76.9 d	3.81[a]
-3	79.9 s		80.0 s	
-4	74.5 t	4.12 d (9.6)	74.7 t	3.84 d (9.3)
		3.74 d (9.6)		3.49 d (9.3)
-5	65.1 t	3.77 d (11.1)	65.0 t	3.45 d (11.1)
		3.67 d (11.1)		3.38 d (11.1)
Rha-1	100.8 d	4.52 d (1.4)	101.0 d	4.36[a]
-2	70.7 d	3.74 dd (1.4, 3.1)	70.5 d	3.53[a]
-3	−81.3 d	3.55 dd (9.3, 3.1)	81.6 d	3.36[a]
-4	71.7 d	3.44 t (9.3)	71.6 d	3.30[a]
-5	68.3 d	3.56 m	68.7 d	3.39[a]
-6	17.0 q	1.18 d (6.2)	18.6 q	1.04 d (5.8)
Xyl-1	105.4 d	4.34 d (7.2)	106.1 d	4.25 d (7.6)
-2	74.2 d	3.25 dd (9.3, 7.2)	74.5 d	3.02[a]
-3	76.5 d	3.33 dd (9.0, 9.3)	76.6 d	3.11[a]
-4	70.1 d	3.50 ddd (11.3, 9.0, 5.0)	70.1 d	3.26[a]
-5	65.8 t	3.79 dd (11.3, 5.0)	66.3 t	3.64[a]
		3.12 dd (11.3, 10.4)		2.99[a]

[a] Multiplicities are unclear due to overlapping with other signals.

(1→Agly-3). Consequently, the structure of **1** was established as 3-*O*-{[β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→6)][β-D-apiofuranosyl-(1→2)]}-β-D-galactopyranosyl kaempferol, a new natural compound. To our knowledge, other tetraglycosides of flavonols have been reported in a few species,[14] and only once in the genus *Astragalus* (3-*O*-α-L-rhamnopyranosyl-(1→6)-[α-L-rhamnopyranosyl-(1→2)]-β-D-galactopyranosyl-7-*O*-α-L-rhamnopyranosyl kaempferol).[9]

Compound **2** was obtained as a major saponin from the methanolic extract of the dried roots of the plant. The HRFABMS (positive-ion mode) of **2** exhibited a pseudomolecular ion peak at m/z 807.4534 $[M + Na]^+$ (calcd 807.4507), consistent with a molecular formula of $C_{41}H_{68}O_{14}$. The ESIMS (positive-ion mode) exhibited a quasimolecular ion peak at m/z 807 $[M + Na]^+$. Other fragment ion peaks at m/z 645 $[M + Na - 162]^+$ and 675 $[M + Na - 132]^+$ indicated the respective elimination of one terminal hexosyl and one terminal pentosyl moiety. Acid hydrolysis of **2** with 2 N CF_3COOH yielded glucose and xylose (identified by co-TLC with authentic samples). The aglycon was identified as 20,25-epoxycycloartane-3β,6α,16β,24α-tetrol from the ^{1}H NMR, ^{13}C NMR, DEPT, $^{1}H-^{1}H$ COSY, HMBC, and HMQC spectra of **2** (see Table 2). Most of the signals were assigned

Table 2. ^{13}C and ^{1}H NMR Data for Compound **2** (125 and 500 MHz, pyridine-d_5, δ ppm)

position	DEPT	δ_C	δ_H (J Hz)
sapogenin			
-1	CH_2	31.5	*a*
-2	CH_2	30.0	*a*
-3	CH	88.4	3.47
-4	C	41.2	
-5	CH	54.9	2.00
-6	CH_2	70.4	3.75
-7	CH_2	32.0	*a*
-8	CH	46.5	1.55
-9	C	21.1	
-10	C	29.0	
-11	CH_2	27.1	*a*
-12	CH_2	34.1	*a*
-13	C	46.5	
-14	C	47.0	
-15	CH_2	49.0	2.25[a]
			2.70[a]
-16	CH	74.4	4.86[a]
-17	CH	60.8	2.08[a]
-18	CH_3	21.2	1.63, s
-19	CH_2	30.0	0.55[a]
			0.20[a]
-20	C	79.6	
-21	CH_3	29.0	1.52, s
-22	CH_2	27.0	1.15[a]
			1.45[a]
-23	CH_2	26.8	1.30[a]
			1.45[a]
-24	CH	78.7	3.85[a]
-25	C	75.0	-
-26	CH_3	28.2	1.20, s
-27	CH_3	29.0	1.38, s
-28	CH_3	25.9	1.30, s
-29	CH_3	15.4	0.99, s
-30	CH_3	20.0	1.03, s
Xyl-1	CH	107.7	4.82 d (7.5)
-2	CH	75.7	4.00[a]
-3	CH	78.7	4.22[a]
-4	CH	72.0	4.13[a]
-5	CH_2	67.5	4.30[a]
			3.75[a]
Glc-1	CH	100.9	4.86 d (7.4)
-2	CH	74.7	4.00[a]
-3	CH	78.7	4.13[a]
-4	CH	71.4	4.22[a]
-5	CH	78.7	3.95[a]
-6	CH_2	63.0	4.28[a]
			4.55[a]

[a] Signal pattern was unclear due to overlapping.

Table 3. $^{1}H-^{13}C$ Long Range Correlations of Compound **2** in Pyridine-d_5

H	correlated carbon
0.99 (Me-29)	25.9 (C-28)
1.03 (Me-30)	46.5 (C-8), 47.0 (C-14), 49.0 (C-15)
1.20 (Me-26)	75.0 (C-25), 29.0 (C-27)
1.30 (Me-28)	88.4 (C-3), 41.2 (C-4), 15.4 (C-29)
1.38 (Me-27)	28.2 (C-26), 75.0 (C-25),
1.63 (Me-18)	34.1 (C-12), 46.5 (C-13), 60.8 (C-17)
4.82 (Xyl H-1)	88.4 (C-3)
4.86 (Glc H-1)	78.7 (C-24)

through $^{2}J_{H-C}$ and $^{3}J_{H-C}$ couplings of the seven methyls (see Table 3) and were in good agreement with literature data.[10]

From the HMQC experiment, it was confirmed that compound **2** contains two sugar units. Thus, the anomeric protons at δ 4.82 and 4.86 gave correlations to the anomeric carbon signals at δ 107.7 and 100.9, respectively. Evaluation of spin−spin couplings and chemical shifts allowed the identification of one β-xylopyranose (Xyl) and one

β-glucopyranose (Glc), respectively. The common D-configuration for Glc and Xyl was assumed.

The point of attachment of the xylose to the aglycon was deduced from the HMBC spectrum of **2**, which showed a cross-peak between the Xyl H-1 at δ 4.82 (d, $J = 7.5$ Hz) and the C-3 of the aglycon at δ 88.4. This attachment was confirmed by observation of a reverse correlation between the Agly H-3 at δ 3.47 and the Xyl C1 at δ 107.7. For the determination of the attachment of the Glc, the observation of a long-range correlation between the Glc H-1 at δ 4.86 (d, $J = 7.4$ Hz) and the C-24 of the aglycon at δ 78.7 together with a reverse correlation proved the glucose unit to be attached at C-24 OH.

On the basis of the above results, the structure of **2** was determined as 3-*O*-(β-D-xylopyranosyl)-24-*O*-(β-D-glucopyranosyl)-20,25-epoxycycloartane-3β,6α,16β,24α-tetrol. This compound is similar to cyclocephaloside I isolated from the roots of *A. microcephalus* except that the Glc was linked at C-24 of the aglycon instead of at C-6.[10] According to an updated literature search, **2** is a new natural compound.

Experimental Section

General Experimental Procedures. Optical rotations were taken with a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Kontron UVIKON 860 spectrophotometer. IR spectra (KBr disk) were recorded on Perkin-Elmer 281 spectrophotometer. The 1D and 2D NMR spectra (DEPT, HSQC-TOCSY, COSY, HMQC, HMBC) were recorded using a Brucker DRX 500 NMR spectrometer that was equipped to allow inverse detection (500 MHz for ^{1}H and 125 MHz for ^{13}C). All chemical shifts (δ) are given in ppm, and the samples were solubilized in CD_3OD (δ 49.0) or in DMSO-d_6 (δ 39.5) for **1** and in pyridine d_5 for **2**. ESIMS data (positive-ion mode) were recorded on a Hewlett-Packard 1100 MSD system. The HR-FABMS was conducted in the positive-ion mode (thioglycerol matrix) on a ZAB2-SEQ instrument. TLC and HPTLC employed precoated Si gel 60F 254 plates (Merck), cellulose plates (Merck), and polyamide plates (Macherey Nagel). For column chromatography, polyamide SC6 (Macherey Nagel), microcrystalline cellulose (Merck), and Sephadex LH20 (Pharmacia) were used. The HPLC was performed on a Kontron liquid chromatograph (autosampler 360, pump 322) coupled to a diode array detector (Waters 991). The MPLC separations were performed on a system equipped with a Gilson pump M 303, a 25SC head pump, a M 802 manometric module, a Büchi column (460 × 25 mm), and a Büchi precolumn (110 × 15 mm), with the stationary phase silica gel 60 (15−40 μm, Merck).

Plant Material. Leaves and roots of *A. caprinus* were collected at Jemmal, Monastir, Tunisia, in April 1998. A voucher specimen of the plant (No. 98/0039) was deposited in the Herbarium of the Faculty of Pharmacy of Monastir, Tunisia.

Extraction and Isolation. Dried, powdered leaves (200 g) were extracted twice in MeOH (70%) (500 mL) for 24 h at room temperature, and after partial evaporation, the aqueous solution was partitioned between *n*-BuOH (3 × 300 mL) and water. The residue from the *n*-BuOH layer was separated by successive column chromatography on cellulose (5% EtOH), polyamide (5% EtOH), and Sephadex LH-20 (50% MeOH), yielding pure compound **1** (500 mg). The purity control was monitored by HPLC using the following system (a): reversed-phase C_{18} Ultrabase (5 μm) column (200 × 4.6 mm i.d.), linear gradient from 0 to 28% of MeCN−H_2O (8:2) with 2% AcOH for 110 min, flow rate 0.8 mL min^{-1}, detection wavelength between 230 and 410 nm. Dried, powdered roots (220 g) were submitted to successive extractions in a Soxhlet with cyclohexane (2 L, 24 h), CH_2Cl_2 (2 L, 24 h), and MeOH (3 L, 48 h). After evaporation under reduced pressure of the solvent, 2.2 g of a cyclohexane extract, 0.8 g of a CH_2Cl_2 extract, and 37 g of a MeOH extract were obtained. The MeOH extract was dissolved in 1 L of H_2O and partitioned with H_2O-saturated *n*-BuOH (2 × 500 mL). The *n*-BuOH-soluble portion (7.6 g) was precipitated with diethyl ether (3 × 200 mL), and a part of this subjected (4.7 g) to repeated column chromatography on Sephadex LH-20 (MeOH) and medium-pressure liquid chromatography (MPLC) using Si gel eluted with $CHCl_3$−MeOH−H_2O (65:35:10) (lower phase) to yield compound **2** (44 mg).

Compound 1: yellow powder; $[a]^{20}_D$ −80.0° (*c* 0.150, MeOH); UV λ_{max} (MeOH) 349, 266 nm; (MeOH + $AlCl_3$) 398, 351, 275 nm; (MeOH + $AlCl_3$ + HCl) 398, 346, 275 nm; (MeOH + NaOAc) 369, 273 nm; (MeOH + NaOAc + H_3BO_4) 349, 268 nm; (MeOH + NaOH) 394, 321, 273 nm; IR (KBr) ν_{max} 3420 (OH), 1650 (C=O), 1620 (C=C), 1100 (C−O); ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 859 $[M + H]^+$ (100), 727 $[M - 132 + H]^+$ (10), 581 $[M - 132 - 146 + H]^+$ (1), 449 $[M - 132 - 146 - 132 + H]^+$ (2.4), 287 $[M - 132 - 146 - 132 - 162 + H]^+$ (12); positive HRFABMS *m/z* 881.2347 $[M + Na]^+$ (calcd for $C_{37}H_{46}O_{23}$, 881.2327); R_f 0.43 (cellulose, BuOH−AcOH−H_2O, 4:1:5, upper phase); TLC R_f 0.80 (polyamide, H_2O−BuOH−Me_2CO−dioxane, 18:3:2:1) (purple fluorescence); R_t 47.8 min (HPLC C_{18}, system a).

Compound 2: white amorphous powder; $[\alpha]^{20}_D$ −7.0° (*c* 0.100, MeOH); IR (KBr) ν_{max} 3400 (OH), 2930 (CH), 1050 (C−O−C); ^{1}H and ^{13}C NMR data, see Table 2; long-range correlations in the HMBC spectrum used for defining the aglycon, see Table 3; ESIMS *m/z* 807 $[M + Na]^+$ (100), 645 $[M + Na - 162]^+$ (25), 675 $[M + Na - 132]^+$ (30); positive HRFABMS *m/z* 807.4534 $[M + Na]^+$ (calcd for $C_{41}H_{68}O_{14}Na$, 807.4507); R_f 0.54 (Si gel, $CHCl_3$−MeOH−H_2O, 65:35:10, lower phase, detection: Komarowsky reagent, a mixture (5:1) of *p*-hydroxybenzaldehyde (2% in MeOH) and 50% H_2SO_4).

Acid Hydrolysis of Compounds 1 and 2. A solution of **1** (1 mg) in 2 N HCl (1.5 mL) was refluxed on a water bath for 1 h. After extraction with Et_2O, the aqueous layer was repeatedly evaporated to dryness with MeOH until neutral and then analyzed on TLC by comparison with standard sugars (Si 60, Me_2CO−H_2O, 9:1, detection by spraying with aniline malonate and heating).[13] A solution of **2** (3 mg) in 2 N CF_3-COOH (5 mL) was refluxed on a water bath for 2 h. After extraction with $CHCl_3$, the aqueous layer was neutralized as described above and then analyzed on TLC by comparison with standard sugars (Si 60, $CHCl_3$−MeOH−H_2O, 64:40:8, detection by spraying with the diphenylaminephosphoric acid reagent and heating).

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