New Flavonol Tetraglycosides from *Astragalus caprinus*

Nabil SEMMAR,*[,a] Bernard FENET,[b] Katia GLUCHOFF-FIASSON,[a] Gilles COMTE,[c] and Maurice JAY[a]

[a] *Laboratoire de Phytochimie et Biologie Micromoleculaire, UFR de Biologie, Université Lyon 1;* [b] *Centre de RMN, UCB-ESCPE, UPRESA CNRS 5012, Université Lyon 1; and* [c] *Laboratoire des Produits Naturels,UMR CNRS 5013, UFR de Chimie-Biochimie, Université Lyon 1; 43 Bd. 11 Novembre, 69622 Villeurbanne, France.*

Received February 1, 2002; accepted March 18, 2002

A new flavonol tetraglycoside, together with four acylated derivatives, were isolated from the leaves of *Astragalus caprinus*. Their structures were elucidated by spectroscopic methods, mainly 2D NMR, as kaempferol-3-*O*-{[β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)][α-L-rhamnopyranosyl(1→2)]}-β-D-galactopyranoside (1), its 3^{Gal}-*p*-coumaric (2) and 3^{Gal}-ferulic (3) esters, and its 4^{Gal}-*p*-coumaric (4) and 4^{Gal}-ferulic (5) esters.

Key words *Astragalus caprinus*; Fabaceae; acylated flavonol tetraglycoside; flavonol tetraglycoside; kaempferol

Astragalus caprinus MAIRE (Fabaceae) is an endemic of North Africa, the leaves of which are used as an antihaemorrhoidal in Tunisian folk medicine. Our previous paper describing the structure of a new 3-*O*-tetraglycoside of kaempferol from this plant was the first phytochemical report on this species.[1)] Here we report the isolation and structural elucidation of five new flavonol glycosides.

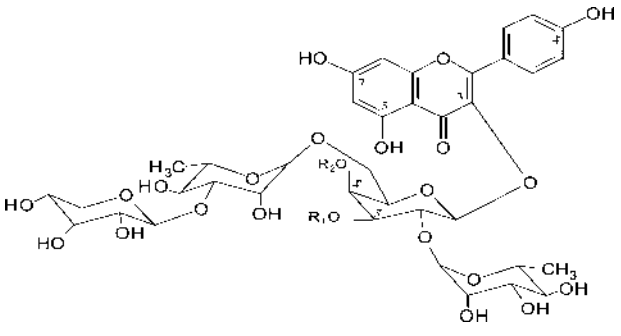
Results and Discussion

The methanolic extract of dried leaves from *A. caprinus*, once prepurified, was fractionated by repeated column and preparative thin-layer chromatography to give **1**—**5**.

Analysis of the ^{1}H- and ^{13}C-NMR spectra of compound **1** (Table 1; all assignments based on heteronuclear single quantum coherence-total correlation spectroscopy (HSQC-TOCSY) and heteronuclear multiple bond correlation (HMBC) experiments) showed the presence of one aromatic system and four sugar moieties. The ^{1}H-NMR resonances of two *meta*coupled doublets at δ 6.19 and 6.38 ppm (1H, J=1.9 Hz), correlated with the carbons at 99.8 and 94.7 ppm, respectively, in the HSQC spectrum, characterized the 6- and 8-protons of a flavonoid 5,7 dihydroxy A-ring.[2)] Ring B was assigned as a 1,4-substituted benzene ring (δ H 8.07, d, 2H, J=8.8 Hz; 6.89, d, 2H, J=8.8 Hz) from a HMBC experiment. Thus, the aglycone of **1** was identified as 3,5,7,4′-tetrahydroxyflavone (kaempferol), as suggested by its UV spectral properties. A HSQC-TOCSY experiment was performed to identify the spin systems of sugar units, starting from anomeric protons at δ 5.57 (d, J=7.8 Hz), 5.22 (s), 4.52 (s) and 4.32 (d, J=7.4 Hz); on the basis of the chemical shifts, multiplicity of the signals and values of the coupling constants, the sugars were identified as β-galactopyranosyl (Gal), α-rhamnopyranosyls (Rha a and Rha b) and β-xylopyranosyl (Xyl).[3)] The common D-configuration for Gal and Xyl, and the L-configuration for Rha were assumed according to those most often encountered among the plant glycosides. HMBC experiments showed long-range correlations between Gal H-1 (δ 5.57) and Kaempferol C-3 (δ 134.5), Rha a H-1/C-1 (δ 5.22/102.6) and Gal C-2/H-2 (δ 77.5/3.93), Rha b H-1/C-1 (δ 4.52/101.8) and Gal C-6/H-6 (δ 67.5/3.68), Xyl H-1/C-1 (δ 4.32/106.4) and Rha b C-3/H-3 (δ 82.3/3.55). Thus, compound **1** was identified as kaempferol-3-*O*-{[β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)][α-L-rhamnopyranosyl(1→2)]}-β-D-galactopyranoside. This identification was corroborated by electrospray ionization mass spectrometry (ESI-MS) which displayed a quasimolecular ion peak $(M+H)^+$ at m/z 873 and fragment ion peaks corresponding to successive losses of four sugar moities, namely a rhamnosyl, a pentosyl, a rhamnosyl and an hexosyl, respectively; the high resolution (HR)-FAB-MS was in agreement with the corresponding formula $C_{38}H_{48}O_{23}$. Acid hydrolysis of **1** yielded kaempferol and sugars, which were identified as galactose, rhamnose and xylose by co-TLC with authentic samples.[4)]

The ^{1}H- and ^{13}C-NMR spectra of compound **2** (Table 1) showed the same signals as those observed for **1**, but with significant downfield shifts of H-3 (δ 5.06) and C-3 (δ 77.7) of the galactose, as well as concomitant upfield shifts of the adjacent carbons C-2 and C-4.[3)] One additional 1,4-substituted benzene ring (δ H 6.82, d, 2H, J=8.8 Hz; 7.79 , d, 2H, J=8.8 Hz), together with two olefinic protons doublets (J=15.8 Hz) at δ 6.45 and 7.73, as well as one ester carbonyl carbon at δ 168.4, indicated the presence of a *trans-p*-coumaroyl moiety. Its linkage to Gal C-3 was corroborated by the long range coupling of the ester carbonyl carbon with Gal H-3 in the HMBC spectrum.

The ^{1}H- and ^{13}C-NMR spectra of compound **3** (Table 1) differed from those of **2** only by the signals of the aromatic part of the acyl moiety: the occurrence in the ^{1}H spectrum of three coupled aromatic protons (δ 7.22, d, J=1.9 Hz, δ 6.81, d, J=8.5 Hz and δ 7.12, dd, J=1.9, 8.5 Hz) characterized a



	R_1	R_2
1	H	H
2	*p* -coumaroyl	H
3	feruloyl	H
4	H	*p* -coumaroyl
5	H	feruloyl

* To whom correspondence should be addressed. e-mail: nabilsemmar@hotmail.com

Table 1. ^{13}C- and ^{1}H-NMR Data of Compounds **1**—**5** in CD_3OD (^{13}C: 125 MHz, ^{1}H: 500 MHz, δ ppm, J Hz)

Position	**1**		**2**		**3**		**4**		**5**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
Kaempferol										
2	158.5		158.9		158.9		159.2		159.2	
3	134.5		134.2		134.3		134.1		134.1	
4	179.4		179.3		179.2		179.3		179.3	
5	163.2		163.2		163.2		163.1		163.2	
6	99.8	6.19 d (1.9)	99.9	6.20 d (2.2)	99.9	6.19 d (2.2)	99.9	6.3 d (2.0)	99.9	6.21 d (2.0)
7	165.6		165.7		165.7		165.6		165.7	
8	94.7	6.38 d (1.9)	94.8	6.39 d (2.2)	94.8	6.38 d (2.2)	94.8	6.3 d (2.0)	94.8	6.41 d (2.0)
9	158.8		158.5		158.5		158.5		158.5	
10	105.9		105.9		105.9		105.9		105.9	
1′	123.1		123.0		123.0		123.2		123.2	
2′—6′	132.3	8.07 d (8.8)	132.3	8.09 d (8.8)	132.3	8.09 d (9.1)	132.3	8.14 d (9.0)	132.3	8.12 d (9.1)
3′—5′	116.2	6.89 d (8.8)	116.2	6.92 d (8.8)	116.2	6.91 d (9.1)	116.1	6.96 d (9.0)	116.1	6.95 d (9.1)
4′	161.3		161.4		161.4		161.4		161.4	
Galactose										
Gal 1	100.9	5.57 d (7.8)	100.7	5.78 d (7.5)	100.8	5.80 d (7.6)	100.6	5.69 d (7.1)	100.6	5.69 d (7.0)
Gal 2	77.5	3.93 dd (7.8, 9.4)	75.7	4.20[a]	75.7	4.19 dd (7.6, 10.1)	77.7	3.94[a]	77.8	3.95[a]
Gal 3	75.7	3.71 dd (9.4, 3.6)	77.7	5.06[a]	77.8	5.08[a]	74.0	3.98[a]	74.0	3.97[a]
Gal 4	70.8	3.76 dd (3.6, 1.0)	68.0	4.06[a]	68.0	4.07[a]	72.0	5.35[a]	72.1	5.36[a]
Gal 5	75.6	3.66 m	75.3	3.82[a]	75.2	3.83[a]	73.9	3.88[a]	73.9	3.87[a]
Gal 6	67.5	3.68 m	67.0	3.70[a]	67.0	3.71[a]	67.3	3.53[a]	67.0	3.49[a]
		3.50 t (12.2)		3.50[a]		3.48[a]		3.27[a]		3.27[a]
Rhamnose a at Galactose C-2										
Rha a 1	102.6	5.22 d (1.4)	102.8	4.99 d (1.3)	102.8	5.07 d (1.6)	102.8	5.20 d (1.3)	102.8	5.19 d (1.5)
Rha a 2	72.4	3.8 dd (1.4, 3.0)	72.5	3.81[a]	72.5	3.83 dd (1.6, 3.2)	72.3	4.01[a]	72.4	4.00[a]
Rha a 3	72.3	4.0 m	72.3	3.75[a]	72.3	3.76[a]	72.3	3.82[a]	72.3	3.81[a]
Rha a 4	74.1	3.35 t (9.8)	73.9	3.32[a]	73.9	3.31[a]	74.0	3.83[a]	74.0	3.36[a]
Rha a 5	69.9	4.07 dd (6.3, 9.8)	70.2	4.06[a]	70.1	4.07[a]	70.0	4.16[a]	70.0	4.12[a]
Rha a 6	17.6	0.99 d (6.3)	17.6	0.98 d (6.0)	17.6	0.99 d (6.3)	18.0	1.05 d (6.2)	18.0	1.00 d (6.2)
Rhamnose b at Galactose C-6										
Rha b 1	101.8	4.52 d (1.5)	101.7	4.53 d (1.6)	101.8	4.54 d (1.6)	102.0	4.46 d (1.7)	102.0	4.46 d (1.5)
Rha b 2	71.7	3.72[a]	71.7	3.76[a]	71.7	3.78[a]	71.7	3.72[a]	71.7	3.71[a]
Rha b 3	82.3	3.55 dd (3.0, 9.4)	82.3	3.57[a]	82.3	3.58[a]	82.3	3.54 dd (3.5, 9.8)	82.3	3.57[a]
Rha b 4	72.7	3.42 t (9.4)	72.7	3.44 t (9.4)	72.8	3.43[a]	72.5	3.66[a]	72.5	3.38[a]
Rha b 5	69.4	3.55 dd (6.7, 10.5)	69.5	3.56[a]	69.4	3.55[a]	69.6	3.43[a]	69.6	3.42[a]
Rha b 6	18.0	1.16 d (6.7)	18.0	1.17 d (6.2)	18.0	1.18 d (6.3)	17.6	1.05[a]	17.6	1.05[a]
Xylose at Rhamnose b C-3										
Xyl 1	106.4	4.32 d (7.4)	106.4	4.34 d (7.9)	106.4	4.34 d (7.3)	106.4	4.37 d (7.4)	106.5	4.36 d (7.8)
Xyl 2	75.2	3.22 dd (7.4, 9.0)	75.2	3.23 dd (7.9, 9.1)	75.3	3.22 dd (7.3, 9.1)	75.2	3.22 t (8.8)	75.2	3.22[a]
Xyl 3	77.5	3.30 t (8.6)	77.5	3.30[a]	77.6	3.29[a]	77.5	3.31[a]	77.5	3.29[a]
Xyl 4	71.1	3.48 m	71.1	3.48[a]	71.1	3.46[a]	71.0	3.47[a]	71.0	3.47[a]
Xyl 5	66.9	3.77 dd (5.4, 12.2)	66.9	3.78[a]	66.9	3.75[a]	66.8	3.77[a]	66.9	3.75[a]
		3.00 t (12.2)		3.10[a]		3.10[a]		3.12[a]		3.11[a]
Coumaric acid										
Cou 1			127.2				127.2			
Cou 2—6			131.5	7.50 d (8.8)			131.5	7.51 d (9.0)		
Cou 3—5			116.8	6.82 d (8.8)			117.0	6.86 d (9.0)		
Cou 4			161.5				161.4			
Cou α			147.6	7.73 d (15.8)			147.4	7.64 d (15.7)		
Cou β			114.8	6.45 d (15.8)			116.0	6.35 d (15.7)		
Cou γ			168.4				168.7			
Ferulic acid										
Fer 1					127.7				127.7	
Fer 2					112.0	7.22 d (1.9)			111.5	7.25 d (1.9)
Fer 3					148.3				149.5	
Fer 4					149.4				150.7	
Fer 5					116.5	6.81 d (8.5)			116.6	6.86 d (8.5)
Fer 6					124.4	7.12 dd (1.9, 8.5)			124.7	7.11 dd (1.9, 8.5)
Fer α					147.8	7.72 d (15.8)			147.7	7.66 d (15.7)
Fer β					115.1	6.48 d (15.8)			115.1	6.41 d (15.7)
Fer γ					168.3				168.7	
Fer OMe					56.5	3.90 s			56.6	3.94 s

a) Obscured by other signals.

1,3,4-substituted benzene ring. An additional methoxy group (δ H 3.90, 3H, s) was correlated in the HMBC spectrum with C-3 of this aromatic ring. These data identified a *trans*-feruloyl moiety.

Compounds **4** and **5** differed from **2** and **3**, respectively, only by the site of acylation which was assigned to C-4 of the galactose from the NMR data (Table 1).

Furthermore, ^{1}H-NMR spectra of compounds **2**—**5** showed an additional weaker series of signals with a similar coupling pattern, except for those of the olefinic protons whose J=13 Hz indicated a *cis*-configuration for the acyl moiety.[5] Thus, each compound had been isolated as a mixture of *cis*/*trans* isomers (ratio 1/5 from integrals). Only NMR allowed us to detect this mixture since the size of the glycosidic sequence shielded the acyl moiety and hindered its interactions with both phases of the chromatographic systems. It was to be noted that generally the overlapping of sugar signals of the two isomers precluded precise measurements of coupling constants with one-dimensional (1D) ^{1}H experiments. The analysis of ^{1}H–^{13}C cross peaks (intensity, multiplicity, total width) allowed an unambiguous determination of proton configuration and finally the sugar identification. There was no hint that the *cis* isomers were not artifacts which appeared during the long processes of extraction and purification.

Consequently, the structures of compounds **2**—**5** were established as kaempferol-3-*O*-{[β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)][α-L-rhamnopyranosyl-(1→2)]}-β-D-galactopyranoside (**1**), kaempferol-3-*O*-{[β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)][α-L-rhamnopyranosyl (1→2)]}-β-D-3-*trans*-*p*-coumaroylgalactopyranoside (**2**), kaempferol-3-*O*-{[β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)][α-L-rhamnopyranosyl(1→2)]}-β-D-3-*trans*-feruloylgalactopyranoside (**3**), kaempferol-3-*O*-{[β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)][α-L-rhamnopyranosyl(1→2)]}-β-D-4-*trans*-*p*-coumaroylgalactopyranoside (**4**) , and kaempferol-3-*O*-{[β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)][α-L-rhamnopyranosyl(1→2)]}-β-D-4-*trans*-feruloylgalactopyranoside (**5**).

These identifications were corroborated by ESI-MS which displayed quasimolecular ion peaks at *m/z* 1019 $[M+H]^+$ for **2** and **4**, *m/z* 1049 $[M+H]^+$ for **3** and **5**, and fragment ion peaks indicating the successive elimination of one rhamnosyl, one pentosyl, one rhamnosyl and one hydroxycinnamoylated hexosyl moiety; the HR-FAB-MS was in agreement with the corresponding formulae $C_{47}H_{54}O_{25}$ for **2** and **4**, and $C_{48}H_{56}O_{26}$ for **3** and **5**. Alkaline hydrolysis of compounds **2**—**5** yielded, besides **1**, coumaric or ferulic acids.[4] To our knowledge, this is only the second report of acylated flavonol tetraglycosides in the plant kingdom;[6] as for acylated flavonol pentaglycosides, a single report has been published.[7]

Experimental

General Procedures The 1D and 2D NMR spectra (HSQC, HSQC-TOCSY, HMBC) were performed in CD_3OD, using a Bruker DRX 500 NMR spectrometer equipped to allow inverse detection (500 MHz for ^{1}H and 125 MHz for ^{13}C). ESI-MS data were obtained on a Hewlett-Packard 1100 MSD spectrometer (100 and 150 eV) in the positive-ion mode. TLC was performed on polyamide (DC6, Macherey-Nagel), mobile phase toluene–MeOH–MeCOEt (4 : 3 : 3) (system *p*); HPLC was conducted on a Kontron LC set (autosampler 360, pump 322) coupled to a diode array detector (Waters 991) on a reversed-phase C_{18} Ultrabase (5 μm) column (200×4.6 mm i.d.), with a linear gradient from 10 to 45% of MeCN in H_2O with 2% AcOH for 118 min, a flow rate of 0.8 ml min^{-1}, and a detection range 230—410 nm (system *hp*). Medium pressure liquid chromatography (MPLC) separations were performed on a Büchi system equipped with a B-688 pump coupled to a B-687 gradient programmer, (110×15 mm) precolumn, (460×26 mm) column filled with stationary phase C_{18} (40—63 μm, Merck), solvent MeOH gradient in water, flow rate 10 ml min^{-1} (system *rmp*) and (460×15 mm) column filled with the stationary phase Lichroprep-diol (40—63 μm, Merck), gradient of *i*-PrOH in $CHCl_3$ (linear 10 to 30% in 30 min, then isocratic to 150 min), at a flow rate of 6 ml min^{-1} (system *smp*). Elution was monitored with a Knauer K-2501 UV detector set at 350 nm. Purification was carried out over Sephadex (LH-20, Pharmacia).

Plant Material The leaves of *Astragalus caprinus* were collected during April–May 1998 at Monastir, Tunisia. A voucher specimen (No. 98/0039) is deposited at the Faculty of Pharmacy of Monastir, Tunisia.

Extraction and Isolation of Compounds 1—5 Dried, powdered leaves (200 g) were macerated twice with (20 ml g^{-1}) MeOH 70% for 24 h. After partial evaporation, the aqueous solution was extracted with *n*-BuOH. The residue from the *n*-BuOH layer was pre-purified on a cellulose column (5% EtOH), then separated on a polyamide column (5 to 40% EtOH). The fraction eluted with 5% EtOH was subjected to MPLC (system *rmp*): the subfraction containing **1** (elution: MeOH 25%) was chromatographed on Sephadex with MeOH 50% to yield pure **1**. The fraction eluted from polyamide with 25% EtOH was subjected to MPLC (system *smp*): the subfractions containing a **2**+**3** mixture, then a **4**+**5** mixture (elution: 30% *i*-PrOH) were submitted to prep. TLC (system *p*, thrice); isolated compounds were finally purified on Sephadex in MeOH. Purity was monitored by HPLC (system *hp*). Compounds **1**—**5** were obtained as pale yellow amorphous powders (14 mg for **1**; 4 mg for **2**; 8 mg for **3**; 10 mg for **4**; 7 mg for **5**).

Kaempferol-3-*O*-{[β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)][α-L-rhamnopyranosyl(1→2)]}-β-D-galactopyranoside (**1**): $[\alpha]_D^{20}$ −97.3° (c=0.38, MeOH); UV λ_{max} (MeOH) (log ε) 266 (4.41), 300 (sh), 349 (4.30) nm; (MeOH+$AlCl_3$) 274, 305, 352, 398 nm; (MeOH+$AlCl_3$+HCl) 275, 303, 348, 397 nm; (MeOH+NaOAc) 274, 385 nm; (MeOH+NaOAc+H_3BO_3) 268, 356 nm; (MeOH+NaOH) 275, 326, 401 nm; IR ν_{max} (KBr) 3400, 2920, 1650, 1605, 1505, 1450, 1360, 1275, 1205, 1175, 1125, 1080, 1045 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table 1; ESI-MS *m/z* 873 $[M+H]^+$ (100), 727 $[M+H-146]^+$ (30), 595 $[M+H-146-132]^+$ (10), 449 $[M+H-146-132-146]^+$ (20), 287 $[M+H-146-132-146-162]^+$ (30); positive HR-FAB-MS *m/z* 895.2488 $[M+Na]^+$ (Calcd for $C_{38}H_{48}O_{23}Na$ 895.2484).

Kaempferol-3-*O*-{[β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)][α-L-rhamnopyranosyl(1→2)]}-β-D-3-*trans*-*p*-coumaroylgalactopyranoside (**2**): $[\alpha]_D^{20}$ −94.4° (c=0.09, MeOH); UV λ_{max} (MeOH) (log ε) 268 (4.38), 316 (4.52), 350 (sh) nm; IR ν_{max} (KBr) 3400, 2920, 1700, 1650, 1600, 1510, 1450, 1360, 1270, 1170, 1125, 1085, 1050 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table 1; ESI-MS *m/z* 1019 $[M+H]^+$ (100), 873 $[M+H-146]^+$ (35), 741 $[M+H-146-132]^+$ (5), 733 (15), 595 $[M+H-146-132-146]^+$ (10), 287 $[M+H-146-132-146-308]^+$ (30); positive HR-FAB-MS *m/z* 1041.2867 $[M+Na]^+$ (Calcd for $C_{47}H_{54}O_{25}Na$ 1041.2852).

Kaempferol-3-*O*-{[β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)][α-L-rhamnopyranosyl(1→2)]}-β-D-3-*trans*-feruloylgalactopyranoside (**3**): $[\alpha]_D^{20}$ −87.5° (c=0.40, MeOH); UV λ_{max} (MeOH) (log ε) 267 (4.35), 300 (sh), 330 (4.50) nm; IR ν_{max} (KBr) 3410, 2920, 1705, 1650, 1590, 1510, 1450, 1360, 1270, 1210, 1175, 1120, 1085, 1050 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table 1; ESI-MS *m/z* 1049 $[M+H]^+$ (100), 903 $[M+H-146]^+$ (30), 771 $[M+H-146-132]^+$ (5), 763 (20), 625 $[M+H-146-132-146]^+$ (10), 287 $[M+H-146-132-146-338]^+$ (15); positive HR-FAB-MS *m/z* 1071.2970 $[M+Na]^+$ (Calcd for $C_{48}H_{56}O_{26}Na$ 1071.2957).

Kaempferol-3-*O*-{[β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)][α-L-rhamnopyranosyl(1→2)]}-β-D-4-*trans*-*p*-coumaroylgalactopyranoside (**4**): $[\alpha]_D^{20}$ 149.0° (c=0.50, MeOH); UV λ_{max} (MeOH) (log ε): 268 (4.41), 315 (4.52), 350 (sh) nm; IR ν_{max} (KBr) 3400, 2930, 1695, 1650, 1600, 1510, 1450, 1360, 1265, 1205, 1170, 1130, 1085, 1050 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table 1; ESI-MS *m/z* 1019 $[M+H]^+$ (100), 873 $[M+H-146]^+$ (40), 741 $[M+H-146-132]^+$ (10), 595 $[M+H-146-132-146]^+$ (20), 287 $[M+H-146-132-146-308]^+$ (15); positive HR-FAB-MS *m/z* 1041.2860 $[M+Na]^+$ (Calcd for $C_{47}H_{54}O_{25}Na$ 1041.2852).

Kaempferol-3-*O*-{[β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→6)][α-L-rhamnopyranosyl(1→2)]}-β-D-4-*trans*-feruloylgalactopyranoside (**5**): $[\alpha]^{20}{}_D$ −146.6° (c=0.09, MeOH); UV λ_{max} (MeOH) (log ε): 267 (4.40), 300 (sh), 329 (4.48) nm; IR ν_{max} (KBr) 3405, 2925, 1700, 1650, 1595, 1505, 1450, 1360, 1270, 1175, 1125, 1085, 1050 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table 1; ESI-MS *m/z* 1049 $[M+H]^+$ (100), 903 $[M+H-146]^+$ (20), 771 $[M+H-146-132]^+$ (5), 625 $[M+H-146-132-146]^+$ (10), 287 [M+H−

146−132−146−338]$^+$ (10); positive HR-FAB-MS *m/z* 1071.2965 [M+Na]$^+$ (Calcd for $C_{48}H_{56}O_{26}Na$ 1071.2957).

Acid Hydrolysis of Compounds 1—5 As described previously.[4)]

Alkaline Hydrolysis of Compounds 2—5 Performed using a previously described procedure.[4)]

Acknowledgments This work was supported by a grant and a scholarship from the Rhone-Alpes (Fr)-Monastir (Tn) Research Convention.

References

1) Semmar N., Fenet B., Lacaille-Dubois M. A., Gluchoff-Fiasson K., Chemli R., Jay M., *J. Nat. Prod*, **64**, 656—658 (2001).
2) Markham K. R., Geiger H., "^{1}H Nuclear Magnetic Resonance Spectroscopy of Flavonoids and their Glycosides in Hexadeuterodimethylsulfoxide," ed. by Harborne J. B., Chapman & Hall, 1994, pp. 441—497.
3) Agrawal P. K., Bansal M. C., "Carbon-13 NMR of Flavonoid Glycosides," ed. by Agrawal P. K., Elsevier, 1989, pp. 283—364.
4) Gluchoff-Fiasson K., Fiasson J. L., Favre-Bonvin J., *Phytochemistry*, **37**, 1629—1633 (1994).
5) Liu H., Orjala J., Sticher O., Rali T., *J. Nat. Prod.*, **62**, 70—75 (1999).
6) Nielsen J. K., Norbaek R., Olsen C. E., *Phytochemistry*, **49**, 2171—2176 (1998).
7) Asada Y., Hirayama Y., Furuya T., *Phytochemistry*, **27**, 1497—1501 (1988).