Natural Product Sciences
14(2) : 86-89 (2008)

Two New Flavonol Glycosides from the Aerial Parts of *Lotus lalambensis* Growing in Saudi Arabia

Hanan M. El-Youssef, Brian T. Murphy[1], Masouda E. Amer, Adnan J. Al-Rehaily, Maged S. Abdel-Kader*, and David G. I. Kingston[1]

Department of Pharmacognosy, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia
[1]Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0212, USA.

Abstract – Phytochemical study of the aerial parts of *Lotus lalambensis* Schweinf resulted in the isolation and identification of two new flavonol glycosides; kaempferol 3-*O*-(5''-acetyl)-apioside-7-*O*-α-L-rhamnopyranoside (**1**) and kaempferol 3-*O*-α-[β-D-xylosyl-(1'''' → 2'')-L-rhamnopyranoside]-7-*O*-α-L-rhamnopyranoside (**2**). Structures were determined utilizing different physical, chemical, spectroscopic data including 2D-NMR experiments and HRFABMS.
Keywords – *Lotus lalambensis*; Fabaceae; flavonol glycosides.

Introduction

The genus *Lotus*, belonging to family Fabaceae, contains approximately 100 species distributed throughout the world, especially around the Mediterranean region (Evans, 2002). Lotus genus is represented in Saudi Arabia by twelve species including *Lotus lalambensis* Schweinf (Chaudhary, 2001; Migahid, 1978). In folk medicine, plants of genus *Lotus* are used as contraceptives, prophylactics and treatment of sexually transmitted disorders and peptic ulcers (El Mousallami, *et al.*, 2002). *Lotus halophilus* has a good antimicrobial activity against Gram-positive, Gram-negative bacteria and fungi (Mahasneh, 2002). Flavone and flavonols are the most common constituents of the aerial parts of *Lotus* species (Abdel-Ghani, *et al*., 2001; El Mousallami, *et al.*, 2002; Abdel-Kader, *et al.*, 2007). Previously we reported on the isolation and identification of 20 known compounds including 5 sterols and triterpenes, a long chain alcohol, 3 cyanogenic glycosides, 3 simple glycosides, 7 flavonol derivatives and D-pinitol from *Lotus lalambensis* Schweinf (El-Youssef, *et al.*, 2008). In this paper we present the structures of two new flavonol glycosides isolated from the same plant.

Experimental

General – Melting points were determined in open capillary tubes using Thermosystem FP800 Mettler FP80 central processor supplied with FP81 MBC cell apparatus, and were uncorrected. Ultraviolet absorption spectra were obtained in methanol and with different shift reagents on a Unicum Heyios α UV-Visible spectrophotometer. IR spectra were recorded in KBr using a FTIR-8400S fourier transform infrared spectrophotometer (SHIMADZU). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 (Central Lab at the College of Pharmacy, King Saud University) spectrometer operating at 500 MHz for proton and 125 MHz for carbon, respectively. The chemical shift values are reported in δ (ppm) relative to the internal standard TMS or residual solvent peak, the coupling constants (*J*) are reported in Hertz (Hz). 2D-NMR experiments (COSY, HSQC, HMBC and NOESY) were obtained using standard Bruker program. HRFABMS were measured using a JEOL JMS-HX-110 instrument (Analytical services unite, Department of Chemistry, Virginia Tech., Blacksburg, VA, USA).

Plant material – The aerial parts of *L. lalambensis* Schweinf were collected in 2003 from Aqubat Al-Abnaa Baljorashi, southern region of Saudi Arabia. The plant was identified by Dr. M. Atiqur Rahman, Prof. of Taxonomy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen (#11180) was deposited at the herbarium of the Research Center for Medicinal, Aromatic and Poisonous Plants of the same College.

Extraction and Isolation – Air-dried powdered aerial parts of *Lotus lalambensis* (1.5 kg) were exhaustedly extracted with 90% ethanol (12 L) at room temperature.

*Author for correspondence
Fax: +99614677245; E-mail: mpharm101@hotmail.com

The ethanol extract was evaporated under vacuum to yield 180 g of dark green residue. The residue was dissolved in 0.6 L H_2O/MeOH (1 : 1). The solution was successively extracted with petroleum ether (3 × 0.5 L), $CHCl_3$ (3 × 0.5 L), EtOAc (3 × 0.5 L) and butanol (4 × 0.5 L). The aqueous layer after extraction with butanol was lyophilized to produce 65 g residue.

The $CHCl_3$ fraction (30.0 g) was chromatographed on a silica gel column (400 g, 4.0 cm). Elution started with petroleum ether/$CHCl_3$ mixture (1 : 1), the polarity was increased by increasing $CHCl_3$ ratio, followed by MeOH in a gradient elution technique. Ninety six fractions, 150 ml each were collected, screened by TLC and similar fractions were pooled. Fractions 39 - 42 (0.32 g) eluted with 5% MeOH in $CHCl_3$ were rechromatographed using C_{18} silica gel column (40 g, 1.5 cm) and MeOH/H_2O mixtures as the eluent. Fractions 4 - 10 eluted with MeOH/ H_2O (6 : 4), and after crystallization from methanol afforded 30 mg of **1**.

The butanol fraction (44.0 g) was dissolved in 0.3 L of MeOH, then $CHCl_3$ was added and the solution was filtered. The filtrate was evaporated to give 10.0 g residue that was chromatographed on silica gel column (200 g, 2.5 cm). Elution was started with EtOAc/MeOH mixtures. Fifty eight fractions, 150 ml each, were collected, and similar fractions were pooled according to their TLC behaviour. Fractions 28 - 34 (1.2 g, 15% MeOH in EtOAc) were further chromatographed on silica gel column (50 g, 2.0 cm) eluting with EtOAc/MeOH/H_2O (30 : 5 : 4) to afford 63 mg of **2**.

Kaempferol 3-*O*-(5'''-acetyl)-apioside-7-*O*-α-L-rhamnopyranoside (1) – Yellow matrix; IR ν_{max} (KBr): 3385 (OH stretching), 1750 and 1654 (C = O stretching), 1601 (C = C stretching) cm^{-1}; UV λ_{max} nm: (MeOH) 265, 346, (NaOMe) 274, 320, 392, ($AlCl_3$) 276, 304, 390, ($AlCl_3$/HCl) 277, 304, 389, (NaOAc) 264, 348; ^{1}H- and ^{13}C-NMR: see the Tables 1, 2; HRFABMS *m/z* 607.1642 $[M+H]^+$, (calc.for $C_{28}H_{31}O_{15}$, 607.1663).

Kaempferol 3-*O*-α-[β-D-xylosyl-(1'''' → 2'')-L-rhamnopyranoside]-7-*O*-α-L-rhamnopyranoside (2) – Pale yellow crystals (MeOH); mp, 195 - 196 °C; UV λ_{max} nm: (MeOH) 266, 344, (NaOMe) 246, 268, 387, ($AlCl_3$) 271, 300, 346, 398, ($AlCl_3$/HCl) 274, 298, 343, 393, (NaOAc) 264, 372; ^{1}H- and ^{13}C-NMR: see the Tables 1, 2; HRFABMS *m/z* 711.2124 $[M+H]^+$ (calc. for $C_{32}H_{39}O_{18}$, 711.2136).

Results and Discussion

Extraction of the aerial parts of *L. lalambensis* with 90% ethanol, followed by liquid-liquid partitioning and column chromatography on silica gel, yielded two new flavonol glycosides.

Table 1. ^{1}H-NMR data (multiplicity, *J* in Hz) of **1** and **2** in CD_3OD

Pos.	1	2
6	6.47 (*d*, *J* = 2.0)	6.40 (*d*, *J* = 2.0)
8	6.74 (*d*, *J* = 2.0)	6.66 (*d*, *J* = 2.0)
2'	7.86 (*d*, *J* = 10.5)	7.74 (*d*, *J* = 8.5)
3'	6.92 (*d*, *J* = 10.5)	6.94 (*d*, *J* = 8.5)
5'	6.92 (*d*, *J* = 10.5)	6.94 (*d*, *J* = 8.5)
6'	7.86 (*d*, *J* = 10.5)	7.74 (*d*, *J* = 8.5)
1''	5.70 (*d*, *J* = 2.0)	5.44 (*s*)
2''	4.21 (*d*, *J* = 2.0)	4.25 (br *s*)
3''	–	3.87 (*d*, *J* = 3.5, 9.5)
4''	3.51 (*d*, *J* = 12.0) 3.62 (*d*, *J* = 12.0)	3.52 (*dd*, *J* = 9.5, 9.5)
5''	4.03 (*d*, *J* = 14.5) 4.15 (*d*, *J* = 14.5)	3.64 (*m*)
6''	–	1.28 (*d*, *J* = 6.5)
1'''	5.57 (br *s*)	5.57 (*s*)
2'''	4.05 (*m*)	4.07 (br *s*)
3'''	3.83 (*dd*, *J* = 4.0, 11.0)	3.87 (*d*, *J* = 3.5, 9.5)
4'''	3.48 (*dd*, *J* = 11.5,12.0)	3.37 (*dd*, *J* = 8.0, 10.0)
5'''	3.60 (*dd*, *J* = 7.5,11.5)	3.71 (*dd*, *J* = 6.0, 11.0)
6'''	1.30 (*d*, *J* = 7.5)	1.02 (*d*, *J* = 6.0)
1''''		4.34 (*d*, *J* = 7.5)
2''''		3.25 (*dd*, *J* = 7.5, 8.0)
3''''		3.35 (*m*)
4''''		3.46 br (*dd*, *J* = 5.5, 10.0)
5''''		3.12 (*dd*, *J* = 10.5,11.5) 3.71 (*dd*, *J* = 6.0, 11.0)
6''''		–
$COCH_3$	2.09 (s)	

*Assignments based on COSY and HSQC experiments.

Compounds **1** and **2** gave yellow colour with alkalis and $AlCl_3$. Their UV data (experimental) in MeOH exhibited two maxima at 265, 346 and 266, 344 nm respectively indicating 3-substituted favonol derivatives (Harborne, *et al.*, 1975). The carbon shifts at δ_C 135.3 and 137.0 (Table 2) in **1** and **2** respectively were in full support for a 3-substituted favonol skeleton (Agrawal, 1989). ^{1}H-NMR of **1** and **2** (Table 1) showed two *meta*-coupled doublets each integrated for one proton at δ_H 6.47, 6.74 ($J = 2.0$ Hz) and 6.40, 6.66 ($J = 2.0$ Hz) assigned for H-6 and H-8 in **1** and **2** respectively. Another two *ortho*-coupled doublets each integrated for two proton at δ_H 6.92, 7.86 (J = 10.5 Hz) and 6.94, 7.74 (J =

Table 2. ^{13}C-NMR data of **1** and **2** in CD_3OD

Pos.	1	2
2	160.3	159.6
3	135.3	137.0
4	179.7	179.7
5	163.0	163.5
6	99.87	100.7
7	163.6	162.9
8	95.6	95.7
9	158.2	157.9
10	107.5	107.8
1'	122.7	122.3
2'	132.1	132.0
3'	116.4	116.7
4'	161.7	161.7
5'	116.4	116.7
6'	132.1	132.0
1''	110.7	103.2
2''	79.4	82.6
3''	79.1	72.2
4''	76.6	73.7
5''	67.8	71.3
6''	–	18.2
1'''	100.6	99.9
2'''	71.7	71.7
3'''	72.1	72.0
4'''	73.6	73.5
5'''	71.3	72.0
6'''	18.1	17.7
1''''		107.6
2''''		75.2
3''''		77.8
4''''		71.0
5''''		67.1
6''''		–
CO<u>C</u>H$_3$	20.7	
<u>C</u>OCH$_3$	172.6	

* Assignments based on DEPT, HSQC and HMBC experiments.

8.5 Hz) assigned for H-3', H-5' and H-2', H-6' in a 4'-monosubstitued B-ring in **1** and **2** respectively. In the UV spectra the 46 and 43 nm bathochromic shift with NaOMe and 43 and 49 nm with $AlCl_3$/HCl indicated free C-5 and C-4' hydroxyl groups in **1** and **2** respectively. The failure of NaOAc to produce any shift in band II was diagnostic for a substituted C-7 hydroxyl groups in both **1** and **2** (Harborne, *et al.*, 1975).

In the ^{1}H- and ^{13}C-NMR of **1** (Tables 1, 2) the presence of a methyl doublet at δ_H 1.30 (J = 7.5 Hz) and δ_C 18.1 along with 5 signals in the sugar region including an anomeric proton H-1''' at δ_H 5.57 (br *s*) with its corresponding carbon at δ_C 100.6 were diagnostic for an α-rhamnosyl moiety. In an HMBC experiment correlation was observed between the rhanmosyl anomeric proton H-1''' at δ_H 5.57 and C-7 at δ_C 163.6 indicating the attachment of rhamnose to C-7 hydroxyl group of the flavonol skeleton. In the ^{1}H-NMR of **1** (Tables 1) another anomeric proton signal at δ_H 5.70 (d, J = 2.0 Hz) correlated to a carbon signal at δ_C 110.7 along with 4 O-CH carbon signals were assigned for a second pentose moiety. The IR spectrum of **1** displayed ester carbonyl absorption at 1750 cm^{-1}. The methyl singlet at δ_H 2.09, δ_C 20.7 and carbonyl absorption at δ_C 172.6 were assigned for an acetyl group. Comparison of the proton and carbon signals with the literature indicated that the second sugar is C-5'' acylated apiose (Imperato, 1996). The position of the acetyl group at C-5'' was confirmed by the HMBC correlations of H-5'' protons (δ_H 4.03, d, J = 14.5 Hz; 4.15, d, J = 14.5 Hz) and both the carbonyl and C-3'' signals at δ_C 172.6 and 79.1, respectively. HMBC experiment also confirmed the position of apiosyl moiety at C-3 of the aglycone through correlation between H-1'' at δ_H 5.70 and C-3 at δ_C 135.3. The appearance of the molecular ion peak at *m/z* 607.1624 $[M + H]^+$ in the positive HRFABMS supported the assigned structure. Acid hydrolysis of **1** afforded rhamnose and apiose as confirmed by TLC and GC comparison with authentic samples. Consequently, **1** was identified as kaempferol 3-*O*-(5''-acetyl)-apioside-7-*O*-α-L-rhamnopyranoside. A literature survey indicated that **1** is a new natural product.

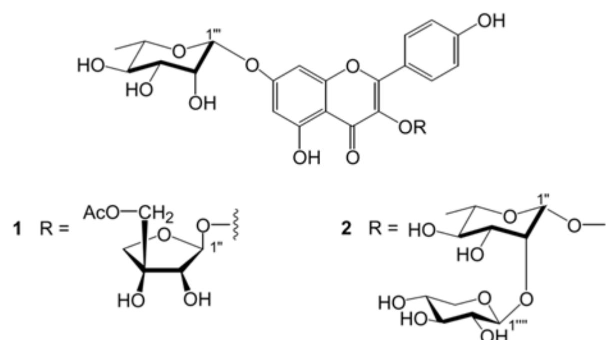

Fig. 1. Structures of compounds **1** and **2**.

HRFABMS of **2** in combination with ^{1}H- and ^{13}C-NMR data (Tables 1, 2) suggested molecular formula $C_{32}H_{38}O_{18}$ indicating a trioside with two hexoses and one pentose unite. The two methyl doublets at δ_H 1.28 (d, J = 6.5) and δ_H 1.02 (d, J = 6.0) were assigned to position six

of two rhamnosyl moieties. Complete assignment of the sugar protons and carbons was achieved by detailed study of the COSY and HSQC spectra. HMBC correlations were observed between H-1" of one rhamnosyl residue (δ_H 5.44, s) and C-3 (δ_C 137.0); H-1"' of the second rhamnosyl (δ_H 5.57, *s*) and C-7 (δ_C 162.9) confirming the attachment of the two rhamnosyl moieties to C-3 and C-7 of the aglycone. The downfield shift of C-2" of the C-3 attached rahnmose to δ_C 82.6 indicating that this position is the site of attachment of the remaining pentose moiety. This fact was confirmed by HMBC correlation between H-1"" (δ_H 4.34) and C-2" (δ_C 82.6). ^{1}H- and ^{13}C-NMR data of the additional pentose unite suggested a xylose moiety (Abdel-Kader, *et al.*, 2000). The 7.5 Hz *J* value of H-1"" indicated its β-configuration.

Acid hydrolysis of **2** afforded rhamnose and xylose as confirmed by TLC and GC comparison with authentic samples. The above discussion identified the structure of **2** as Kaempferol 3-*O*-α-[β-D-xylosyl-(1"" → 2")-L-rhamnopyranoside]-7-*O*-α-L-rhamnopyranoside, a previously unreported flavonol triglycoside.

Acknowledgements

We thank the Research center at the College of Pharmacy and KACST for financial support to Dr Hanan M. EL-Youssef. Our thanks are also due to the Analytical Services at the Department of Chemistry, Virginia Polytechnic Institute and State University and the Central Laboratory at the College of Pharmacy, King Saud University for spectral analyses.

References

Abdel-Ghani, A.E., Hafez, S.S., Abdel Aziz, E.M., and El-Shazly, A.M., Phytochemical and biological studies of *Lotus corniculatus* var. *tenuifolius* L. growing in Egypt. *Alex. J. Pharm. Sci.* **15**, 103-108 (2001).

Abdel-Kader, M.S., Bahler, B.D., Malone, S., Werkhoven, M.C.M., Wisse, J.H., Neddermann, K.M., Bursuker, I., and Kingston, D.G.I., Bioactive Saponins from *Swartzia schomburgkii* from the Suriname Rainforest. *J. Nat. Prod.* **63**, 1461-1464 (2000).

Abdel-Kader, M.S., Basoudan, O.A., Alqasoumi, S.I., and Abou-Shoer, M.I., Phytochemical study of *Lotus ornithopodioloides* L. *Nat. Prod. Sci.* **13**, 317-321 (2007).

Agrawal, P.K., *^{13}C-NMR of flavonoids*. Elsevier, Amsterdam, 1989.

Chaudhary, S.A., *Flora of the kingdom of Saudi Arabia*. Ministry of Agriculture and Water, National Agriculture and Water Research Center, Riyadh, KSA, Vol **II** (part II), 2001, pp. 92.

El Mousallami, A.M.D, Afifi, M.S., and Hussein, S.A.M., Acetylated flavanol diglucosides from *Lotus polyphyllos*. *Phytochemistry* **60**, 807-811 (2002).

El-Youssef, H.M., Murphy, B., Amer, M.E., Abdel-Kader, M.S., and Kingston, D.G.I., Phytochemical and biological study of the aerial parts of *Lotus lalambensis* growing in Saudi Arabia. *Saudi Pharmaceutical Journal* **19**, 122-134 (2008).

Evans, W.C., *Trease and Evans' Pharmacognosy.* 15th ed. W. B. Saunders Company Ltd. Edinburhg, London, New York, Philadelphia, St Louis, Sydney, Toronto, 2002.

Harborne, J.B., Mabry, T.J., and Mabry, H., *The flavonoids*. Academic press, New York San Francisco, 1975.

Imperato, F., Kaempferol 3-O-(5"-Feruloylapioside) from *Pteridium aquilinum Phytochemistry* **43**, 1421-1423 (1996).

Mahasneh, A.M., Screening of some indogenous Qatari medicinal plants for antimicrobial activity, *Phytotherapy Research* **16**, 751-753 (2002).

Migahid, A.M., *Flora of Saudi Arabia*. 2nd ed., Riyadh University Publication, Riyadh, Saudi Arabia, 1978, pp. 337.

(Accepted May 15, 2008)