



## ACETYLATED FLAVONOL TRIGLYCOSIDES FROM *AMMI MAJUS* L.

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**Key Word Index**—*Ammi majus*; Apiaceae; aerial parts; acetylated flavonol triglycosides; kaempferol and isorhamnetin 3-*O*-[2''-(4'''-acetyl-rhamnosyl)-6''-glucosyl] glucoside.

**Abstract**—Two new acetylated flavonol triglycosides: kaempferol and isorhamnetin 3-*O*-[2''-(4'''-acetyl-rhamnosyl)-6''-glucosyl] glucosides, were isolated and identified from the aerial parts of *Ammi majus* L. In addition, three known flavonol glycosides namely; isorhamnetin-3-*O*-rutinose, kaempferol-3-*O*-glucoside and isorhamnetin-3-*O*-glucoside were detected. © 1998 Elsevier Science Ltd. All rights reserved

### INTRODUCTION

*Ammi majus* L. is one of the most important medicinal plants in Egypt. The fruits contain several furanocoumarin types which are used for numerous reputed medicinal purposes [1–6], e.g. for the production of xanthotoxin, which is one of the most potent drugs used to treat leukoderma [7–9]. However, the non-fruitle aerial parts of this plant have not been analysed previously. Therefore, it was felt important to investigate these tissues for their phytochemical constituents. The present report deals with the isolation and identification of two novel acetylated flavonol triglycosides as well as three known flavonol glycosides which are reported for the first time from this plant.

### RESULTS AND DISCUSSION

Fractionation of the *n*-butanol aerial parts extract of *A. majus* by a combination of column chromatography and preparative TLC resulted in the isolation of five glycosides 1–5.

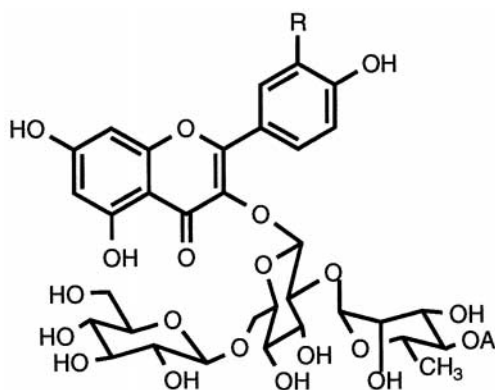
The IR spectrum of 1 showed a band at 3400 cm<sup>-1</sup>(OH) and a characteristic band at 1740 cm<sup>-1</sup> specific for an ester carbonyl group. UV spectral data obtained with usual shift reagents before and after hydrolysis were typical for a 3-*O*-substituted flavonol [10]. Acid hydrolysis followed by TLC comparison with authentic samples revealed the presence of glucose, rhamnose and kaempferol.

The negative-ion FAB mass spectrum of 1 showed a peak at *m/z* 797 [M–H] which was con-

sistent with a molecular formula C<sub>35</sub>H<sub>42</sub>O<sub>21</sub> for a flavonol triglycoside. Fragments at *m/z* 635 [(M–H)–162 (terminal glucose)], 609 [(M–H)–188 (terminal acetyl-rhamnose)] and 285 [(M–H)–162–188–162 kaempferol] indicated the presence of a branched sugar moiety and one acetyl group located on the rhamnose unit; established from the loss of 188 mass unit.

The presence of kaempferol skeleton was further confirmed by comparison of the <sup>1</sup>H and <sup>13</sup>C-NMR signals of 1 with reported values [11].

The presence of three proton signals represented by two doublets at δ 4.21 and 5.49 (*J* = 7.2 and 7.3 Hz), respectively, characteristic for the anomeric protons of two β-D-glucose residues [12,13] and a broad singlet at δ 5.13 characteristic for one anomeric proton of an α-L-rhamnosyl moiety, confirm



1, R = H

2, R = -OCH<sub>3</sub>

the presence of three sugar units [12, 13]. This finding was further confirmed by a DEPT spectrum which revealed the presence of 18 carbon signals, of which three represent anomeric carbon signals at  $\delta$  103.3, 100.9 and 98.5 [12], methine carbon signals in the region  $\delta$  69.8–77.8 and two oxymethylene carbons at  $\delta$  60.8–67.8. The appearance of two extra carbon signals at  $\delta$  20.9 and 169.9 as well as a sharp singlet (three protons) in the  $^1\text{H}$  NMR spectrum at  $\delta$  1.96 supported the presence of an acetyl group. The complete assignments of the sequence of the sugar moieties and the position of the acetyl group, was achieved by HMBC correlations [14]. The attachment of the sugar moiety to the 3-hydroxyl of kaempferol was determined from the correlation between the H-1 anomeric proton of the central glucose at  $\delta$  5.49 and C-3 of kaempferol at  $\delta$  132.6 (Table 1).

The interglucosidic linkage in **1** was determined as 1  $\rightarrow$  6 from the HMBC correlation between the H-1 anomeric proton of the terminal glucose  $\delta$  4.29 and the carbon signal at  $\delta$  67.8 (C-6 of the central glucose). Proof of the (1  $\rightarrow$  2) attachment of rhamnose to the central glucose was obtained from the correlation between the H-1 of rhamnose at  $\delta$  5.13 and the C-2 carbon signal at  $\delta$  77.8 of the central glucose. A proton signal at  $\delta$  4.74 (*t*,  $J$  = 9.8 Hz) correlated with the carbon signal at  $\delta$  73.9 in the HMQC experiment [14], indicating that the hydroxyl group at C-4 of the rhamnose was acetylated. This was further confirmed from the correlation between the anomeric proton of  $\alpha$ -L-rhamnose ( $\delta$  5.13) and carbon signals at  $\delta$  69.0 and

65.7 corresponding to C-3 and C-5 of the rhamnosyl unit. The signal at  $\delta$  69.0 and 65.7 which is shifted upfield by about 3 ppm from than the normal position for C-5 of the rhamnose [15], as well as a correlation between the singlet at  $\delta$  1.98 (3H) and carbon at  $\delta$  73.9 pointed to an acetyl group at C-4 of the rhamnose. Thus, **1** could be identified as kaempferol 3-*O*-(2''-(4''-acetylramnosyl)(1  $\rightarrow$  2)-6''-glucosyl] glucoside.

UV spectral analysis of **2** before and after hydrolysis gave data which were typical for a 3-*O*-substituted flavonol [10]. The negative-ion FAB mass spectrum of **2** showed a peak at  $m/z$  827 [M–H] which was consistent with a molecular formula  $\text{C}_{36}\text{H}_{44}\text{O}_{22}$  for the flavonol triglycoside. Other significant peaks were observed, 665 [(M–H)–162 (terminal hexose)], 639 [(M–H)–188 (terminal deoxyhexose containing an acetyl group)], 315 [(M–H)–162 + 188 + 162(isorhamnetin)]. Therefore, **2** was considered to be an isorhamnetin 3-*O*-triglycoside which has a branched sugar moiety containing an acetyl group located on the deoxyhexose unit. The  $^1\text{H}$  NMR spectrum was very similar to **1**, except the signals corresponding to the aglycone moiety showed five kinds of aromatic protons [ $\delta$  6.21 (1H, *d*,  $J$  = 1.98) 6.20 (1H, *d*,  $J$  = 1.98), 6.19 (1H, *d*,  $J$  = 1.98), 6.44 (1H, *d*,  $J$  = 1.98), 7.91 (1H, *d*,  $J$  = 1.98), 6.93 (1H, *d*,  $J$  = 8.55), 7.55 (1H, *dd*,  $J$  = 1.98, 8.55) and  $-\text{OCH}_3$  at 3.84 (3H, *s*) due to isorhamnetin. Additionally, two anomeric protons in the  $\beta$ -D- form at  $\delta$  4.64 and 5.65 ppm with  $J$  = 7.19, 7.21, respectively and another one in the  $\alpha$ -L-form at  $\delta$  5.22 ppm as a broad singlet. An acetyl group was depicted from a sharp singlet at  $\delta$  1.98 (3H) and one characteristic methyl doublet signal at  $\delta$  0.98 ( $J$  = 6.5). Acid hydrolysis yielded isorhamnetin, rhamnose and glucose. From the above data the proposed structure of **2** is isorhamnetin 3-*O*-(2''-(4''-acetylramnosyl)(1  $\rightarrow$  2)-6''-glucosyl] glucoside.

The other known compounds were identified by direct TLC comparison and comparison of their spectral data with literature values [10, 11, 13, 16–18].

## EXPERIMENTAL

### General

Mps are uncorr.  $^1\text{H}$ ,  $^{13}\text{C}$  NMR and 2D NMR spectra ( $\delta$ , ppm,  $J$  in Hz) were recorded with JEOL-EX 270/67 MHz and JEOL-JNM 500/125 MHz in  $\text{DMSO}-d_6$  with TMS as int. standard. FAB-MS were registered in negative ion mode in thioglycerol matrix, using a JASCO AUTOSPEC MS. UV spectra were recorded in MeOH using standard procedures [10]. IR spectra were recorded in KBr discs. TLC was carried out on silica gel G

Table 1.  $^{13}\text{C}$  NMR spectral data of compound **1** (125 MHz,  $\text{DMSO}-d_6$ , ppm from TMS as internal standard)\*

C	ppm	C	ppm
<b>Kaempferol</b>			
C4	177.1	Rha.	
C7	164.2	C1''	100.9
C5	161.2	C2''	70.8
C4'	159.8	C3''	69.0
C9	156.7	C4''	73.9
C2	156.3	C5''	65.7
C3	132.6	Me	16.7
C2', 6'	130.8	COMe	20.9
C1'	120.8	COMe	169.9
C3', 5'	115.2	Glc. (terminal)	
C10	104.5	C1'''	103.3
C6	98.7	C2'''	73.8
C8	93.8	C3'''	76.6
		C4'''	70.4
		C5'''	75.9
		C6'''	60.8
<b>Glc. (central)</b>			
C1''	98.5		
C2''	77.8		
C3''	75.5		
C4''	69.8		
C5''	76.8		
C6''	67.8		

\*All carbons were assigned by  $^{13}\text{C}$  NMR, DEPT and 2D NMR (HMBC and HMQC).

60 F<sub>254</sub> (Merck), cellulose (without fluorescent indicator) Merck.

#### Plant material

Aerial parts of *Ammi majus* L. were collected from the Delta region, Egypt in June 1995 and identified by Professor Nabil El-Haidi, Faculty of Science, Cairo University, Egypt. A voucher specimen has been deposited in the Herbarium of the Department of Pharmacognosy (No. 1121), Faculty of Pharmacy, Al-Azhar University.

#### Extraction and isolation

Air-dried aerial parts of *Ammi majus* L. (1/2 kg) were defatted with petrol then percolated successively with 70% MeOH and the combined MeOH extracts concentrated in *vacuo* at 45°C. Water was added to the solid residue and the mixture partitioned with (1) EtOAc and (2) *n*-BuOH. The *n*-BuOH residue (15 g) was fractionated over a silica gel column (500 g) using CHCl<sub>3</sub>/MeOH with increasing polarity. Fractions which were positive for flavonoids were combined (**I–III**).

Fraction **I** (1.5 g) gave 2 major spots on TLC. Rechromatography on silica gel CC using CHCl<sub>3</sub>/MeOH (8:3) yielded 35 mg isorhamnetin 3-glucoside(**5**) and 10 mg of kaempferol 3-glucoside(**4**), *R<sub>f</sub>* 0.65 and 0.62 in CHCl<sub>3</sub>/MeOH (7:3), respectively.

Fraction **II** (400 mg) afforded isorhamnetin 3-rutinoside (**3**), *R<sub>f</sub>* 0.55 CHCl<sub>3</sub>/MeOH (7:3).

Fraction **III** (250 mg) was purified by CC on Sephadex LH-20 with 70% MeOH as eluent. The flavonoids containing fraction gave only one spot on TLC using several different solvent systems. However, TLC on cellulose in EtOAc/HCOOH/H<sub>2</sub>O (33:7:10) gave a major spot and one minor spot. Final separation was performed using this system. The two main bands were eluted with MeOH, and purified through Sephadex-LH-20 (90% aq. MeOH), to yield **1** (22 mg) and **2** (3 mg).

#### Acid hydrolysis of **1–5**

Each compound (1 mg) was refluxed in 0.1 N H<sub>2</sub>SO<sub>4</sub> (10 ml) for 4 h. The aglycones were extracted with EtOAc and identified by comparison with authentic samples of isorhamnetin and kaempferol on (TLC silica gel, CHCl<sub>3</sub>/MeOH 9:1, EtOAc/Petrol 1:1). The aq. layer was neutralized with NaHCO<sub>3</sub>. After freeze dried sugars were extracted with pyridine, the solvent evapd, the residue dissolved in a small amount of MeOH and run on silica gel TLC in EtOAc/MeOH/H<sub>2</sub>O/HOAc (57:13:13:17) using *p*-anisidine phthalate as detection agent.

Kaempferol 3-*O*-[2''-(4''-acetyl)rhamnosyl](1 → 2)-6''-glucosyl] glucoside (**1**); amorphous yellow powder, m.p.: 196–198°C (MeOH/EtOAc 1:3) IR  $\gamma_{\max}^{\text{cm}^{-1}}$ : 3400, (OH); 1740 (MeCO); 1650 (C=O); UV  $\lambda_{\max}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 352 (3.98), 271 *sh* (4.01), 267

(4.31) (AlCl<sub>3</sub>) 407, 368, 303 *sh*, 273 (AlCl<sub>3</sub>+HCl)411. 348, 300, 274 (NaOMe) 388, 327, 277 (NaOAc) 371, 302, 274 (NaOAc + H<sub>3</sub>BO<sub>3</sub>) 352, 268; negative-ion FAB-MS [glycerol] *m/z* (rel. int.):797(10) [M–H], 755(3) [M–acetyl group–H]<sup>–</sup>, 635(6) [M–glc–H]<sup>–</sup>,609(4) [M–acetyl rhamnose–H]<sup>–</sup>, 285(30) [kaempferol–H]<sup>–</sup>, [M–glc–acetyl rha–glc–H]<sup>–</sup>; <sup>1</sup>H NMR(500 MHz, DMSO-*d*<sub>6</sub>, TMS, ppm):  $\delta$  7.93 (2H, *d*, *J* = 8.8 Hz, H-2', H-6'), 6.81 (2H, *d*, *J* = 8.8 Hz, H-3', H-5'), 6.33 (1H, *d*, *J* = 1.95, H-8) and 6.12 (1H, *d*, *J* = 1.95, H-6), 4.21 (1H, *d*, *J* = 7.2 Hz, Glc H-1), 5.49 (1H, *d*, *J* = 7.3 Hz, Glc H-1), 5.13 (1H, *brs*, H-1 Rha), 1.98 (3H, *s*, CH<sub>3</sub>CO). 0.98 (3H, *d*, *J* = 6.5 Me-Rha.), <sup>13</sup>C NMR (Table 1).

Isorhamnetin 3-*O*-[2''-(4''-acetyl)rhamnosyl](1 → 2)-6''-glucosyl] glucoside (**2**). Yellow amorphous powder; UV  $\lambda_{\max}^{\text{MeOH}}$  nm (log  $\epsilon$ ): 355 (3.88), 268 *sh* (3.95), 255 (4.22); (+ AlCl<sub>3</sub>) 407, 370 *sh*, 302, 267, (+ AlCl<sub>3</sub>+HCl) 400, 358, 268; (+ NaOMe) 405, 327, 269; (+ NaOAc) 379, 320, 276; (+ NaOAc + H<sub>3</sub>BO<sub>3</sub>) 355, 255; negative-ion FAB-MS [glycerol] *m/z* (rel. int.):827 (8) [M–H], 665(6) [M–glc–H]<sup>–</sup>, 639(4) [M–acetyl rhamnose–H]<sup>–</sup>, 315 (30) [isorhamnetin–H]<sup>–</sup> [M–glc–acetyl rha–glc–H]<sup>–</sup>; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>, TMS, ppm);  $\delta$  6.21 (1H, *d*, *J* = 1.98) 6.20 (1H, *d*, *J* = 1.98), 6.19 (1H, *d*, *J* = 1.98), 6.44 (1H, *d*, *J* = 1.98), 7.91 (1H, *d*, *J* = 1.98), 6.93 (1H, *d*, *J* = 8.55), 7.55 (1H, *dd*, *J* = 1.98, 8.55) and –OCH<sub>3</sub> at 3.84 (3H,*s*), 4.64 (1H *d*, *J* = 7.19 Hz, Glc H-1), 5.65 (1H *d*, *J* = 7.21 nHz, Glc H-1), 5.22 (1H, *brs*, Rha H-1). 1.98 (3H, *s*, CH<sub>3</sub>CO), 0.98 (1H, *d*, *J* = 6.5, Me-Rha).

#### REFERENCES

- Murry, H. D. R., Mendz, J. and Brown, A. S., *The Natural Coumarins*. John Wiley and Sons, New York, 1982.
- Abu-Mustafa, E. A. and Fayez, M. B. E., *J. Org. Chem.*, 1961, **26**, 161.
- El-Gamal, M. H. A., Abu-Mustafa, E. A. and Shalaby, N. M. M., *Egypt. J. Pharm. Sci.*, 1991, **32**, 389.
- Abu-Mustafa, E. A., El-Bay, F. K. A. and Fayez, M. B. E., *J. Pharm. Sci.*, 1971, **60**, 788.
- Meshal, S. A., Nawar, M. A., Nofal, Z., El-Sherbiny, A. and Abu-Mustafa, E. A., *Int. Conf. Chem. Biotechnol. Biol. Act. Nat. Prod.*, 1981, **3**(11).
- El-Gamal, M. H. A., Shalaby, N. M. M., Duddeck, H. and Hiegemann, M., *Phytochemistry*, 1993, **34**, 819.
- Schonberg, A. and Sina, A., *Nature*, 1948, **161**, 481.
- Fahmy, I. R. and Abu-Shady, H., *Q. J. Pharm. Pharmacol.*, 1948, **21**, 499.
- Pathak, M. A., Worden, L. R. and Kaufman, K. D., *J. Invest. Dermatol.*, 1967, **48**, 103.

10. Mabry, T. J., Markham, K. R. and Thomas, M. B., in *The Systematic Identification of Flavonoids*. Springer, New York, 1970.
11. Markham, K. R., *Techniques of Flavonoids Identification*. Springer, New York, 1970.
12. Tedder, M. J., Nechvatal, A., Murray, W. A. and Carnduff, J., *Basic Organic Chemistry*, Part IV. John Wiley and Sons London, 1972.
13. Harborne, J. B., Mabry, T. J. and Mabry, H., 1975. *The Flavonoids*. Academic Press, London, New York, p. 69.
14. Müller, L., *J. Am. Chem. Soc.*, 1979, **101**, 4481.
15. Guinaudeau, H., Seligmann, O., Wagner, H. and Neszmely, A., *Phytochemistry*, 1981, **20**, 1113.
16. Markham, K. R., Ternai, B., Stanely, R., Geiger, H. and Mabry, T. J., *Tetrahedron*, 1978, **34**, 1389.
17. Bader, G., Tuja, D., Wray, V. and Hiller, V., *Planta Med.*, 1993, **59**, 284.
18. Merfort, I. and Wendisch, D., *Planta Med.*, 1987, **53**, 434.