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An Acylated Kaempferol Glycoside from Flowers of *Foeniculum vulgare* and *F. Dulce*

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Abstract: An acylated kaempferol glycoside, namely kaempferol-3-O-α-L-(2",3"-di-*E*-*p*-coumaroyl)-rhamnoside (**1**) was isolated from the flowers of *Foeniculum vulgare* Mill. and *F. dulce* DC. It is thus isolated for the first time from family Apiaceae. In addition, the different organs of both plants afforded six flavonoid glycosides - namely afzelin (kaempferol-3-O-α-L-rhamnoside) (**2**), quercitrin (**3**), isorhamnetin-3-O-β-D-glucoside (**4**), isoquercitrin (**5**), rutin (**6**), and miquelianin (quercetin-3-O-β-D-glucuronide) (**7**). Structure elucidation of the above mentioned flavonoids was achieved by UV, ^{1}H- and ^{13}C-NMR, ^{1}H-^{1}H COSY, HMQC and EI-MS.

Keywords. *Foeniculum vulgare*, *F. dulce*, acylated kaempferol glycoside, spectroscopy.

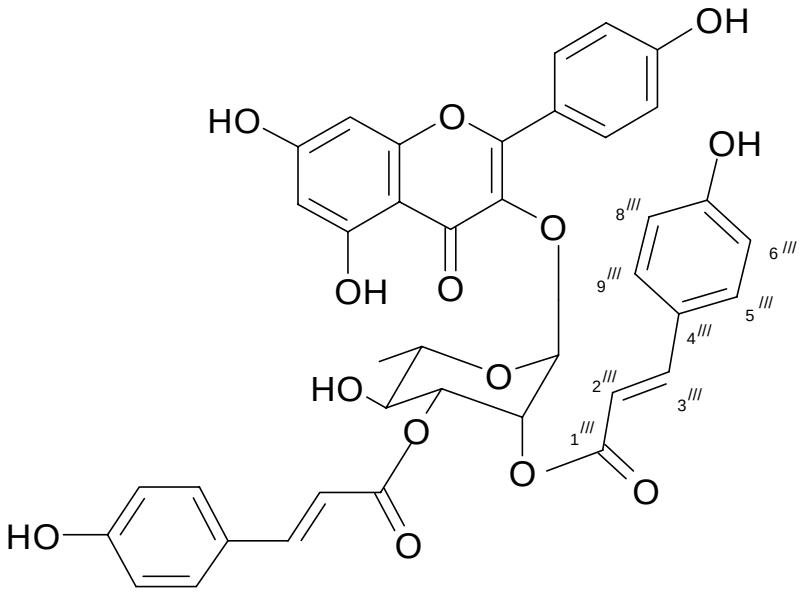
Introduction

Foeniculum vulgare Mill. is used in folk medicine as carminative, digestive, lactagogue and diuretic. The leaves, stalks and fruits are edible [1, 2]. *F. dulce* DC. is an annual herb grown especially for its bulb-like swollen leaf bases which are delicious in salads [3, 4]. Flavonoids are more common throughout the family Apiaceae than other constituents [5]. The presence of flavonol glycoside types

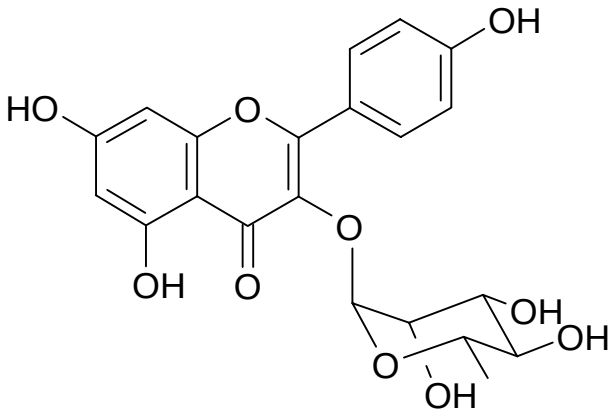
in *fennel* species was based on morphological heterogenicity and variation in flavonol glycosides have been reported [6]. Kaempferol and quercetin were detected in leaves of *F. vulgare* Mill. [7] and the presence of kaempferol-3-O-glucuronide, quercetin-3-O-glucuronide, quercetin-3-arabinoside, kaempferol -3-arabinoside and rutin in leaves and fruits of the same plant was also reported [6, 8-10]. Moreover, the presence of quercetin and isoquercitrin in fruits [6,9,11] and isorhamnetin glycosides in leaves of the same *fennel* species was reported [9]. Since little research has been conducted on the leaf, fruit, and flower of *F. vulgare* and nothing has been found concerning the flavonoids of *F. dulce*, it was of interest to examine the flavonoid patterns in the different organs of the two species.

Results and Discussion

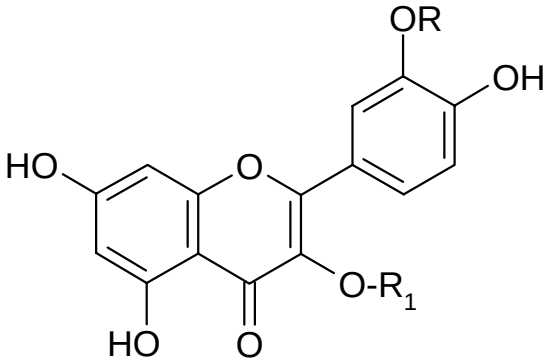
All isolated compounds displayed UV absorption data typical of 3-substituted flavonols. The ^{1}H-NMR spectrum of compound **2** displayed the characteristic signals of the kaempferol nucleus [12,13]: two doublets at δ_H 6.20 and 6.40 ppm (J = 2.1 Hz), assigned to the H-6 and H-8 protons, respectively, and a pair of A_2B_2 aromatic system protons at δ_H 6.93 and 7.77 ppm (J = 8.4 Hz), assigned to H- 3', 5' and H-2', 6' respectively. Acid hydrolysis of **2** gave kaempferol and L-rhamnose which were identified by comparison with authentic samples. ^{1}H-NMR of the sugar moiety suggested the presence of L-rhamnose in the molecule (the anomeric proton at δ_H 5.30 ppm, H-2" at δ_H 3.89 and H-3" at δ_H 3.49 ppm). The compound was identified as afzelin (kaempferol -3-O-α-L-rhamnoside).



1



2



3-7

3 R=H, R_1 = α-L-rhamnose
4 R=OCH_3, R_1=β-D-glucose
5 R=H, R_1=β-D-glucose
6 R=H, R_1 β-D-glucose- α-L-rhamnose
7 R=H, R_1= β-D-glucuronic acid

The UV shifts and ^{1}H-NMR spectra of compounds **3** and **5** -**7** were in agreement with a quercetin skeletal pattern [12,13]. In addition, TLC investigation of the hydrolysis products showed that **3** and **5-7** have the same aglycone moiety. Additionally, the sugar fraction of the hydrolysis product of **3** was L-rhamnose, of **5** was D-glucose, of **6** was L-rhamnose and D-glucose and of **7** was D-glucuronic acid. The identity of D-glucuronic acid was confirmed by ^{13}C-NMR: C-6" was found at δ 172.43 [14,15]. Therefore, **3** was identified as quercitrin, **5** as isoquercitrin, **6** as rutin and **7** as miquelianin (quercetin-3-O-β-D-glucuronide). Compound **4** was identified as isorhamnetin-3-O-β-D-glucoside by comparison of its UV and ^{1}H-NMR data with literature values [12,13].

The characteristic UV spectrum of compound **1**, a shoulder at λ_{max} = 268 nm and a broad band at λ_{max} = 312 nm, suggested a diacylated glycoside [16]. The ^{1}H-NMR spectrum displayed the typical signals of the kaempferol nucleus [13], in addition to two *p*-coumaroyl moieties as indicated by the presence of two pairs of doublets with a relatively large coupling constant (15.9 Hz) [17]. The first pair at δ_H 6.63 and 7.62 ppm and the second at δ_H 7.60 and 6.36 ppm, indicated two pairs of *trans* configured ethylenic protons. Additionally, two A_2B_2 systems, each integrating for two protons, were found at δ_H 7.44, 6.80 and 6.75, 7.37 ppm, respectively. This was also supported by a fragment at m/z 164 (56 %) in the EI-MS. The sugar moiety of **1** was identified as L-rhamnose by acid hydrolysis. ^{1}H-NMR showed the presence of three downfield sugar proton signals at δ_H 5.30 (dd, *J* = 9.7, 3.4 Hz), 5.60 (d, *J* = 1.7 Hz) and 5.82 (d, *J* = 1.7 Hz) which could be ascribed to H-3", H-1" and H-2". This assignment was deduced by the correlation of H-1" with H-2", H-2" with H-1" and H-3", in ^{1}H-^{1}H COSY. The downfield shift of H-2" and H-3", relative to the corresponding positions in compound **2** (kaempferol-3-O-α-L-rhamnoside) proved their acylation by the two *p*-coumaric acid units (+1.84, +1.81 for H-2" and H-3", respectively). The acylation at C-2" and C-3" positions is further confirmed by the upfield shift of the adjacent carbons in ^{13}C (-2.4 ppm for both carbons C-1" and C-4") relative to the corresponding carbons of kaempferol-3-O-L-rhamnoside [14]. The assignments of the chemical shifts of the carbons were deduced by a HMQC experiment. Therefore, compound **1** was identifed as kaempferol-3-O-α-L-(2",3"-*E*-di-*p*-coumaroyl)-rhamnoside, previously reported in *Planatus acerifolia* buds F. Platanaceae[18], but representing a new kaempferol derivative in family Apiaceae. The distrubution of the various isolated compounds in the leaves, flowers and roots of the two species is given in Table 1.

Table 1: Comparison of the flavonoid contents of the different organs of *F. vulgare* and *F. dulce*.

Comp.	**R_f***	***F. vulgare* Mill.**					***F. dulce* DC.**				
		leaf	flower	fruit	stem	root	leaf	flower	fruit	stem	root
1	0.96	-	++	-	-	-	-	++	-	-	-
2	0.89	-	++	-	-	-	-	++	+	-	-
3	0.86	+	-	-	-	-	-	-	-	-	-
4	0.81	-	+	-	-	-	-	+	-	-	-
5	0.79	+	++	-	-	-	-	++	+	-	-
6	0.58	-	+	-	-	-	-	+	-	-	-
7	0.42	+	++	+	-	-	-	++	+	-	-

*Solvent system: ethyl acetate: methanol: water (100: 16.5: 13.5)

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Experimental

General

UV spectra were determined in methanol and after addition of different shift reagents on a Hewlett Packard 8452A diode array spectrophotometer. EI-MS was carried on a Finnigan Mat SSQ 7000 GC/MS instrument at 70 eV. Melting points were determined using a Gallenkamp apparatus. ^{1}H-NMR spectra were recorded in DMSO-*d*6 on a JEOL TMS Route instrument at 300 MHz using TMS as internal standard. ^{1}H-NMR , ^{1}H-^{1}H COSY, ^{13}C-NMR and HMQC spectra of compound **1** were measured in CD_3OD on a Varian 500 instrument operated at 400 (^{1}H-) and 100 (^{13}C-) MHz, respectively. Thin-layer chromatography and preparative thin-layer chromatography were perfomed on silica gel GF_{254} precoated plates (Machery Nagel, Germany) and developed with either 100:16.5:13.5 ethyl acetate/methanol/water (system A), 100:11:11:10 ethyl acetate/formic acid/acetic acid/water (system B), 9:1 chloroform/methanol (system C), 4:1:1 *n*-butanol/acetic acid/water (system D) or 4:5:1 *n*-butanol/acetic acid/water (system E); the compounds were visualized in UV light and $AlCl_3$ spray reagent (flavonoids) or using aniline phthalate spray reagent (sugars). Silica gel 60 (230-400 mesh ASTM, Machery Nagel, Germany), silica gel H for vacuum liquid chromatography (VLC) (Merck), and Sephadex LH-20 (Pharmacia) were used for column chromatography. Reference samples of flavonoid aglycones and sugars were obtained from E. Merck, Darmstadt, Germany and B.D.H., Poole, England.

Plant material

Samples of the different organs of *Foeniculum vulgare* and *Foeniculum dulce* (Family Apiaceae) were collected from February to April 2001 from the Experimental and Research Station of the Faculty of Pharmacy, Giza. Identification of the plants was carried out by Prof. Dr. Nabil El Hadidy, Faculty of Science, Cairo University, Egypt.

Extraction and Isolation

The air-dried flowers of *F. vulgare* (350 g) were extracted with 95% ethanol (5L) to yield 68g of dry residue. Fifty grams of the residue were then suspended in water (250 mL) and partitioned successively with petroleum ether (7.4 g), chloroform (2 g), ethyl acetate (4 g) and *n*-butanol (4 g). The ethyl acetate extract was chromatographed over 40 g Si gel H in a vacuum liquid chromatography column (VLC) (13 x 4 cm). Gradient elution was carried out using chloroform and increasing the polarity with ethyl acetate in 5% stepwise elutions to 100% ethyl acetate (40 x 100 mL) and then with ethyl acetate and increasing the polarity with methanol in 5% stepwise increments to 75% ethyl acetate-25% methanol (15 x 100 mL). Fractions 16 and 17 (99 mg) were combined and purified on a Sephadex LH-20 column (40 x 2 cm) using methanol as eluent to give compound **1** (43 mg). Fractions 28-30 (110 mg) and fractions 35 and 36 (100 mg) were treated separately as described for **1** to yield **2**

(18 mg) and **4** (12 mg) respectively. Fractions 39 and 40 (523 mg) were filtered on a Sephadex LH-20 column (40 x 2 cm) using methanol as eluent and then chromatographed over 25 g Si gel column (25 x 1.5 cm) eluting with 4:1chloroform/methanol to give **5** (25 mg). Fractions 41-48 (590 mg) were filtered on a Sephadex LH-20 column (40 x 2 cm) and then purified by preparative TLC using solvent system A to yield **6** (8 mg). Fractions 52-55 (90 mg) were purified on a Sephadex column (40 x 2 cm) to yield compound **7** (36 mg)

The leaves (600 g) and fruits (400 g) of *F. vulgare*, as well as flowers (250 g) and fruits (700 g) of *F. dulce* were extracted and fractionated as indicated above. The leaves of *F. vulgare* yielded 8 mg of **3,** which was present only in leaves**,** 9 mg of **5,** and 15 mg of **7.** The fruits of *F. vulgare* yielded only 6 mg of **7** while that of *F. dulce* yielded **2** (3 mg), **5** (11 mg) and **7** (10 mg).

Kaempferol-3-O-α-L-(2",3"-E-di-p-coumaroyl)-rhamnoside (**1**).

Yellow amorphous powder (43 mg), mp 190-193°C; R_f 0.96 (A); UV (MeOH): λ_{max} (log ε) 268_{sh} (4.48), 300_{sh} (4.73), 312 (4.76) (MeOH + NaOMe) 276, 312_{sh}, 362 (MeOH + $AlCl_3$) 280_{sh}, 310, 396 (MeOH +$AlCl_3$ + HCl) 280_{sh}, 310, 394 (MeOH + NaOAc) 280_{sh}, 312, 364 (MeOH + NaOAc + H_3BO_3) 268, 300, 310 nm; ^{1}H-NMR (400 MHz, δ_H , CD_3OD): 1.06 (d, *J* = 6.3, H-6"), 3.56 (m, H-5"), 3.64 (t, *J* = 9.7, H-4"), 5.30 (dd, *J* = 9.7, 3.4, Hz, H-3"), 5.60 (d, *J* = 1.7 Hz, H-1"), 5.83 (d, *J* =1.7 Hz, H-2"), 6.21 (d, *J* =1.7 Hz, H-6), 6.28, 6.36 (d, *J* =15.9, H-2"', H-2""), 6.39 (d, *J* = 1.7 Hz, H-8), 6.75, 6.80 (d, *J* = 8.7, H-6"',8"', H-6"",8""), 6.99 (d, *J* = 8.7, H-3',5'), 7.37, 7.44 (d, *J* = 8.4, H-5"',9"', H-5"",9""), 7.60, 7.62 (d, *J* = 15.9, H-3"', H-3""), 7.86 (d, *J* = 8.7, H-2',6') ppm; ^{13}C-NMR (100 MHz, δ_H, CD_3OD): 179.2 (C-4), 168.4, 167.7 (C-1"', C-1""), 165.7 (C-7), 163.1 (C-5), 161.5, 161.3 (C-7"', C-7""), 161.1(C-4'), 158.9 (C-2), 158.3 (C-9), 147.6, 146.9 (C-3"', C-3""), 135.3 (C-3), 131.8 (C-2', 6'), 131.3, 131.1 (C-5"', 9"', C-5"", 9""), 127,126.9 (C-4"', C-4""), 122.3 (C-1'), 116.8, 116.7 (C-6"', 8"', C-6"", 8""), 116.67 (C-3', 5'), 114.8, 114.3 (C-2"', C-2""), 105.9 (C-10), 100.1 (C-1"), 99.9 (C-6), 94.8 (C-8), 72.9 (C-3"), 72.1 (C-5"), 70.9 (C-4"), 70.8 (C-2"), 17.7 (C-6") ppm; EIMS (70 ev) m/z (%): 432 (32), 286 (100), 164 (55.7), 163 (18.3), 153 $[A_1+H]^+$ (4), 152 $[A_1]^+$ (0.9), 147 (88.4), 134 $[B_1]^+$ (3.6), 121 $[B_2]^+$ (24), 120 (52).

Hydrolysis of the isolated compounds

A few mg of the glycosides were refluxed with 10% HCl in 50% methanol for 3 hrs. The aglycones and sugar fractions were identified by chromatographic comparison with authentic samples.

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Sample availability: Samples of compounds **1** (2 mg), **3** (5mg), **5** (5mg) and **7** (5 mg) are available from the authors.