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ACYLATED KAEMPFEROL GLYCOSIDES FROM THE FLOWERS OF *DELPHINIUM FORMOSUM*

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Key Word Index—*Delphinium formosum*; Ranunculaceae; acetylated flavonol glycosides; kaempferol 3-(4″,6″-diacetylglucoside)-7-rhamnoside; kaempferol 3-(6″-acetylglucoside)-7-rhamnoside.

Abstract—One new flavonol glycoside, kaempferol 3-(4″,6″-diacetylglucoside)-7-rhamnoside and three other known kaempferol glycosides: kaempferol 3-glucoside-7-rhamnoside, kaempferol 3-(6″-acetylglucoside)-7-rhamnoside, and kaempferol 7-rhamnoside, were isolated and characterised from flowers of *Delphinium formosum*. The structures were elucidated by spectral and chemical methods. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The genus *Delphinium* is represented by 26 species in Turkey [1, 2]. *Delphinium* species are used in folk medicine as antirheumatics, pediculicides and insecticides for cattle [3]. These plants are rich sources of alkaloids and a study on alkaloids from the roots of *Delphinium formosum* Boiss. et Huet. have been reported in a previous paper [4]. To date, there have been no reports of acylated flavonols in the flowers of *D. formosum*. In this study, we report the isolation and structural elucidation of four kaempferol glycosides (**1–4**) including one new compound (**3**), which was the major flavonol constituent of this tissue.

RESULTS AND DISCUSSION

Compounds **1–4** were isolated from the ethyl acetate soluble portion of the methanolic extract of the flowers of *D. formosum*.

It was observed that the acetylated compounds **2** and **3** were easily hydrolysed in methanolic solution to give **1** and **2**, respectively. Therefore, the isolation of these compounds was carried out in a neutral solvent or with decreased proportions of protic solvents (weakly acidic solvents). Acidic hydrolysis of **1** gave kaempferol, rhamnose and glucose, and acetylation of **1** gave a nona-acetate derivative, which was confirmed by analysis of FAB mass and ^{1}H NMR spectra (see in Experimental). The partial hydrolysis of **1** gave kaempferol 7-rhamnoside and 3-glucoside. These products were identified by TLC and HPLC comparison with authentic markers [5]. Compound **4** was similarly identified as kaempferol 7-rhamnoside and the structure confirmed by FAB mass and NMR spectral analysis (Tables 1 and 2). The UV spectra of **1**, **2** and **3** in methanol gave absorption peaks at 266 nm (band II) and 349 nm (band I), indicating a C3-*O*-substituted flavonol skeleton [5]. The bathochromic shift of band I with aluminum trichloride-hydrochloric acid (about 45 nm) is a characteristic feature of a 5-hydroxy-3-*O*-substituted flavonol. The addition of sodium methoxide produced a bathochromic shift about 40–65 nm of band I with an increase in intensity, which is diagnostic for the presence of a free 4′-hydroxyl group [5, 6]. The lack of a shift of band II with sodium acetate supported that the C-7 hydroxyl was substituted. In addition, the absence of a bathochromic shift of band I with boric acid in the presence of sodium acetate indicated that there were no *o*-dihydroxyl groups on the A ring [5]. Therefore, **1**, **2** and **3** were presumed to have a kaempferol 3-glucoside-7-rhamnoside basic structure. The IR spectra of these compounds showed a strong absorption band at 1650 cm^{-1} corresponding to a ring carbonyl group. Compound **3** exhibited two characteristic absorption peaks for carbonyl groups at 1710 and 1720 cm^{-1}, indicating a diacylated flavonol glucoside. On the other hand, the IR spectrum of **2** showed only one

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Table 1. ^{1}H NMR data or flavonols **1–4** isolated from the flowers of *Delphinium formosum* (500 MHz, DMSO-d_6, TMS as internal standard)

H	1	2	3	4
Kaempferol				
6	6.45 *d* (2.1)	6.46 *d* (2.1)	6.46 *d* (2.1)	6.50 *d* (2.1)
8	6.84 *d* (2.1)	6.85 *d* (2.1)	6.85 *d* (2.1)	6.85 *d* (2.1)
2′,6′	8.09 *d* (8.9)	8.05 *d* (8.8)	8.05 *d* (8.9)	8.17 *d* (9.0)
3′,5′	6.90 *d* (8.9)	6.89 *d* (8.8)	6.91 *d* (8.9)	7.02 *d* (9.0)
Glucose A				
1	5.49 *d* (7.3)	5.37 *d* (7.3)	5.45 *d* (7.6)	
2	3.21 *t* (8.0)	3.23 *t* (7.9)	3.33 *t* (8.2)	
3	3.35 *m*	3.35 *m*	3.49 *t* (9.2)	
4	3.09 *m*	3.13 *t* (8.9)	4.65 *t* (9.8)	
5	3.35 *m*	3.30 *m*	3.60 *dd* (2.4, 5.2)	
6a	3.37 *m*	3.97 *dd* (6.1, 11.9)	3.82 *dd* (2.4, 11.9)	
6b	3.57 *dd* (3.4, 11.3)	4.11 *dd* (2.1, 11.9)	3.92 *dd* (5.2, 11.9)	
Rhamnose				
1	5.56 *d* (1.5)	5.56 *d* (1.5)	5.56 *d* (1.5)	5.65 *d* (1.5)
2	3.85 *br s*	3.85 *br s*	3.84 *br s*	3.5–4.1
3	3.64 *d* (8.9)	3.64 *dd* (3.1, 9.2)	3.63 *dd* (3.4, 9.5)	3.5–4.1
4	3.31 *m*	3.35 *m*	3.30 *t* (9.5)	3.5–4.1
5	3.43 *dd* (6.1, 9.5)	3.42 *dd* (6.1, 9.5)	3.42 *dd* (6.4, 9.5)	3.5–4.1
6(CH_3)	1.12 *d* (6.1)	1.12 *d* (6.1)	1.11 *d* (6.4)	1.20 *d* (6.2)
Acetyl I				
CH_3		1.73 *s*	1.75 *s*	
Acetyl II				
CH_3			2.01 *s*	

Coupling constants (J in Hz) in parentheses.

carbonyl absorption. The detailed structures of **1**, **2** and **3** were elucidated by FAB mass and NMR spectral measurements (Tables 1 and 2). The FAB mass spectra of **1**, **2** and **3** gave peaks at 595 m/z $[M+H]^+$ (Calcd for M^+; $C_{27}H_{30}O_{15}$ 594.16), 659 m/z $[M+Na]^+$ (Calcd for M^+; $C_{29}H_{32}O_{16}$ 636.17), and 679 m/z $[M+H]^+$ (Calcd for M^+; $C_{31}H_{34}O_{17}$ 678.18), respectively. These results indicate that **1** contains one molecule each of kaempferol, glucose and rhamnose, **2** has the same structure as **1** with an additional molecule of acetic acid, and **3** has the same structure as **1** with two additional acetic acid. By analysis of ^{1}H-^{1}H COSY spectrum of **3**, six proton signals of kaempferol were assigned as shown in Table 1. The proton signals of the sugar and acetyl units were determined by ^{1}H-^{1}H COSY and negative NOE difference (DIFNOE) spectra [7]. The ^{13}C signals of **3** were correlated with proton signals by HMQC spectrum [8], and assigned as shown in Table 2. Since the signals of an anomeric proton of glucose appeared at δ 5.45 with coupling constant $J = 7.6$ Hz, this glucose moiety must have a β-D-glucopyranose form. The chemical shifts of methylene protons (δ 3.82, *dd*, $J = 2.4$, 11.9 Hz and δ 3.92, *dd*, $J = 5.2$, 11.9 Hz) and H-4 proton (δ 4.65, *t*, $J = 9.8$ Hz) of the glucose moiety were shifted to the lower magnetic field supporting the acetylation of the 6- and 4-hydroxyls. The anomeric proton of rhamnose moiety was observed at δ 5.56 (*d*, $J = 1.5$ Hz) and the results of NOE experiments in the rhamnose moiety showed it to have the α-L-rhamnopyranose configuration.

In order to determine the attachments and/or positions of the sugar and acyl units in **3**, DIFNOE spectra were measured. By irradiation at H-1 of rhamnose, strong NOEs were observed at H-6 and H-8 of kaempferol. Therefore, rhamnose is attached to the 7-hydroxyl of kaempferol through a glycosidic bond. However, by irradiation at H-1 of glucose moiety, no NOE was observed on the kaempferol positions, but NOEs of H-2, 3, 4 and 5 of glucose moiety were observed, indicating that the 3-hydroxyl of kaempferol is glycosylated with glucose. By analysis of the HMBC spectrum of **3**, the two long distance correlations between the carbonyl carbon (δ 169.8) of acetic acid I and H-6 (δ 3.82 and 3.92) of glucose, and the carbonyl carbon (δ 169.8) of acetic acid II and H-4 (δ 4.65) of glucose were observed, leading to the conclusion that the first acetic acid molecule was

Table 2. ^{13}C NMR data for flavonols **1–4** isolated from the flowers of *Delphinium formosum* (125.78 MHz, DMSO-d_6)

C	1	2	3	4*
Kaempferol				
2	156.7	157.1	157.5	160.8
3	133.5	133.3	133.1	137.4
4	177.6	177.5	177.6	177.4
5	160.9	160.8	161.0	161.9
6	99.4	99.4	99.6	100.0
7	161.6	161.6	161.8	163.2
8	94.5	94.6	94.8	95.7
9	156.0	156.0	156.1	157.5
10	105.7	105.5	105.7	106.1
1′	120.8	120.6	120.6	123.3
2′	131.0	130.9	131.1	131.0
3′	115.1	115.1	115.3	116.7
4′	160.9	160.8	160.5	160.8
5′	115.1	115.1	115.3	116.7
6′	131.0	130.9	131.1	131.0
Glucose A				
1	100.7	101.0	101.0	
2	74.2	74.1	74.2	
3	77.6	76.1	73.4	
4	69.8	69.8	70.6	
5	76.4	73.9	71.7	
6	60.8	62.7	61.8	
Rhamnose				
1	96.4	98.3	98.5	100.1
2	70.2	70.2	69.9	71.5
3	70.1	70.1	70.4	72.0
4	71.6	71.6	71.4	73.4
5	69.9	69.8	70.2	71.4
6	17.9	17.9	18.0	18.7
Acetyl I				
CO		169.8	169.8	
CH_3		20.2	20.3	
Acetyl II				
CO			169.8	
CH_3			21.0	

* Recorded at 100 MHz (δ values).

bonded to the 6-hydroxyl of the glucose and the second linked to the 4-hydroxyl. Therefore, **3** is determined to be kaempferol 3-*O*-(4″,6″-*O*-diacetyl-β-D-glucopyranoside)-7-*O*-α-L-rhamnopyranoside, which is a new flavonol glycoside [9–11].

The structures of **1** and **2** were elucidated by analysis of their NMR spectra in a similar manner to **3** (Table 1 and 2). The ^1H NMR spectrum of **2** was superimposable on that of **3** except for the signals of the glucose moiety (Table 1 and Fig. 1). Two characteristic protons of **2** being shifted to a lower magnetic field were assigned to be the methylene protons of glucose (δ 3.97 and 4.11) suggesting the 6-hydroxyl of

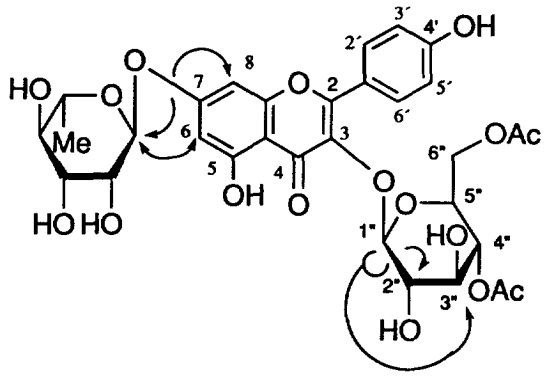

Fig. 1. Compound **3**. Main observed NOEs were indicated by arrows.

glucose is esterified with acetic acid. However, the H-4 of glucose was not shifted to a lower magnetic field (δ 3.13). Therefore, **2** is determined to be kaempferol 3-*O*-(6″-acetyl-β-D-glucopyranoside)-7-*O*-α-L-rhamnopyranoside. The ^1H NMR spectrum of **1** was also superimposable on that of **2** except for the signals of the methylene protons of glucose (δ 3.37 and 3.57) indicating that the 6-hydroxyl of glucose of **1** was free of acetic acid (Table 1). Thus, the structure of **1** is kaempferol 3-*O*-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside. The structures of **1** and **2** were confirmed by ^{13}C NMR spectral analysis (Table 2).

Compounds **1** and **2** have been found previously in *Equisetum telmateje* and *Equisetum silvaticum* [12, 13], but **2** is a very rare plant constituent [9].

EXPERIMENTAL

Mps were uncorr. The chemical shifts were given in δ values (ppm). NMR spectra were recorded on a VARIAN UNITY instrument (^1H at 400 MHz, ^{13}C at 100 MHz) and JEOL JNM GX-500 instrument (^1H at 500 MHz, ^{13}C at 125.78 MHz) in DMSO-d_6. FAB-MS spectra were recorded on a VG ANALYTICAL ZAB-2F spectrometer and JEOL JMS SX-102 spectrometer in glycerol matrix. Identification of flavonols was based on the standard methods of TLC, UV and NMR spectroscopy [5, 14]. HPLC was performed on an Inertsil ODS-2 column (4.6 × 250 mm) at 35° with a flow rate of 0.8 ml min^{-1} monitoring at 360 nm. A solvent system used was as follows: a linear gradient elution for 40 min from 25 to 85% B (1.5% H_3PO_4, 20% HOAc, 25% MeCN in H_2O) in A (1.5% H_3PO_4).

Plant material

The flowers of *Delphinium formosum* Boiss. *et* Huet. were collected in August, 1991 from the Santa Plateau of Gümüshane in Turkey. The dried flowers were stored in an airy place. The material was identified by Prof. Seckin Özden, Faculty of Pharmacy, Ankara University, Turkey and by Dr Kenjiro Toki, Lab-

oratory of Floriculture, College of Horticulture, Minami-Kyusyu University, Japan. A voucher specimen is available for inspection at the herbarium of Ankara University.

Extraction and isolation

Dried flowers were exhaustively extracted with MeOH. The dried extract was redissolved in H_2O and successively extracted with petrol, $CHCl_3$, Et_2O, EtOAc and *n*-BuOH. The dried Et_2O extract was dissolved in 2% $NaHCO_3$ soln to remove the plant acids and re-extracted with Et_2O. Compound **4** was crystallised from the concd. Et_2O extract, which also yielded **1**, **2**, and **3**. The purification of **1–3** was carried out using prep. TLC (Si gel; $CHCl_3$–MeOH–H_2O, 75:25:3) and HPLC (HCOOH solvent system).

Acid hydrolysis of glycosides: The glycoside dissolved in a minimum amount of 6% aq. HCl was refluxed for 1 h and the aglycone was extracted with EtOAc. The aq. soln was neutralized with powdered $BaCO_3$, filtered, the water was distilled off *in vacuo* and the residue was examined by TLC on cellulose. The identification of sugars was carried out by comparison with authentic sugar markers on cellulose plates using BAW (*n*-BuOH–HOAc–H_2O, 4:1:5) and *i*-PrOH–*n*–BuOH–H_2O (7:1:2). The acetylation of **1** was performed as described in the literature [14].

Kaempferol 3-β-glucoside-7-α-rhamnoside (**1**). Yellow amorphous powder, mp 238–40° (dec.) UV λ_{max} MeOH: 266, 349; +NaOMe: 267, 401; +$AlCl_3$: 274, 301, 353, 394; +$AlCl_3$+HCl: 274, 300, 349, 394; +NaOAc: 266, 356; +NaOAc+H_3BO_3: 266, 350 nm. TLC R_f values: $CHCl_3$–MeOH–H_2O (75:25:3) 0.28, EtOAc–MeOH–H_2O (100:13.5:10) 0.38. FAB-MS: *m/z* 617 $[M+Na]^+$, 595 $[M+H]^+$, 433 [M−glucosyl+H]$^+$, 287 [M−rhamnosyl−glucosyl+H]$^+$.

Kaempferol 3-(6″-acetyl-β-glucoside)-7-α-rhamnoside (**2**). Yellow amorphous powder, mp 216–18° (dec.). UV λ_{max} MeOH: 266, 349; +NaOMe: 245, 273, 392; +$AlCl_3$: 274, 302, 355, 395; +$AlCl_3$+HCl: 274, 301, 351, 391; +NaOAc: 267, 402; NaOAc+H_3BO_3: 267, 352 nm. TLC R_f values: $CHCl_3$–MeOH–H_2O (75:25:3) 0.43, EtOAc–MeOH–H_2O (100:13.5:10) 0.46. FAB MS: *m/z* 659 $[M+Na]^+$, 433 [M−acetylglycosyl+H]$^+$, 287 [M−rhamnosyl−acetylglucosyl+H]$^+$.

Kaempferol 3-(4″,6″-diacetyl-β-glucoside)-7-α-rhamnoside (**3**). Yellow amorphous powder, mp 225° (dec.). UV λ_{max} (nm) MeOH: 266, 349; +NaOMe: 244, 273, 406; +$AlCl_3$: 274, 301, 354, 398; +$AlCl_3$+HCl: 275, 299, 350, 398; +NaOAc: 267, 399; +NaOAc+H_3BO_3: 266, 352. TLC R_f values: $CHCl_3$–MeOH–H_2O (75:25:3) 0.61, EtOAc–MeOH–H_2O (100:13.5:10) 0.71. FAB-MS: *m/z* 679 $[M+H]^+$, 433, 287, 165, 153, 121.

Kaempferol 7-α-rhamnoside (**4**). Yellow amorphous powder, mp 228–30° (dec.). UV λ_{max} (nm) MeOH: 265, 323; +NaOMe: 246, 267, 424; +$AlCl_3$: 267, 354, 427; +$AlCl_3$+HCl: 267, 352, 427; +NaOAc: 275, 299, 349, 397; +NaOAc+H_3BO_3: 261, 375. FAB MS: *m/z* 433 $[M+H]^+$, 287 [M−rhamnosyl+H]$^+$.

Nona-acetyl kaempferol 3-glucoside-7-rhamnoside. Yellow amorphous powder, mp 134° (dec.). TLC R_f values: Ether–petrol (50:10) 0.27. FAB MS: *m/z* 973 $[M+H]^+$. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (2H, *d*, *J* = 8.8 Hz, H-2′, 6′), 7.47 (1H, *d*, *J* = 2.4 Hz, H-8), 7.32 (2H, *d*, *J* = 8.8 Hz, H-3′, 5′), 7.01 (1H, *d*, *J* = 2.4 Hz, H-6), 5.99 (1H, *br s*, H-1 of rha), 5.59 (1H, *d*, *J* = 7.8 Hz, H-1 of Gl), 5.41 (1H, *t*, *J* = 9.5 Hz, H-4 of Gl), 5.38 (1H, *br s*, H-2 of rha), 5.32 (1H, *dd*, *J* = 3.7, 10.3 Hz, H-3 of Gl), 5.00 (1H, *dd*, *J* = 5.1, 10.3 Hz, H-2 of Gl), 4.99 (1H, *dd*, *J* = 2.9, 9.8 Hz, H-3 of rha), 4.90 (1H, *t*, *J* = 9.3 Hz, H-4 of rha), 3.94 (1H, *m*, H-5 of rha), 3.91 (2H, *m*, H-5 and H-6a of Gl), 3.82 (1H, *d*, *J* = 10.0 Hz, H-6b of Gl), 2.36 (3H, *s*, acetyl), 2.31 (3H, *s*, acetyl), 2.16 (3H, *s*, acetyl), 2.06 (3H, *s*, acetyl), 2.05 (3H, *s*, acetyl), 1.98 (3H, *s*, acetyl), 1.96 (3H, *s*, acetyl), 1.95 (3H, *s*, acetyl), 1.83 (3H, *s*, acetyl), 1.10 (3H, *d*, *J* = 6.1 Hz, H-6 of rha).

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