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Flavonol glycosides from flowers of *Crocus speciosus* and *C. antalyensis*

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Abstract

From the flower extracts of *Crocus speciosus* and *C. antalyensis* nine flavonol glycosides have been isolated. One of these products is a new flavonol glycoside identified as kaempferol 3-*O*-α-(2,3-*di*-*O*-β-D-glucopyranosyl)rhamnopyranoside by UV, mass and NMR spectroscopy. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Crocus speciosus; *C. antalyensis*; Iridaceae; Flavonol glycosides; Kaempferol 3-*O*-α-(2,3-*di*-*O*-β-D-glucopyranosyl)rhamnopyranoside; ^{1}H; ^{13}C; 2D NMR

1. Introduction

In a chemotaxonomic study on the genus *Crocus*, we have isolated five new flavonol 3-*O*-α-(2-*O*-β-D-glucosyl)rhamnoside-7-*O*-β-D-glucosides (Nørbæk, Nielsen, & Kondo, in press). We now report on identification of a new modified kaempferol 3-*O*-α-(2-*O*-β-D-glucosyl)rhamnoside, which was linked by glucose at OH-3 of the rhamnose unit. Other investigations on the flavonoids of *Crocus* have been restricted to chromatographic methods. From *Crocus laevigatus*, *C. heuffelianus* and *C. aureus* some flavone and flavonol glycosides based on 6-hydroxyluteolin, scutellarein, scutellarein 7-methyl ether and kaempferol have been isolated; in addition the aglycones acacetin and tricin have been identified (Harborne & Williams, 1984). Isorhamnetin 3,4′-diglucoside has been isolated from *Crocus cv Sir John Bright* (Kuhn & Low, 1944) and a C-glycosylflavone has been isolated from *Crocus reticulatus* (Sergeyeva, 1977).

There is no evidence that the present nine flavonoids contribute to flower color.

It has previously been suggested that flavone and flavonol glycosides from three *Crocus* species give no contribution to yellow petal color which is probably based on carotenoids (Harborne & Williams, 1984). In addition anthocyanins have been found in *Crocus* species (Price, Robinson, & Robinson, 1938; Nørbæk & Kondo, 1998; Nørbæk & Kondo, 1999).

2. Results and discussion

Perianth segments of *Crocus speciosus* and *C. antalyensis* were extracted with aqueous acetonitrile containing 0.5% trifluoroacetic acid. Nine flavonoids were isolated by column chromatography on Amberlite XAD-7 with subsequent preparative HPLC. A new flavonol glycoside, kaempferol 3-*O*-α-(2,3-di-*O*-β-D-glucopyranosyl)rhamnopyranoside (**1**) was isolated, together with eight known flavonol glycosides, kaempferol 3-*O*-α-(2-*O*-β-D-glucopyranosyl)rhamnopyranoside (**2**) (Markham, Geiger, & Jaggy, 1992), 3-*O*-β-D-(2-*O*-α-rhamnopyranosyl)glucopyranosides of kaempferol (**3**) (Esperanza et al., 1998; Carotenuto et al., 1997) and

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Table 1
Analytical HPLC retention times (R_t) and FAB–MS of **1**–**9**. FAB–MS spectra were obtained in a positive mode using glycerol (1 drop of HCl aq. was added) as a matrix

Flavonoid	HPLC (R_t (min))	FAB–MS ($[M]^+$, fragment ions)
1	29.5	757 $[C_{33}H_{41}O_{20}]^+$, 595, 433, 287
2	31.9	595 $[C_{27}H_{31}O_{15}]^+$, 433, 287
3	24.7	595 $[C_{27}H_{31}O_{15}]^+$, 449, 287
4	25.2	625 $[C_{28}H_{33}O_{16}]^+$, 479, 317
5	21.6	611 $[C_{27}H_{31}O_{16}]^+$, 449, 287
6	15.6	627 $[C_{27}H_{31}O_{17}]^+$, 465, 303
7	19.0	611 $[C_{27}H_{31}O_{16}]^+$, 449, 287
8	17.0	627 $[C_{27}H_{31}O_{17}]^+$, 465, 303
9	44.8	449 $[C_{21}H_{21}O_{11}]^+$, 287

isorhamnetin (**4**) (Dandapani & Nagarajan, 1989; Vidal-Ollivier et al., 1989), 3-*O*-β-D-(2-*O*-β-D-glucopyranosyl)glucopyranosides of kaempferol (**5**) (Budzianowski, 1990) and quercetin (**6**) (Harborne, 1963), 3,4′-*di*-*O*-β-D-glucopyranosides of kaempferol (**7**) (Stein & Zinsmeister, 1990) and quercetin (**8**) (Krauze-Baranowska & Cisowski, 1996) and kaempferol 3-*O*-β-D-glucopyranoside (**9**) (Chaurasia & Wichtl, 1987; Stein & Zinsmeister, 1990).

The FAB (positive-ion mode) mass spectrum of **1** showed a quasi molecular ion peak at *m*/*z* 757 $[M+H]^+$ (Table 1). The fragments at *m*/z 595 $[(M+H)\text{-}162]^+$, *m*/*z* 433 $[(M+H)\text{-}162\text{–}162]^+$ and *m*/*z* 287 $[(M+H)\text{-}162\text{–}162\text{–}146]^+$ correspond to the subsequent losses of two hexose units and one deoxyhexose unit. Fragment at *m*/*z* 287 corresponds to kaempferol and this observation was supported by UV, ^1H and ^{13}C NMR spectra. The UV spectral analysis in the presence of customary shift reagents (see Section 3) showed the presence of free hydroxyl groups at position 5, 7 and 4′ of kaempferol (Mabry, Markham, & Thomas, 1970); hence a trisaccharide must be attached to hydroxyl group at position 3 of kaempferol.

^1H-NMR and/or ^{13}C-NMR spectra assigned by

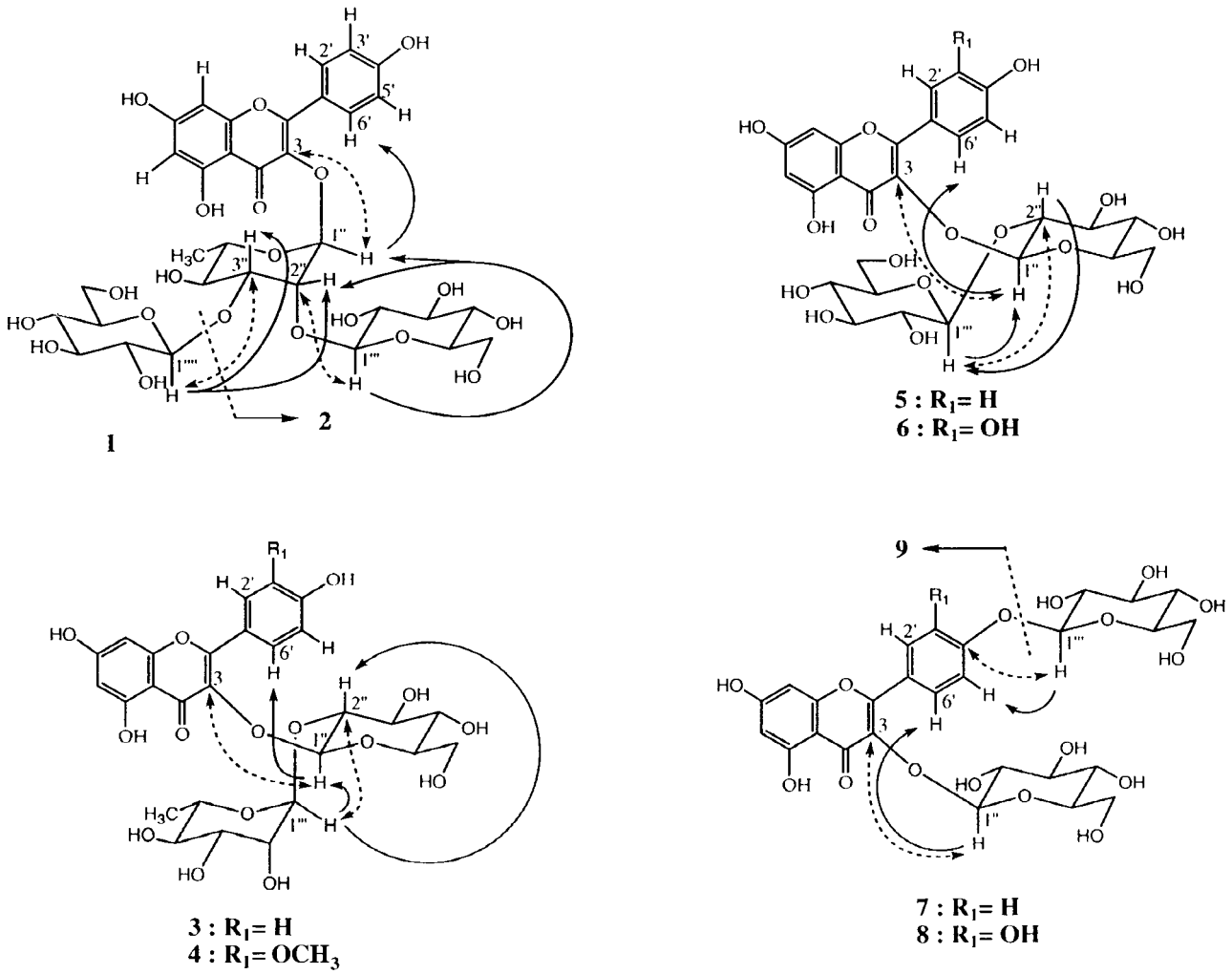

Fig. 1. ← NOE, ← - - → HMBC. Only important correlations was described.

Table 2
^{1}H-NMR spectral data of carbohydrate moieties of **1**–**9** in DMSO-d_6–10% TFA-*d*. Coupling constants (J in Hz) given in parentheses

	H	Sugar units at C-3		Sugar unit at C-4′	
		3-Glc	(2→1)Glc	(3→1)Glc	4′Glc
1	1	5.65 br s	4.51 d(7.8)	4.42 d(7.2)	
	2	4.46 m	3.17 t(9.0)	3.15 t(9.6)	
	3	3.71 m	3.21 t(9.0)	3.27 t(9.0)	
	4	3.39 t(9.0)	3.01 t(9.0)	3.09 t(9.0)	
	5	3.41 m	3.09 m	3.32 m	
	6	0.93 d(6.0)	3.53 m	3.58 dd(6.0;11.4)	
			3.53 m	3.79 m	
2	1	5.63 br s	4.31 d(7.8)		
	2	4.14 m	3.22 t(9.0)		
	3	3.60 dd(3.6;9.9)	3.20 t(9.0)		
	4	3.17 t(9.0)	3.06 t(9.0)		
	5	3.35 m	3.08 m		
	6	0.91 d(6.6)	3.53 m		
			3.53 m		
		3-Glc	(2 → 1)Rha		
3	1	5.65 d(7.8)	5.07 br s		
	2	3.42 t(9.0)	3.72 dd(4.2;9.6)		
	3	3.37 t(9.0)	3.46 dd(3.0; 9.6)		
	4	3.08 t(9.0)	3.12 t(9.0)		
	5	3.07 m	3.71 m		
	6	3.27 m	0.76 d(6.0)		
		3.53 m			
4	1	5.75 d(7.2)	5.03 br s		
	2	3.48 t(9.0)	3.71 dd(4.2; 9.6)		
	3	3.36 t(9.0)	3.42 dd(3.0; 9.3)		
	4	3.09 t(9.6)	3.12 t(9.0)		
	5	3.12 m	3.67 m		
	6	3.28 dd(4.8; 11.7)	0.65 d(6.6)		
		3.56 m			
		3-Glc	(2 → 1)Glc		
5	1	5.58 d(7.3)	4.62 d(7.3)		
	2	3.52 t(8.8)	3.21 t(8.8)		
	3	3.51 t(8.8)	3.25 t(8.8)		
	4	3.19 t(8.8)	3.12 t(8.8)		
	5	3.10 m	3.16 m		
	6	3.48 m	3.60 m		
		3.32 dd(4.4; 12.1)	3.49 dd(4.4; 12.1)		
6	1	5.67 d(7.2)	4.60 d(7.2)		
	2	3.52 t(8.4)	3.17 t(8.4)		
	3	3.49 t(8.4)	3.20 t(8.4)		
	4	3.15 t(8.4)	3.10 t(8.4)		
	5	3.08 m	3.14 m		
	6	3.28 m	3.54 m		
		3.29 dd(6.0; 12.0)	3.47 m		
7	1	5.47 d(7.2)			5.00 d(7.2)
	2	3.23 t(9.0)			3.25 t(9.0)
	3	3.18 t (9.6)			3.29 t(9.0)
	4	3.09 t (9.6)			3.18 t(9.0)
	5	3.10 m			3.37 m
	6	3.56 m			3.67 m
		3.33 m			3.46 dd(4.8; 11.7)
8	1	5.48 d(7.2)			4.84 d(7.8)

(continued on next page)

Table 2 (*continued*)

	H	Sugar units at C-3		Sugar unit at C-4′	
		3-Glc	(2→1)Glc	(3→1)Glc	4′Glc
	2	3.23 t(9.0)			3.29 t(9.0)
	3	3.22 t(9.6)			3.31 t(9.6)
	4	3.10 t(9.0)			3.17 t(9.0)
	5	3.11 m			3.36 m
	6	3.59 m			3.68 dd(6.0; 11.7)
		3.35 m			3.45 dd(6.0; 11.7)
9	1	5.39 d(7.2)			
	2	3.10 t(9.0)			
	3	3.21 t(9.0)			
	4	3.13 t(9.0)			
	5	3.24 m			
	6	3.54 m			
		3.34 dd(4.8; 12.0)			

HSQC and HMBC confirmed the aglycone of **1** as kaempferol (Section 3 and Tables 2 and 3). 1D-HOHAHA, ^{1}H–^{1}H-COSY and homodecoupling spectra of **1** showed the presence of two hexose units and one deoxyhexose unit. The signals at δ_H 5.65 (br s, H-1″), 4.46 (m, H-2″), 3.71 (m, H-3″), 3.39 (t, $J=9.0$ Hz, H-4″), 3.41 (m, H-5″) and 0.93 (d, $J=6.0$ Hz, H-6″) indicate existence of a rhamnopyranosyl unit in the trisaccharide. The signals of two other sugar units appeared at δ_H 4.51 (d, $J=7.8$ Hz, H-1‴) and 4.42 (d, $J=7.2$ Hz, H-1⁗), and 3.0–3.7 ($J_{2,3}=J_{3,4}=J_{4,5}=$ca 9.0 Hz). Thus, the remaining two sugar units must be β-D-glucopyranose.

The glycosidic linkages of **1** were determined by NOE difference spectra. By irradiation of H-1″, strong negative NOE was observed on H-1‴ and weaker on H-6′, but not on H-3″ and H-5″, indicating that rhamnose is directly linked to OH-3 of the flavonol and in α-configuration. By irradiation of H-1‴, strong negative NOEs appeared to H-2″ and H-1″ indicating that a glucosyl unit is linked to OH-2″. The β 1→3 linkage was confirmed by irradiation of H-1⁗ which showed a strong NOE effect on H-3″ and H-2″ (Fig. 1). Furthermore, C-3″ was lowfield-shifted by ca 10 ppm more than other glucosylrhamnosyl units (Table 3) (Markham et al., 1992). Finally the linkages of the sugar units were directly confirmed by HMBC since correlations were observed between C-3(δ_c 134.0) and H-1″(δ_H 5.65), C-2″(δ_c 78.6) and H-1‴(δ_H 4.51) and between C-3″(δ_c 80.5) and H-1⁗(δ_H 4.42) (Fig. 1 and Table 3). Thus, **1** were identified as kaempferol 3-*O*-α-(2,3-*di*-*O*-β-D-glucopyranosyl)rhamno-pyranoside, a new natural product.

FAB–MS of **2** and **3** showed $[M+H]^+$ at m/z 595 supporting the molecular formula $C_{27}H_{31}O_{15}$ but with different fragments corresponding to kaempferol 3-rhamnoside (m/z 433) and kaempferol 3-glucoside (m/z 449), respectively. In addition **2** and **3** showed different HPLC retention times (Table 1). ^{1}H NMR signals of **2** and **3** were assigned by using 1D-HOHAHA and ^{1}H–^{1}H-COSY. The anomeric proton signals of 3-*O*-glucosylrhamnosyl moiety of **2** appeared at δ_H 5.63 (br s, H-1″ of rhamnosyl) and δ_H 4.31 (d, $J=7.8$ Hz, H-1‴ of glucosyl) while the corresponding anomeric proton of glucosyl unit in the 3-*O*-rhamnosylglucoside moiety of **3** appeared at δ_H 5.65 (d, $J=7.8$ Hz, H-1″ of glucosyl) and H-1‴ of rhamnosyl appeared at δ_H 5.07. This indicates the difference in sugar sequence (Markham et al., 1992) and was confirmed by HSQC and HMBC (Fig. 1). The structures were established as kaempferol 3-*O*-α-(2-*O*-β-D-glucopyranosyl)rhamnopyranoside (**2**) and kaempferol 3-*O*-β-D-(2-*O*-α-rhamnopyranosyl)glucopyranoside (**3**), respectively (Fig. 1). All data were consistent with those previously reported (Markham et al., 1992; Carotenuto et al., 1997; Esperanza et al., 1998) (Tables 2 and 3).

Compound **4** gave the characteristic UV spectrum of isorhamnetin (Dandapani & Nagarajan, 1989). The $[M+H]^+$ was 30 mass units higher than that of **3**. Fragments were observed at m/z 479 [(M + H)-rhamnosyl]$^+$ and 317 [isorhamnetin]$^+$. The ^{1}H-NMR spectrum showed the expected signals of isorhamnetin in the aromatic region; the methoxy protons appeared at δ_H 3.85 (br s) (Section 3).

The ^{1}H and ^{13}C NMR spectra of the sugar units were almost identical to that of **3** and the structure was confirmed using our NMR methods (Tables 2 and 3). Thus, **4** is isorhamnetin 3-*O*-β-D-(2-*O*-α-rhamnopyranosyl)glucopyranoside (Fig. 1).

UV spectra and FAB–MS (Harborne, 1963; Budzianowski, 1990) of **5** and **6** suggested that these flavonoids may be diglucosides of kaempferol and quercetin, respectively (Table 1). ^{1}H NMR showed that all glucose units were β-linked to the other glucose unit or to the aglycone since $J_{1,2}$ was ca 7 Hz. The anomeric protons of **5** and **6** at δ 5.58 and 5.67

Table 3
^{13}C-NMR spectral data of **1**–**7** and **9** in DMSO-d_6–10% TFA-*d*. Assignments have been confirmed by 2D techniques (^{1}H–^{1}H COSY, HSQC or HMBC), but carbons having almost the same chemical shifts may be reversed. ^{13}C-NMR of **8** has not been measured because of limited amounts

C	1	2	3	4	5	6	7	9
2	157.0	157.5	156.5	156.4	156.3	156.7	156.0	156.4
3	134.0	135.0	133.2	132.9	132.8	133.5	134.2	133.3
4	177.6	178.1	177.7	177.6	177.5	177.9	177.9	177.4
5	161.0	161.5	161.4	161.3	160.9	161.6	161.4	161.0
6	98.6	99.0	98.9	98.9	98.4	98.5	100.3	98.4
7	164.0	164.5	164.3	164.3	163.8	164.4	164.5	163.9
8	93.6	94.1	94.0	93.9	93.3	93.8	94.0	93.4
9	156.5	157.0	156.7	156.7	156.1	156.0	156.9	156.4
10	104.5	104.6	104.5	104.4	103.9	104.5	104.5	104.0
1′	120.4	120.9	121.3	121.5	120.9	122.2	124.1	120.9
2′	130.5	131.0	131.1	113.8	130.6	116.5	130.9	130.7
3′	115.3	116.2	115.3	147.1	115.0	145.1	118.1	117.5
4′	160.0	160.4	160.2	149.5	159.6	148.8	159.6	159.8
5′	115.3	116.2	115.3	115.4	115.0	115.8	118.1	117.5
6′	130.5	131.0	131.1	122.1	130.6	121.6	130.9	130.7
OMe				55.9				
Sugar at C-3								
1″	100.4	101.3	98.7	98.7	98.4	99.0	101.2	101.2
2″	78.6	81.7	78.0	78.0	81.8	83.1	73.5	74.0
3″	80.5	70.5	77.7	77.6	76.0	76.9	76.6	76.1
4″	69.4	72.0	70.9	70.8	69.4	69.9	69.8	69.6
5″	69.3	70.8	77.5	77.3	76.5	77.8	77.4	77.0
6″	17.2	17.6	61.1	60.7	60.5	61.0	60.9	60.6
1‴	104.5	106.5	101.0	101.0	103.5	104.4		
2‴	69.7	69.6	70.5	70.3	73.9	74.6		
3‴	75.7	76.5	70.8	70.8	76.0	76.8		
4‴	73.7	74.1	72.2	72.0	69.1	69.8		
5‴	76.4	76.9	68.7	68.6	76.4	77.1		
6‴	60.5	60.8	17.5	17.2	60.2	60.9		
1⁗	104.1							
2⁗	73.7							
3⁗	76.4							
4⁗	69.9							
5⁗	76.8							
6⁗	60.8							
Sugar at C-4′								
1‴							99.0	
2‴							74.4	
3‴							76.7	
4‴							70.1	
5‴							77.8	
6‴							61.1	

were shift-correlated with signals of C-3 at δ 132.8 and 133.5, respectively. The anomeric protons signals at δ 4.62 and 4.60 correlated unambiguously with C-2″ at δ 81.8 and 83.1, respectively, indicating β 1 → 2 linkage. These results were consistent with NOE difference spectra, since NOEs between H-1″ and H-6′ and between H-1″ and H-1‴, were observed (Fig. 1). Thus, **5** and **6** are 3-*O*-β-D-(2-*O*-β-D-glucopyranosyl)glucopyranosides of kaempferol and quercetin, respectively (Fig. 1).

FAB–MS suggested that **7** and **8** were diglucosides of kaempferol and quercetin, respectively (Table 1). ^{1}H NMR showed that the hexoses were β-linked glucopyranose because all vicinal coupling constants were 7.2–9.6 Hz. Connection was directly determined by NOE difference and by HSQC and HMBC (compound **7** only). UV data were similar to those reported (Stein & Zinsmeister, 1990; Krauze-Baranowska & Cisowski, 1996). Thus, **7** and **8** were determined to be 3,4′-di-*O*-β-D-glucopyranosides of kaempferol and quercetin, respectively (Fig. 1).

Compound **9** was kaempferol 3-*O*-β-D-glucopyranoside according to UV (Vidal-Ollivier et al., 1989; Harborne, 1963), FAB–MS, ^{1}H NMR and ^{13}C NMR data (Tables 1–3). Correlations found by NOE difference, HSQC and HMBC spectra are shown in Fig. 1.

3. Experimental

3.1. Plant material

Field grown flowers of the *Crocus speciosus* and *C. antalyensis* were collected in Noordwijk, Holland, in March 1996 and identified by Professor N. Jacobsen, Royal Veterinary and Agricultural University, Copenhagen.

3.2. Isolation of flavonoids

Freeze-dried perianth segments of *speciosus* (50 g) and *C. antalyensis* (40 g) were extracted with 50% aq. CH_3CN containing 0.5% TFA at room temp. for 1 h. The conc extracts were adsorbed on an Amberlite XAD-7 column, washed with 0.5% TFA aq. soln and then eluted stepwise from 4 to 20% aq. CH_3CN containing 0.5% TFA. The flavonoids were further purified by prep. ODS-HPLC (20ϕ × 250 mm, Develosil ODS-HG-5, Nomura Chemicals) in the same solvent system; flow rate 7 ml min^{-1}; monitoring at 280 nm. Evaporation of solvents *in vacuo* gave TFA salts which were stored at −80°. From *C. speciosus* **1** (15 mg) and **2** (15 mg) were obtained; from *C. antalyensis* **3** (15 mg), **4** (10 mg), **5** (10 mg), **6** (60 mg), **7** (17 mg), **8** (7 mg) and **9** (10 mg) were isolated.

3.3. Analysis of flavonoids

About 1 g of the freeze-dried perianth segments of *Crocus speciosus* and *C. antalyensis* were extracted with 13 ml 50% aq. CH_3CN containing 3.0% TFA. After filtration the extract was analyzed by ODS-HPLC (4.6ϕ × 250 mm, Develosil ODS-HG-5, Nomura Chemicals) at 40°, monitoring on a 3D diode-array detector at 280–360 nm. Solvent A (H_2O–TFA, 99:1) and solvent B (CH_3CN–H_2O–TFA, 60:140:1) were used in elution profile: 0 min 16% B, 3 min 38%

B, 10 min 44% B, 20 min 50% B, 25 min 67% B, 40–50 min 100% B; flow rate 1.5 ml min^{-1}.

3.4. Kaempferol 3-O-α-L-(2,3-di-O-β-D-glucopyranosyl)rhamnopyranoside (1)

UV λ_{max} (nm): 268, 315 sh, 347; +NaOH: 272, 326, 409; +$AlCl_3$: 269, 305 sh, 348, 397; +$AlCl_3$+HCl: 269, 302 sh, 349, 392; +NaOAc: 279, 398; +NaOAc+H_3BO_3: 268, 352. ^{1}H NMR (aglycone): δ 7.83 (2H, d, J=9.0 Hz, H-2′, H-6′), 6.97 (2H, d, J=9.0 Hz, H-3′, H-5′), 6.46 (1H, d, J=1.8 Hz, H-8), 6.26 (1H, d, J=2.4 Hz, H-6).

3.5. Kaempferol 3-O-α-L-(2-O-β-D-glucopyranosyl)rhamnopyranoside (2)

UV data similar to that of **1**. ^{1}H NMR (aglycone): δ 7.81 (2H, d, J=8.4 Hz, H-2′, H-6′), 6.97 (2H, d, J=8.4 Hz, H-3′, H-5′), 6.46 (1H, d, J=1.8 Hz, H-8), 6.26 (1H, d, J=2.4 Hz, H-6).

3.6. Kaempferol 3-O-β-D-(2-O-α-rhamnopyranosyl)glucopyranoside (3)

UV data similar to that of **1**. ^{1}H NMR (aglycone): δ 8.01 (2H, d, J=9.0 Hz, H-2′, H-6′), 6.86 (2H, d, J=9.0 Hz, H-3′, H-5′), 6.41 (1H, d, J=2.4 Hz, H-8), 6.19 (1H, d, J=2.4 Hz, H-6).

3.7. Isorhamnetin 3-O-β-D-(2-O-α-rhamnopyranosyl)glucopyranoside (4)

^{1}H NMR (aglycone): δ 7.95 (1H, d, J=2.4 Hz, H-2′), 7.47 (1H, dd, J=2.4; 9.0 Hz, H-6′), 6.89 (1H, d, J=9.0 Hz, H-5′), 6.43 (1H, d, J=2.4 Hz, H-8), 6.20 (1H, d, J=1.8 Hz, H-6), 3.85 (3H, br s, OMe).

3.8. Kaempferol 3-O-β-D-(2-O-β-D-glucopyranosyl)glucopyranoside (5)

^{1}H NMR (aglycone): δ 7.99 (2H, d, J=8.8 Hz, H-2′, H-6′), 6.88 (2H, d, J=8.8 Hz, H-3′, H-5′), 6.19 (1H, d, J=2.2 Hz, H-8), 6.16 (1H, d, J=2.2 Hz, H-6).

3.9. Quercetin 3-O-β-D-(2-O-β-D-glucopyranosyl)glucopyranoside (6)

^{1}H NMR (aglycone): δ 7.58 (1H, d, J=2.3 Hz, H-2′), 7.45 (1H, m, H-6′), 6.87 (1H, d, J=7.8 Hz, H-5′), 6.39 (1H, d, J=1.8 Hz, H-8), 6.17 (1H, d, J=2.4 Hz, H-6).

3.10. Kaempferol 3,4′-di-O-β-D-glucopyranoside (7)

^{1}H NMR (aglycone): δ 8.10 (2H, d, J=9.0 Hz, H-2′, H-6′), 7.14 (2H, d, J=9.0 Hz, H-3′, H-5′), 6.44 (1H, d, J=1.8 Hz, H-8), 6.21 (1H, d, J=1.8 Hz, H-6).

3.11. Quercetin 3,4′-di-O-β-D-glucopyranoside (8)

^{1}H NMR (aglycone): δ 7.64 (1H, d, J=1.8 Hz, H-2′), 7.60 (1H, dd, J=1.8; 10.8, H-6′), 7.19 (1H, d, J=9.0 Hz, H-5′), 6.43 (1H, d, J=1.8 Hz, H-8), 6.20 (1H, d, J=2.4 Hz, H-6).

3.12. Kaempferol 3-O-β-glucopyranoside (9)

^{1}H NMR (aglycone): δ 7.98 (2H, d, J=9.0 Hz, H-2′, H-6′), 6.85 (2H, d, J=9.0 Hz, H-3′, H-5′), 6.38 (1H, d, J=1.8 Hz, H-8), 6.18 (1H, d, J=2.4 Hz, H-6).

FAB–MS spectra were obtained in a positive mode using glycerol (1 drop of HCl aq. was added) as a matrix.

NMR: 600 MHz (JNM alpha 600, JEOL) (^{1}H, ^{1}H–^{1}H-COSY, 1D-HOHAHA, homodecoupling, NOE difference, ^{13}C, HSQC and HMBC) in DMSO-d_6–10%TFA-d; CD_2HOD (3.326 ppm) int. standard. 1D HOHAHA, homodecoupling and 2D spectra were obtained using a pulse sequence supplied from JEOL.

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References

Budzianowski, J. (1990). *Phytochemistry*, *29*, 3643.
Carotenuto, A., Fattorusso, E., Lanzotti, V., Magno, S., De Feo, V., & Cicala, C. (1997). *Phytochemistry*, *44*, 949.
Chaurasia, N., & Wichtl, M. (1987). *Planta Medica*, *53*, 432.
Dandapani, M., & Nagarajan, S. (1989). *Indian Journal of Chemistry*, *28B*, 606.
Esperanza, J. C. G., Luyengi, L., Lee, S. K., Zhu, L. F., Zhou, B. N., Fong, H. H. S., Pezzuto, J. M., & Kinghorn, A. D. (1998). *Journal of Natural products*, *61*, 706.
Harborne, J. B. (1963). *Experientia*, *19*, 7.
Harborne, J. B., & Williams, C. A. (1984). *Zeitschrift fur Naturforschung*, *39c*, 18.
Krauze-Baranowska, M., & Cisowski, W. (1996). *Polish Journal of Chemistry*, *70*, 430.
Kuhn, R., & Low, I. (1944). *Chemische Berichte*, *77*, 196.
Mabry, T. J., Markham, K. R., & Thomas, M. B. (1970). *The Systematic Identification of Flavonoids*. Heidelberg: Springer.
Markham, K. R., Geiger, H., & Jaggy, H. (1992). *Phytochemistry*, *31*, 1009.

Nørbæk, R., & Kondo, T. (1998). *Phytochemistry*, *47*, 861.
Nørbæk, R. & Kondo, T. (1999) *Phytochemistry, 50,* 325.
Nørbæk, R., Nielsen, J. K., & Kondo, T. *Phytochemistry* (in press).
Price, J. R., Robinson, G. M., & Robinson, R. (1938). *J. Chem. Soc*., 281.
Sergeyeva, N. Y. (1977). *Khim. Prir. Soedin*, 124.
Stein, W., & Zinsmeister, H. D. (1990). *Zeitschrift fur Naturforschung*, *45C*, 25.
Vidal-Ollivier, E., Elias, R., Faure, F., Babadjamian, A., Crespin, F., Balansard, G., & Boudon, G. (1989). *Planta Medica*, *55*, 73.