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ACYLATED FLAVONOIDS FROM *BLEPHARIS CILIARIS*

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Key Word Index—*Blepharis ciliaris*; Acanthaceae; aerial parts; flavone; flavanone; apigenin 7-*O*-(3″-acetyl-6″-*E*-*p*-coumaroyl-β-D-glucopyranoside); naringenin 7-*O*-(3″-acetyl-6″-*E*-*p*-coumaroyl-β-D-glucopyranoside); electrospray MS; two-dimensional NMR.

Abstract—From the ethanolic extract of aerial parts of *Blepharis ciliaris* one novel flavone, apigenin 7-(3″-acetyl-6″-*E*-*p*-coumaroylglucoside), and one novel flavanone, naringenin 7-(3″-acetyl-6″-*E*-*p*-coumaroylglucoside), were isolated. Their structures were elucidated mostly based on electrospray mass spectrometry and homo- and hetero-nuclear two-dimensional NMR techniques. No flavonoid has previously been identified with aliphatic and aromatic acyl groups on the same monosaccharide in the 3- and 6-positions, respectively. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The genus *Blepharis* (Acanthaceae) is represented in Egypt and Saudi Arabia only by *B. ciliaris* L. [1]. The plant extract and also the crushed roasted seed exhibit significant antibacterial activity [2, 3], and have been reported to have several medical applications [4]. It has previously not been examined for flavonoids, however, seeds of *B. sindica* have been reported to contain apigenin, apigenin 7-*O*-(6″-*p*-coumaroylglucoside) and naringenin 7-*O*-(6″-*p*-coumaroylglucoside) [5]. The present paper describes the isolation and structure elucidation of two novel acylated flavonoid glycosides **1** and **2** from *B. ciliaris*.

RESULTS AND DISCUSSION

The two flavonoid glycosides, compounds **1** and **2**, were obtained by column fractionation (silica gel) of a 95% ethanolic extract of powdered air-dried aerial parts of *B. ciliaris*. In order to identify the aglycones, the sugar and acyl moieties, and to elucidate the points of attachment between the units, it was necessary to apply mass spectrometry experiments and several NMR techniques in tandem.

The number of carbon resonances of compound **1** was determined to be 28 by a coupling modulated spin echo NMR experiment (SEFT). The SEFT experiment also indicated the number of protons attached on each carbon [6]. The 4H AA′XX′ system at δ 7.93 (*d*, H-2′,6′) and δ 6.91 (H-3′,5′), the 2H *meta*-coupled AX system at δ 6.49 (*d*, H-6) and δ 6.83 (H-8) together with the 1H singlet at δ 6.82 (H-3) were in accordance with an apigenin aglycone [7]. The assignment of the aglycone carbon signals found in the SEFT spectrum were performed using the one-bond (HSC) and long-range (COLOC) heteronuclear shift correlation experiments (Table 1). The proton signal at δ 12.97 belonging to the 5-hydroxyproton showed an unsubstituted 5-position on the aglycone [7].

The spectral region between δ 60 and δ 80 in the SEFT spectrum of compound **1** showed five resonances which, together with the anomeric carbon, identified one hexose. The sugar protons were assigned using the anomeric ^{1}H signal as the entry point in the two-dimensional double quantum filtered homonuclear correlation experiment (DQF-COSY). The shift values of the corresponding sugar carbons were found using the one-bond HSC experiment (Table 1). The large ^{1}H–^{1}H coupling constants of the sugar moiety (Table 2) showed that all the sugar ring protons were positioned di-axial to each other in accordance with a β-D-glucopyranoside in a $^4C_1$ chair conformation [8].

The 3H singlet at δ 2.06 found in the one-dimensional ^{1}H spectrum, together with its two-bond correlation (COLOC) to the carbonyl carbon signal at δ 169.71 (Fig. 1) were in agreement with the resonances of an acetyl group. The 2H AX system at δ 6.33 (*d*, H-α) and δ 7.49 (H-β) together with the 4H AA′XX′ system at δ 7.38 (*d*, H-2‴,6‴) and δ 6.68 (H-3‴,5‴) identified the other acyl group as *p*-coumaroyl. The large coupling

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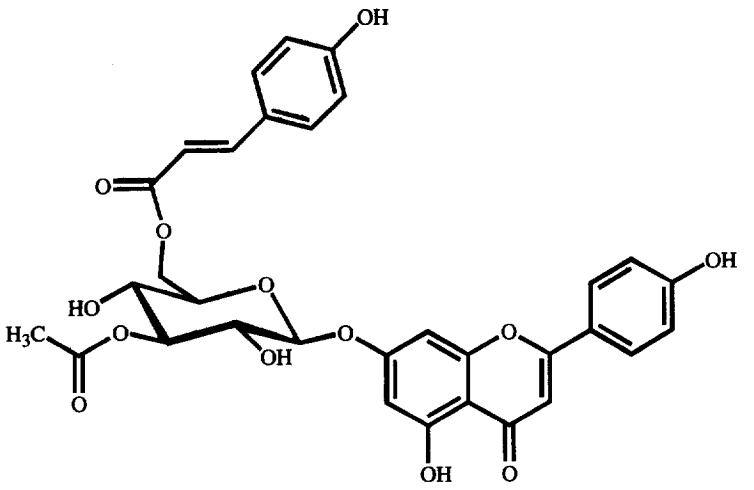

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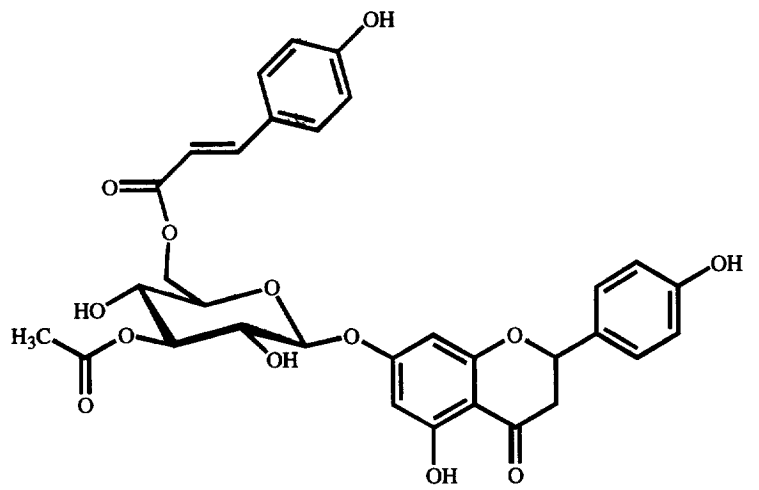

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constant between the H-α and H-β ($J = 15.9$ Hz) showed the *E*-configuration for the double bond.

The COLOC cross peak at δ 5.33/162.38 (H-1″/C-7) together with the NOESY cross peaks between the glucose H-1″ and both H-6 and H-8 on the aglycone (Fig. 1), revealed the connection point between the sugar moiety and the aglycone to be at C-7. To assign the points of attachment of the two acyl groups, their respective carbonyl signals were used as entry points in the COLOC spectrum. The carbonyl signal at δ 169.71 showed a correlation to the acetyl protons at δ 2.06 and to the H-3″ of the glucosyl unit (δ 4.96), while the carbonyl signal at δ 166.36 showed a correlation with the *p*-coumaroyl H-β (δ 7.49) and to H-6″A (δ 4.44) of the glucosyl unit (Fig. 1). The acylation pattern was also supported by the observation in the DQF-COSY spectrum of only two proton signals (2-OH and 4-OH) representing the sugar hydroxyl groups.

The mass spectrum of compound **1** showed a $[M + H]^+$ of *m/z* 619 and a fragment ion of *m/z* 269. This compound was thus identified as the novel flavone, apigenin 7-*O*-(3″-acetyl-6″-*E*-*p*-coumaroyl-β-D-glucopyranoside).

The one-dimensional ^{1}H spectrum of compound **2** showed many similarities with the corresponding spectrum of compound **1**. However, the change in the shift value of H-2′,6′ (δ 7.28), the lack of a singlet around δ 6.8 together with the appearance of three new signals at δ 5.45 (1H, *dd*, H-2), δ 3.33 (1H, *dd*, H-3A) and 2.71 (1H, *dd*, H-3B) showed compound **2** to be the flavanone analogue of apigenin, naringenin [7]. The ^{1}H, ^{13}C and $^3J_{(HH)}$ values of the sugar moiety were in accordance with a β-D-glucopyranoside, while the two acyl groups of compound **2** were identified as acetyl and *p*-*E*-coumaroyl, respectively (Tables 1 and 2).

The resonance at δ 12.07 in the ^{1}H spectrum of

Table 1. The ^{13}C data of the 7-*O*-(3″-acetyl-6″-*p*-*E*-coumaroyl-β-D-glucopyranoside) of apigenin (**1**) and naringenin (**2**)

	1		**2**	
Position	δ ^{13}C	SEFT*	δ ^{13}C	SEFT*
2	164.3	↑	78.6	↓
3	103.0	↓	42.0	↑
4	181.9	↑	197.3	↑
5	161.4	↑	163.0	↑
6	99.4	↓	96.3	↓
7	162.4	↑	164.7	↑
8	94.8	↓	95.5	↓
9	156.9	↑	157.8	↑
10	105.5	↑	103.4	↑
1′	120.9	↑	128.6	↑
2′,6′	128.5	↓	128.4	↓
3′,5′	116.0	↓	115.2	↓
4′	161.2†	↑	162.7	↑
β-D-glucopyranoside				
1″	99.0	↓	98.7	↓
2″	70.9	↓	70.8	↓
3″	77.1	↓	77.0	↓
4″	67.8	↓	67.6	↓
5″	73.5	↓	73.4	↓
6″	63.0	↑	62.8	↑
6″-*p*-*E*-coumaroyl				
COO	166.4	↑	166.4	↑
α	113.7	↓	113.8	↓
β	145.0	↓	145.0	↓
1‴	124.9	↑	125.0	↑
2‴,6‴	130	↓	130.3	↓
3‴,5‴	115.6	↓	115.8	↓
4‴	159.8†	↑	159.9	↑
3″-Acetyl				
COO	169.7	↑	169.7	↑
CH_3	21.1	↓	21.1	↓

*SEFT = coupling modulated spin echo. Arrows are showing the phase: ↑ corresponds to C_q and CH_2; ↓ corresponds to CH and CH_3.

†Assignments may be interchanged.

compound **2** again indicated an unsubstituted 5-OH. The correlation in the inverse heteronuclear multiple-bond correlation (HMBC) spectrum between the H-1″ and C-7, showed the linkage point between the sugar and the aglycone. Because of the correlations between the carbonyl carbon of the acetyl moiety and both H-3″ (δ 4.92) and the acetyl protons (δ 2.05), the acetyl unit was placed in the 3″-position of the glucosyl unit. The carbonyl carbon in the *p*-*E*-coumaroyl moiety showed correlations with four protons; H-α (δ 6.36), H-β (δ 7.52), H-6″A (δ 4.39) and H-6″B (δ 4.17) revealing the 6″-position of the glucosyl unit as the linkage point.

The mass spectrum of compound **2** showed a $[M + H]^+$ of *m/z* 621 and a fragment ion of *m/z* 271. Thus compound **2** was identified as the novel compound naringenin 7-*O*-(3″-acetyl-6″-*E*-*p*-coumaroyl-β-D-glucopyranoside).

Acylation of the 3-position of a sugar moiety is very rare in flavonoids [9–12], and is reported here for the first time for a naringenin derivative. The identification of the 7-*O*-(3″-acetyl-6″-*E*-*p*-coumaroyl-β-D-glucopyranosides) of apigenin and naringenin is, to our knowledge, the first report of flavonoids acylated with aliphatic and aromatic acyl groups on the same monosaccharide in the 3- and 6-positions, respectively.

EXPERIMENTAL

Plant material. Blepharis ciliaris L. was collected in October 1993 and May 1994 from the Qassim province, Saudi Arabia. The identification of the plant was confirmed by the Botany Department, College of Science, King Saud University. Voucher specimens are deposited in the Department of Veterinary Medicine, King Saud University, Saudi Arabia and in the Department of Pharmacognosy, College of Pharmacy, University of Alexandria, Egypt.

Extraction and isolation. Powdered air-dried aerial parts of the plant (80 g) were extracted several times with 95% EtOH at room temp. The residue (6 g) was chromatographed by CC on silica gel (E. Merck, 170 g,

Table 2. The ^{1}H shift values and the ^{1}H–^{1}H coupling constants of the 7-*O*-(3″-acetyl-6″-*p*-*E*-coumaroyl-β-D-glucopyranoside) of apigenin (**1**) and naringenin (**2**)

	1		**2**	
Position	δ ^{1}H	$J_{(HH)}$ (Hz)	(δ ^{1}H)	$J_{(HH)}$ (Hz)
2			5.45	2.7; 12.4
3A	6.82		3.33	12.4; 17.3
3B			2.71	3.3; 17.3
5-OH	12.97		12.07	
6	6.49	2.1	6.20	2.2
8	6.83	2.1	6.16	2.2
2′,6′	7.93	8.8	7.28	8.7
3′,5′	6.91	8.8	6.77	8.7
β-D-glucopyranoside				
1″	5.33	7.7	5.24	7.6
2″	3.51	7.7; 9.3	3.46	7.6; 9.2
2″-OH	5.72		5.67	
3″	4.96	9.3	4.92	9.2; 9.2
4″	3.48	9.3; 9.1	3.45	9.2; 9.2
4″-OH	5.59		5.57	
5″	3.98	u	3.92	u
6″A	4.44	u	4.39	u
6″B	4.20	12.0; 6.7	4.17	11.9; 6.0
6″-*p*-*E*-coumaroyl				
α	6.33	15.9	6.36	16
β	7.49	15.9	7.52	16
2‴,6‴	7.38	8.6	7.50	8.7
3‴,5‴	6.68	8.6	6.78	8.7
3″-Acetyl				
CH_3	2.06		2.05	

u = Unresolved.

40–63 μm) packed in hexane. Elution started with pure hexane followed by increasing amounts of EtOAc (0–100%). The flavone (**1**) and the flavanone (**2**) were eluted in different frs with 80% EtOAc in hexane, and then crystallized (26 and 271 mg, respectively) from the same solvent.

TLC. Analyt. TLC was carried out on microcrystalline cellulose (F1440/LC254, Schleicher & Schüll)

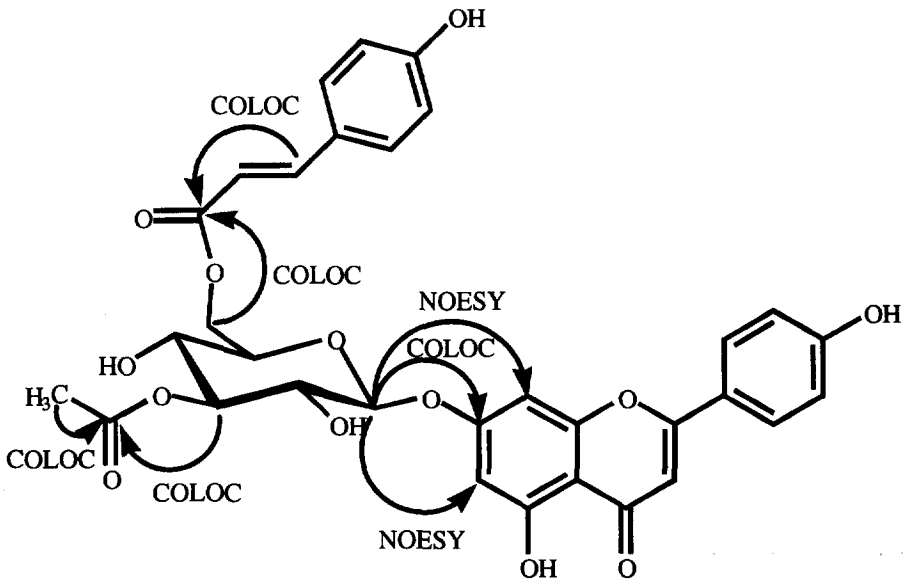

Fig. 1. The most important correlations in the following two-dimensional experiments on apigenin 7-*O*-(3″-acetyl-6″-*E*-*p*-coumaroyl-β-D-glucopyranoside): COLOC, the heteronuclear long-range correlations with arrow heads positioned at the carbon atoms; NOESY, the ^{1}H–^{1}H NOE correlations.

with the solvents 15% HOAc (A) and BAW (*n*-BuOH–HOAc–H_2O, 4:1:5, upper phase) (B) as eluents. Spray reagent: 1:1 mixture of 1% diphenyl–boric acid–ethanolamine complex in MeOH (Naturstoffreagenz A) and 5% polyethyleneglycol 4000 in EtOH. TLC R_f: 0.01 (A), 0.81 (B) for **1**; 0.07 (A), 0.85 (B) for **2**; 0.17 (A), 0.49 (B) for apigenin 7-glucoside and 0.56 (A), 0.35 (B) for quercetin 3-rutinoside.

Spectroscopy. The NMR experiments were obtained at 25° at 400.13 MHz and 100.62 MHz for ^1H and ^{13}C, respectively, on a Bruker DMX-400 instrument, and at 600.13 MHz and 150.92 MHz for ^1H and ^{13}C, respectively, on a Bruker DRX-600 instrument and at 599.92 MHz and 150.86 MHz for ^1H and ^{13}C, respectively, on a Varian Unity-600 instrument. The residual ^1H signal of the solvent (DMSO-d_6) and the ^{13}C signal were used as secondary reference (δ 2.49 and δ 39.5 from TMS, respectively). The experiments were performed using a 5 mm ^{13}C–^1H dual probe (DMX-400), or a 5 mm broad-band probe (DRX-600). The HMBC experiment on DRX-600 was performed on a 5-mm inverse gradient probe. The data in the two-dimensional experiments were collected with 2K complex data points. A sweep width of δ 13.5–1.5 for the ^1H-axis were chosen, while a ^{13}C sweep width of δ 200–10 and δ 150–10 were used for the long-range and the one-bond correlation experiments, respectively. The mass spectra were obtained on a Fisons MD 800 LCD-EI instrument with electrospray (negative mode) interface using MeOH–H_2O (1:1 (v/v) adjusted to pH 6.7 with ammoniumacetate buffer) as the mobile phase. Shift reagents for UV spectral analysis were prepared and used according to Markham [13].

Apigenin 7-O-(3″-acetyl-6″-E-p-coumaroyl-β-D-glucopyranoside) (**1**). Mp 223–225°. UV λ_{MeOH} 269, 317, 335sh; +NaOMe 245sh, 264sh, 310sh, 371; +$AlCl_3$ 277, 299, 320, 348sh, 382; +$AlCl_3$/HCl 277, 299, 320, 348sh; +NaOAc 266, 320sh, 379; NaOAc/H_3BO_3 268, 319.

Naringenin 7-O-(3″-acetyl-6″-E-p-coumaroyl-β-D-glucopyranoside) (**2**). Mp 154–156°. UV λ_{MeOH} 285, 313; +NaOMe 239sh, 284, 307sh, 360; +$AlCl_3$ 282sh, 307, 327sh, 379sh; +$AlCl_3$/HCl 282sh, 307, 327sh, 379sh; +NaOAc 285, 318sh, 360sh; NaOAc/H_3BO_3 285, 312.

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