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NMR SPECTRAL ANALYSIS OF FLAVONOIDS FROM *Chrysanthemum coronarium*

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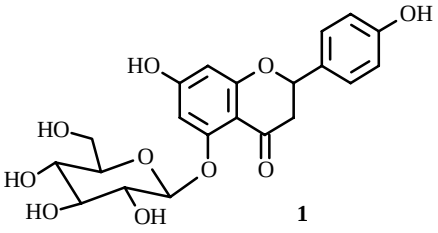
Naringenin 5-O-glucoside, apigenin 7-O-glucoside, luteolin 7-O-glucoside, kaempferol 3-O-glucoside, quercetin 3-O-glucoside, apigenin, luteolin, kaempferol, and quercetin, nine flavonoid derivatives, were isolated for the first time from the aqueous methanolic extract of the aerial parts of Chrysanthemum coronarium. *Their structures were elucidated on the basis of chemical and spectroscopic (UV, ^{1}H, ^{13}C NMR) analyses. 1- and 2-dimensional NMR spectroscopy of the rare naringenin 5-O-glucoside have been recorded and assigned for the first time. The flavonoid glucosides from* Chrysanthemum coronarium *showed week activity against Poliovirus I and Adenovirus type 7.*

Key words: naringenin, NMR, *Chrysanthemum coronariu*m, antiviral activity.

Chrysanthemum coronarium L. (Compositae) is regarded in East Asia as a healthy vegetable because the edible portion contains abundant nutrients [1] as well as a fresh flavor [2]. This species is occasionally used as folk medicine [3] and has been found to have biologically active substances [4–7]. The elucidation of the bioactive substances from this plant is of a great importance to increase its vegetable value. It is used in Egyptian traditional medicine [8]. It was reported that the plant contained terpenes [4], sterols, polyacetylenes [6], anthraquinones [9], and flavonoids [9, 10]. In the course of our continuous studies on this plant, naringenin 5-*O*-glucoside (**1**) [11, 12] as a rare flavanone glucoside was isolated with two flavone glucosides, apigenin 7-*O*-*β*-glucoside (**2**) and luteolin 7-*O*-*β*-glucoside (**3**); two aglycones, apigenin (**4**) and luteolin (**5**); two flavonol glucosides, kaempferol 3-*O*-*β*-glucoside (**6**) and quercetin 3-*O*-*β*-glucoside (isoquercitrin) (**7**) [11, 13]; and two aglycones, kaempferol (**8**) and quercetin (**9**).



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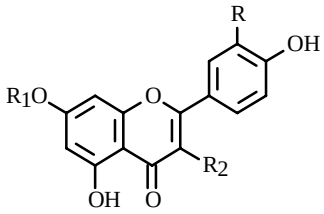

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2: R = R_2 = H, R_1 = Glc; **3:** R = OH, R_1 = Glc, R_2 = H
4: R = R_1 = R_2 = H; **5:** R = OH, R_1 = R_2 = H
6: R = R_1 = H, R_2 = OGlc; **7:** R_1 = H, R = OH, R_2 = OGlc
8: R = R_1 = H, R_2 = OH; **9:** R = OH, R_1 = H, R_2 = OH

Ground dry shrublets of *C. coronarium* were exhaustively extracted with 70% ethanol/water, and the extract was concentrated under reduced pressure. The aqueous residue was extracted with solvents of increasing polarity. The fractions were subjected to repeated column chromatography to yield nine compounds.

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TABLE 1. ^{1}H (300 MHz) and ^{13}C NMR (125.7 MHz) Data of Naringenin 5-*O*-*β*-glucoside (**1**) in DMSO-d_6 with Shifts as δ Values, Coupling Constants in [Hz]

Atom C/H	^{13}C	^{1}H	^{13}C	HMBC
2	78.4	5.33 (dd, 5,11)	78.4	C-2′, C-6′
3_A	42.0	2.58 (d, 14)	44.8	C-2, C-4, C-1′
3_B	42.0	3.02 (d, 14)	44.8	
4	196.2		190.2	
5	163.6		160.7	
6	95.6	7.37 (d, 2)	99.5	C-8, C-10, C-5, C-7
7	166.7		166.5	
8	95.0	6.03 (d, 2)	98.0	C-6, C-10, C-7, C-9
9	162.9		164.2	
10	101.8		104.7	
1′	128.9		129	
2′	128.2	7.28 (d, 8)	128.2	C-4′, C-6′
3′	115.2	6.77 (d, 8)	115.1	C-1′, C-5′
4′	157.8		157.7	
5′	115.2	6.77 (d, 8)	115.1	C-1′, C-5′
6′	128.2	7.28 (d, 8)	128.2	C-4′, C-2′
1″		4.64 (d, 7.5)	103.6	C-3″, C-5″, C-5
2″		3.28	73.4	C-3″, C-1″
3″		3.27	75.5	C-2″, C-4″
4″		3.21	69.5	C-3″, C-5″, C-6″
5″		3.31	77.5	C-6″
$6''_A$		3.73 (dd, 11.5, 3)	60.6	C-4″
$6''_B$		3.54 (dd, 11.5, 3)	60.6	C-5″

Flavonoids were detected on paper chromatography by spraying with alcoholic aluminum chloride, color reactions, and UV spectral data.

Compound **1** was isolated as white crystals appearing as a fluorescent light blue spot on paper chromatography and silica gel TLC under UV light, and changing into fluorescent blue-green with ammonia vapor, indicating a flavonoid with free 4′- and substituted 5-hydroxyl groups [14]. UV spectral data in methanol and after addition of the diagnostic shift reagents suggested a flavanone with free 4′-, 7-, and substituted 5-hydroxyl groups.

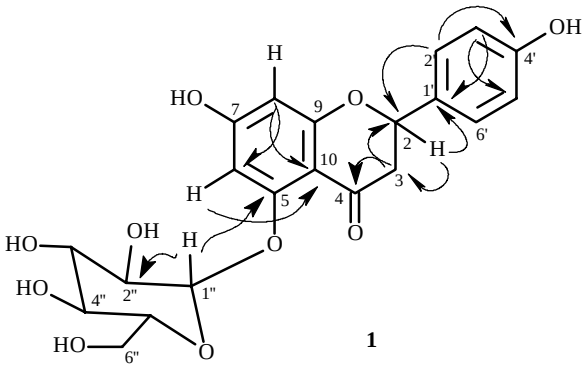
Complete acid hydrolysis of **1** yielded naringenin (UV and EI-MS) as the flavanone component together with glucose. The two components were co-chromatographed with authentic samples.

In the ^{1}H NMR spectrum of **1** (Table 1), the absence of the chelated signal of 5-OH with the 4-keto group indicated that the 5-hydroxyl group was replaced. In the sp2 region, the four proton signals of the *B*-ring appearing as two doublets at δ 7.25 and 6.77 with J = 8.0 Hz due to *ortho*-coupling were assigned to H-2′,6′ and H-3′,5′, and the two *meta*-coupled protons at δ 6.37 and 6.04 with J = 2 Hz were assigned to H-8 and H-6. The aliphatic region of **1** also delivered two aliphatic resonances at δ 2.58 and 3.02 (d, J = 14 Hz) and 5.33 (dd, J = 5, 11) from the methylene protons at C-3 and the methine proton at C-2, respectively, as well as a doublet at δ 4.64 with coupling constant 7.5 Hz of the anomeric proton of a glucosyl residue, indicating the *β*-configuration of the glucose moiety located at C-5 of naringenin aglycone. the other proton resonances were in the range δ 3.21–3.73.

This was fully confirmed by the ^{13}C NMR and HMQC spectra of **1** (Table 1), which exhibited 19 individual carbon signals. Comparison of the carbon chemical shift of naringenin [15] with that of **1** showed similarity. There was, however, an upfield shift (Δδ = 2.2) of the C-5 carbon resonance of **1** compared to the same carbon of the naringenin and an accompanying downfield shift of the resonances of the adjacent carbons, C-6 and C-10 (Δδ = 3 and 1.4, respectively).

The structure elucidation of compound **1** was finally achieved by HMBC experiments (Table 1). the most important HMBC correlations are the ^{2}J correlations of the methine proton at δ_H 5.33 (H-2) with the quaternary carbon at δ_C 129 (C-1′) and the methylene protons at δ_H (2.58 and 3.02) (3-CH_2) with the carbonyl carbon at δ_C 190.2 (C-4), confirming ring-A of the flavanone moiety. The HMBC correlations further confirmed that the glucose moiety is connected by an acetal linkage *via* the

5-*O* position of naringenin, as the anomeric proton at δ_H 4.64 (H-1″) correlated with the carbon signal at δ_C 160.7 (C-5). Thus, compound **1** is identified as naringenin 5-*O*-*β*-glucopyranoside, a rare isolated flavonoid compound.



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The antiviral activity of our tested pure compounds was weak against poliovirus I (virus titre is 4×10^6 pfu/mL). The reduction factor (RF) ranged from RF 1 to RF 10. There was no antiviral activity against adenovirus type 7 (virus titre is 5×10^5 $TCID_{50}$/mL).

EXPERIMENTAL

UV: Perkin–Elmer Lamda 15 UV/VS Spectrophotometer, ^{1}H NMR spectra: Varian Unity 300 (300 MHz), Bruker AMX 300 (300 MHz), Varian Inova 500 (499.8 MHz). ^{13}C NMR spectra: Varian Unity 300 (75.5 MHz). Chemical shifts were measured relative to tetramethylsilane as internal standard. 2D-NMR spectra: HMBC, HMQC and NOSY. EI MS: Finnigan MAT 731 (70 eV). R_f-values were measured on Polygram SIL G/UV_{254} (Macherey-Nagel & Co., Duren, Germany). Solvent mixtures, BAW (*n*-butanol–acetic acid–water 4:1:5 upper phase). Paper chromatography, Whatman No. 3 MM (46×57 cm).

Plant Material. Fresh shrublets of *Chrysanthemum coronarium* L. were collected from El-Omayed, A North Coast (2005) and authenticated by Dr. Salwa Kawshty, Phytochemistry and Plant systematics, National Research Centre, Cairo, Egypt.

Isolation and Identification. As a preliminary step, the dried powdered aerial parts (whole plants) of *Chrysanthemum coronarium* (500 g) were extracted with 70% ethanol–water (three extractions each for 9 hours at 40–60°C). The aqueous extracts were concentrated and the aqueous residue was extracted with solvents of different polarity. The alcoholic and aqueous fractions were applied to a polyamide 6S column and eluted with H_2O followed by EtOH–H_2O mixtures of decreasing polarities to yield nine fractions. The ethanol fraction was further purified by paper chromatography (3 MM) using BAW as gradient followed by successive columns of Sephadex LH-20 using methanol to yield **1** (3.4 mg); paper chromatography (3 MM) with 15% acetic acid-water yielded compounds **2–5** (5.6, 3.8, 6.4, and 8.9 mg), while the aglycones **6–9** (2.5, 4.9, 5.3, and 1.7 mg) were isolated by paper chromatography with BAW as eluent.

Naringenin 5-*O*-*β*-glucoside (1): $C_{21}H_{20}O_{10}$, white crystals, PC R_f 0.65 (BAW), 0.34 (H_2O) and 0.55 (15% HOAc); UV (λ_{max}, MeOH): 281, 318 sh; (NaOMe) 245, 326; ($AlCl_3$) 280, 314 sh; ($AlCl_3$/HCl) 280, 313; (NaOAc) 278, 315 sh; (NaOAc/H_3BO_3) 279, 314 and ^{1}H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (125.7 MHz, DMSO-d_6), see Table 1.

Antiviral Testing. The *in vitro* antiviral screening method was used to estimate the inhibition of the cytopathic effect (CPE) of the pure compounds on BGM and HEP-2 cell monolayers infected with poliovirus I and adenovirus type 7 using the end-point titration technique (EPTT) [16]. Confluent monolayers of BGM and HEP-2 cells were grown in 96-well microtiter plates, which were infected with serial tenfold dilutions of a poliovirus I and adenovirus type 7 suspensions respectively. The viruses were allowed to adsorb for 60 min at 37°C, after which serial twofold dilutions of the test compounds in maintenance medium, supplemented with 2% serum and antibiotics, were added. The plates were incubated at 37°C, and the viral cytopathic effect was recorded by light microscopy after 2 to 8 days. Virus suspensions are characterized by their virus titres, which are expressed as the smallest amount of virus capable of producing a reaction in the host cells. The antiviral activity is expressed as a reduction factor (RF), being the ratio of the viral titres in the virus control and in the presence of the maximal non-toxic dose of test substance.

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