Studies on the Constituents of *Cyclanthera pedata* Fruits: Isolation and Structure Elucidation of New Flavonoid Glycosides and Their Antioxidant Activity

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The isolation of six flavon glycosides (**1**−**6**), among them four new natural compounds (**1**−**4**), from the $CHCl_3$/MeOH extract of the fruits of *Cyclanthera pedata* is reported. All of the structures were elucidated by spectroscopic methods, including the concerted application of one-dimensional (^1H, ^1H TOCSY, ^{13}C, and ^{13}C DEPT-NMR) and two-dimensional NMR techniques (DQF-COSY, HSQC, and HMBC). For all of the isolated compounds the antioxidant activity was determined by measuring the free radical scavenging activity, using the Trolox equivalent antioxidant capacity (TEAC) method, and the coupled oxidation of β-carotene and linoleic acid.

Keywords: *Cyclanthera pedata; Cucurbitaceae; flavone glycosides; NMR; antioxidant activity*

INTRODUCTION

Cyclanthera pedata Scrabs (Caigua), a plant cultivated by ancient Peruvians, is largely used to make salad soup and has the reputation of being antiinflammatory, hypocholesterolemic, and hypoglycemic. In previous papers (*1*, *2*) we reported the isolation and structure determination of six new cucurbitacin glycosides from the seeds and six new triterpene saponins from the fruits of *C. pedata*. We have now investigated the fruits of this plant to determine their flavone glycoside content and the antioxidant activity of the isolated compounds.

Natural antioxidants are dietary elements responsible for protective effects against the risk of many physiological and pathological processes such as cancer, aging, and cardiovascular diseases. Natural antioxidants, such as flavonoids and other polyphenolic compounds, may have one or more of the following functions: free radical scavengers; reducing agents; protection against lipid peroxidation; and quenchers of reactive oxygen species (ROS), such as superoxide anion (*3*−*5*).

The $CHCl_3$/MeOH extract of *C. pedata* showed antioxidant activity in two preliminary assays, conducted to characterize free radical scavenging activity and inhibition of the oxidation of linoleic acid. This prompted us to search for antioxidant compounds from this plant. Bioassay-guided fractionation of the $CHCl_3$/MeOH extract led to the isolation of flavonoids **1**−**6** including four new compounds (**1**−**4**). In this investigation, we report the isolation, structure elucidation, and antioxidant activities of the flavonoids **1**−**6**.

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MATERIALS AND METHODS

Material. The fruits of *C. pedata* Scrabs were supplied by the Instituto Peruano Investigaciones Fitoterapica Andina (IPIFA) and were collected in Peru in 1997. A voucher sample is deposited at the Herbario del Museo de Historia Natural J. Prado, Lima (Peru), and a voucher sample is deposited at the Dipartimento di Scienze Farmaceutiche, Università di Salerno (n.5), Salerno (Italy). 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), trolox, potassium persulfate, linoleic acid, Tween 20, butylhydroxytoluene (BHT), and β-carotene were obtained from Sigma Aldrich (Milano, Italy). Nanopure water was prepared by a Milli-Q apparatus.

Apparatus. A Bruker DRX-600 spectrometer (Spectroscopin AG, Fallanden, Switzerland) operating at 599.19 MHz for ^1H and at 150.858 for ^{13}C, using the UXNMR software package, was used for NMR experiments. Samples were dissolved in CD_3OD. The distortionless enhancement by polarization transfer (DEPT) experiments were performed using a transfer pulse of 135° to obtain positive signals for CH and CH_3 and negative ones for CH_2. $^1H-^1H$ double quantum filtered correlate spectroscopy (DQF-COSY) (*6*, *7*), $^1H-^{13}C$ heteronuclear single quantum coherence (HSQC) (*8*, *9*), and heteronuclear multiple bond correlation (HMBC) (*10*) experiments were obtained using the conventional pulse sequences as described in the literature; 1D total correlation spectroscopy (TOCSY) spectra (*11*) were acquired using a waveform generator-based Gauss shaped pulse, mixing time ranging from 60 to 100 ms, and a MLEV-17 spin−lock field of 10 kHz preceded by a 2.5 ms trim pulse. Spectrophotometric analyses were performed by using a Perkin-Elmer λ-12 UV−vis spectrophotometer. HPLC separations were performed on an HP 1100 series HPLC, equipped with a photodiode array detector, from Agilent Technologies (Palo Alto, CA).

Optical rotation were determined on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and a 10 cm microcell.

Electrospray ionization mass spectrometry (ESIMS) in the positive ion mode was performed using a Finnigan LC-Q Deca instrument from Thermoquest (San Jose, CA) equipped with Excalibur software. Samples were dissolved in MeOH and infused in the ESI source by using a syringe pump; the flow

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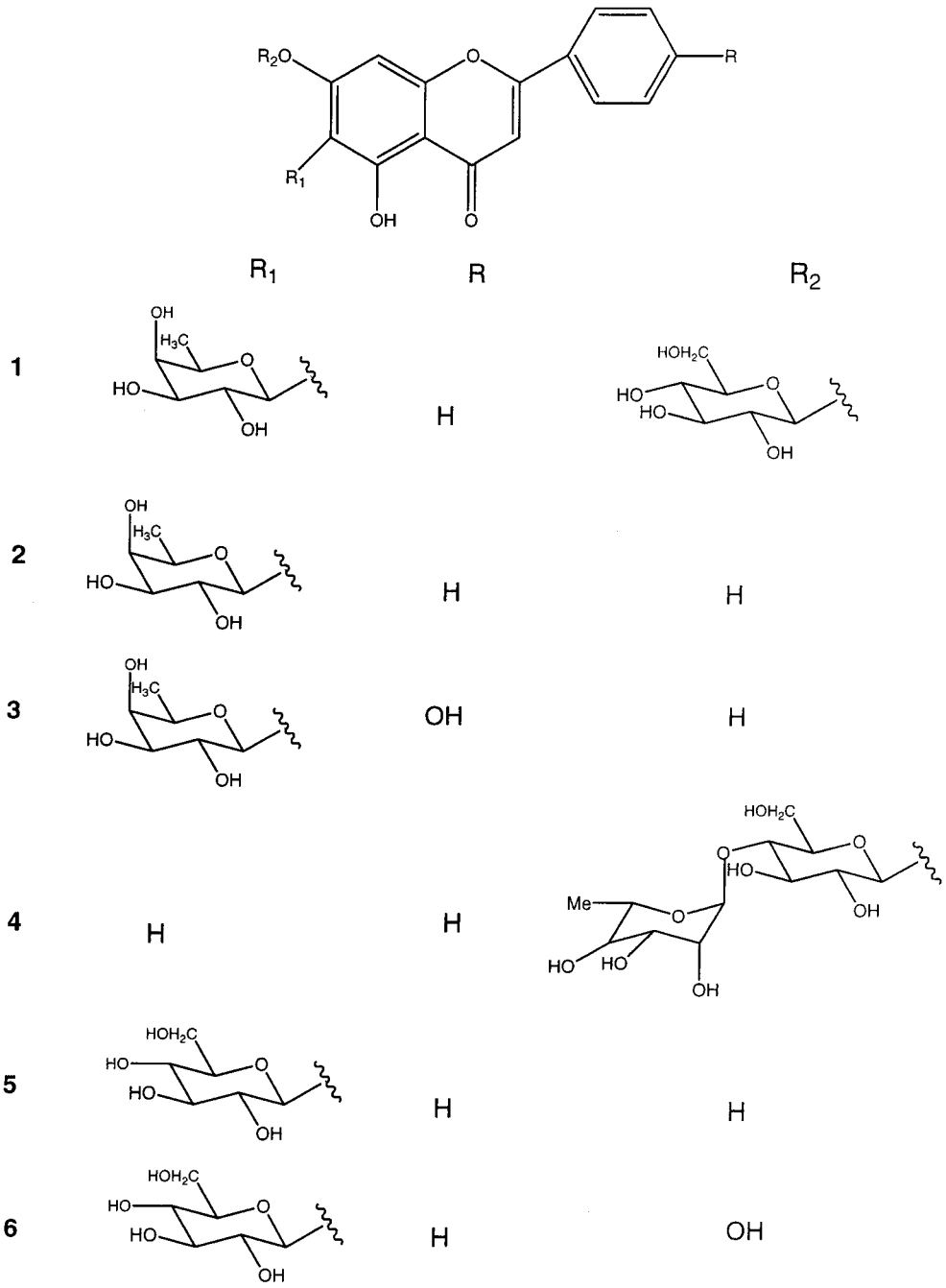

Figure 1. Structures of flavonoid glycosides from *C. pedata* fruits.

rate was 3 μL/min. The capillary voltage was at 5 V, the spray voltage at 5 kV, and the tube lens offset at 35 V. The capillary temperature was 220 °C. Data were acquired in the MS1 scanning mode (*m*/*z* 150−700).

Exact masses were measured by a Q-Star Pulsar (Applied Biosystems) triple-quadrupole orthogonal time-of-flight (TOF) instrument. Electrospray ionization was used in TOF mode at 8.500 resolving power.

Samples were dissolved in pure methanol, mixed with the internal calibrant, and introduced directly into the ion source by direct infusion. Calibration was performed on the peaks of cesium iodide and synthetic peptide (TOF positive ion calibration solution, Bachem,) at *m*/*z* 132.9054 and 829.5398, respectively. Sodium-containing molecular ions of analytes were measured.

Extraction and Isolation. The powdered, dried fruits (500 g) were defatted with *n*-hexane (yield = 6.3 g) and $CHCl_3$ (yield = 2.5 g) in a Soxhlet apparatus and extracted successively at room temperature with $CHCl_3$/MeOH (9:1) (yield = 6 g) and MeOH (yield = 30 g). Part of the $CHCl_3$/MeOH extract (2.2 g) was chromatographed on a Sephadex LH-20 column using MeOH as eluent. Fractions (8 mL) were collected, checked by TLC [Si gel plates/*n*-BuOH/HOAc/H_2O (60:15:25)] and combined into five main pools (A−E). Pools D and E contained flavone glycosides. Fractionation of pool D (180 mg) was achieved by RP-HPLC on a Waters μ-Bondapak C-18 column (30 cm × 7.8 mm, flow rate = 2.5 mL/ min) using MeOH/H_2O (1:1) to yield pure compounds **1** (36 mg, t_R =13 min), **3** (12 mg, t_R =15.0 min), **4** (14 mg, t_R =19 min), and **6** (23 mg, t_R =8 min). Pool E (120 mg) was separated by RP-HPLC with MeOH/H_2O (1:1) to yield pure compounds **2** (8 mg, t_R =21 min), **3** (6 mg, t_R =15.0 min), and **5** (10 mg, t_R =12.5 min) (structures are shown in Figure 1).

Compound ***1*** ($C_{27}H_{30}O_{13}$) was obtained as a yellow amorphous powder: $[\alpha]_D^{25}$ −51.3° (MeOH, *c* 0.1); UV (MeOH) λ_{max} 330, 275, and 210 nm; HRESIMS, *m*/*z* 585.1596 $[M + Na]^+$; ESIMS, *m*/*z* 563 $[M + H]^+$, 585 $[M + Na]^+$, and 401 $[M + H - 162]^+$; for ^1H and ^{13}C NMR data see Tables 1 and 2.

Table 1. ^{13}C NMR Data of Compounds 1−4 in CD_3OD[a]

	1 δ_C	**2** δ_C	**3** δ_C	**4** δ_C		**1** δ_C	**2** δ_C	**3** δ_C	**4** δ_C
1					Fuc-1	74.9	76.12	76.3	
2	166.2	166.2	165.5	166.2	-2	70.0	71.1	71.0	
3	107.0	107.0	108.0	107.0	-3	77.1	77.0	77.0	
4	184.6	185.0	184.0	184.6	-4	76.2	74.1	74.1	
5	162.0	162.1	162.0	162.0	-5	73.8	76.7	76.8	
6	112.2	111.9	112.0	99.0	-6	17.5	17.6	17.5	
7	164.8	164.5	164.5	165.3	Glc-1	103.9			102.5
8	96.0	96.5	96.2	96.8	-2	74.6			73.9
9	158.9	158.7	158.7	158.9	-3	77.0			78.0
10	106.8	107.0	104.6	106.8	-4	71.6			79.0
1′	131.7	132.0	132.4	131.6	-5	78.4			77.0
2′	128.0	128.2	128.4	128.0	-6	62.7			62.2
3′	133.3	133.4	116.2	133.2	Rha-1				101.2
4′	130.5	131.0	155.0	130.5	-2				72.1
5′	133.3	133.4	116.2	133.3	-3				73.0
6′	128.0	128.2	128.4	128.0	-4				72.5
					-5				70.0
					-6				18.2

[a] Assignments were confirmed by HSQC and HMBC experiments.

Compound **2** ($C_{21}H_{20}O_8$) was obtained as a yellow amorphous powder: $[\alpha]_D^{25}$ −26.6° (MeOH, *c* 0.1); UV (MeOH) λ_{max} 330, 275, and 210 nm; HRESIMS, *m/z* 401.1236 $[M + H]^+$; ESIMS, *m/z* 401 $[M + H]^+$ and 423 $[M + Na]^+$; for ^1H and ^{13}C NMR data see Tables 1 and 2.

Compound **3** ($C_{21}H_{20}O_9$) was obtained as a yellow amorphous powder: $[\alpha]_D^{25}$ −30.3° (MeOH, *c* 0.1); UV (MeOH); λ_{max} 330, 275, and 210 nm; HRESIMS, *m/z* 417.1189 $[M + H]^+$; ESIMS, *m/z* 417 $[M + H]^+$ and 439 $[M + Na]^+$; for ^1H and ^{13}C NMR data see Tables 1 and 2.

Compound **4** ($C_{27}H_{30}O_{13}$) was obtained as a yellow amorphous powder: $[\alpha]_D^{25}$ −41.2° (MeOH, *c* 0.1); α_d −41.2; UV (MeOH) λ_{max} 350, 275, and 210 nm; HRESIMS, *m/z* 585.1596 $[M + Na]^+$; ESIMS, *m/z* 563 $[M + H]^+$, 585 $[M + Na]^+$, 417 $[M + H - 146]^+$, and 255 $[(M + H^+) - (162 + 146)]$; for ^1H and ^{13}C NMR data see Tables 1 and 2.

Compound **5** ($C_{21}H_{20}O_9$) was obtained as a yellow amorphous powder: $[\alpha]_D^{25}$ −31.3 (MeOH, *c* 0.1); UV (MeOH) λ_{max} 330 and 275 nm; ESIMS, *m/z* 417 $[M + H]^+$ and 439 $[M + Na]^+$; identified as chrysin-6-*C*-glucopyranoside, previously isolated from *Scutellaria baicalensis*, by spectral data (*12*).

Compound **6** ($C_{21}H_{20}O_{10}$) was obtained as a yellow amorphous powder: $[\alpha]_D^{25}$ −45.3 (MeOH, *c* 0.1); UV (MeOH) λ_{max} 350, 275, and 210 nm; ESIMS, *m/z* 433 $[M + H]^+$ and 455 $[M + Na]^+$; identified as isovitexin, previously isolated from *Canephora madagascariensi*, by spectral data (*13*).

Trolox Equivalent Antioxidant Capacity (TEAC) Assay. The TEAC assay is based on the ability of the antioxidant to scavenge the $ABTS^{\bullet+}$ cation radical, by spectrophotometric analysis. The assay was carried out according to the method of Re et al. (*14*). $ABTS^{\bullet+}$ cation radical was produced by the reaction between ABTS (7 mM) in water and potassium persulfate (2.45 mM), kept in the dark at room temperature for 12 h. $ABTS^{\bullet+}$ is a blue-green chromogen with a characteristic absorption at 734 nm. The $ABTS^{\bullet+}$ solution was diluted with phosphate-buffered saline (PBS), pH 7.4, to an absorbance of 0.70 at 734 nm and equilibrated at 30 °C. Samples were diluted with MeOH to give 0.3, 0.5, 1, 1.5, and 2 mM concentration solutions. The reaction was enhanced by the addition of 1 mL of diluted ABTS to 10 μL of each solution of sample or Trolox (standard) or 10 μL of MeOH (blank). The determination was repeated three times for each sample solution. The percentage inhibition of absorbance at 734 nm was calculated for each concentration as a function of the absorbance of the blank. The percentage inhibition was plotted as a function of the concentration of compound or standard. The antioxidant activities of compounds **1**−**6** are expressed as TEAC. This value is defined as the concentration of a standard Trolox solution with the same percentage inhibition as a 1 mM concentration solution of the compound.

Autoxidation of β-Carotene. The antioxidant activity of the compounds, based on coupled oxidation of β-carotene and linoleic acid, was measured following the method described by Pratt (*15*). Twenty milligrams of linoleic acid and 200 mg of Tween 20 were placed in a flask, and then a solution of 2 mg of β-carotene in 10 mL of $CHCl_3$ was added. After removal of the $CHCl_3$, 50 mL of distilled water saturated with oxygen was added. Two hundred microliters of the test compounds, dissolved in ethanol to give a 15 μg/mL solution, was added to each flask with shaking. Samples without dosed compounds were used as a blank, and a sample with BHT as the dosed compound was used as standard. Samples were subjected to oxidation by placing them in an oven at 50 °C for 3 h.

The absorbance was read at 470 nm at regular intervals. The antioxidant activity (AA) was expressed as inhibitory ratio, calculated with the equation $AA = 100\ [1 - (A_0 - A_t)/(A_{00} - A_{0t})]$, where A_0 is the absorbance at the beginning of the incubation, A_t is the absorbance at time *t*, A_{00} is the absorbance at the beginning of the incubation, without compound, and A_{0t} is the absorbance at time *t*, without compound.

RESULTS AND DISCUSSION

Dried and powdered fruits of *C. pedata* were extracted with *n*-hexane, $CHCl_3$, $CHCl_3$/MeOH (9:1), and MeOH. The residue obtained after evaporation of the solvents was fractionated by Sephadex LH-20 gel filtration using MeOH as eluent. Purification of the flavonoid fractions obtained from gel filtration yielded six constituents, including four new derivatives. The known compounds were identified as isovitexin and chrysin-6-*C*-glucopyranoside by comparison with published physical and spectral data (*12*, *16*).

The molecular formula $C_{27}H_{30}O_{13}$ for compound **1** was obtained by ESIMS and ^{13}C and DEPT NMR analyses. Its ESIMS spectrum showed an ion at *m/z* 563 $[M + H]^+$ and 585 $[M + Na]^+$ and a peak at *m/z* 401 $[M + H - 162]^+$ due to the loss of one hexose unit.

Mass spectrometry, ^{13}C, and ^{13}C-DEPT NMR analyses indicated its flavonoid nature and, in particular, 15 carbon atoms ascribable to the aglycon and 12 to the sugar moiety.

In the ^1H NMR spectrum the chemical shifts and coupling constants of the protons indicated the absence of substitution for ring B of the flavone skeleton. The ^{13}C NMR shifts were compatible with chrysin in which either C-6 or C-8 was substituted. This was confirmed by the observation of only two singlets in the ^1H NMR corresponding to H-3 and H-8 or H-6 (δ 6.87 and 7.11, both singlets) (*17*). Two anomeric protons were easily identified in the ^1H NMR spectrum. They resonated at δ 4.94 ($J = 7.5$ Hz) and 4.84 ($J = 7.0$ Hz) and correlated, respectively, with ^{13}C resonances at 103.9 and 74.9. The proton coupling network within each sugar residue was elucidated using a combination of DQF-COSY and 1D-TOCSY experiments. The 1D-TOCSY correlations of two monosaccharide units could be easily interpreted and, at the same time, the type of sugar and its configuration and conformation could be assigned (Table 2). The 1D-TOCSY spectrum obtained by irradiating the anomeric proton at δ 4.94 established that proton as belonging to the glucopyranose unit. Irradiation of the anomeric and methyl signals at δ 4.84 and 1.29, respectively, allowed the identification of a fucopyranose unit. An HSQC experiment correlated all proton resonances in **1** with those of the corresponding carbon atoms (Tables 1 and 2). The absence of any glycosidation shift for both β-D-glucopyranosyl and β-D-fucopyranosyl moieties suggested that both sugars were terminal units. The chemical shifts of H-1_{Fuc} (δ 4.84) and C-1_{Fuc} (δ 74.9) indicated that this sugar unit was involved in a *C*-glycosidic linkage (*18*). The signals of C-6 and C-8 in

Table 2. ^{1}H NMR Shifts and Multiplicities of Compounds 1−4 in CD_3OD[a,b]

	1 δ_H	**2** δ_H	**3** δ_H	**4** δ_H
3	6.87 s	6.85 s	6.87s	6.84
6				6.45
8	7.11 s	7.09 s	7.12 s	6.60
2′	8.06 br d (8.0)	8.06 br d (8.0)	7.87 d (8.5)	8.06 br d (8.0)
3′	7.61 m	7.61 m	6.97 d (8.5)	7.61 m
4′	7.59 m	7.59 m		7.59 m
5′	7.61 m	7.61 m	6.97 d (8.5)	7.61 m
6′	8.06 br d (8.0)	8.06 br d (8.0)	7.87 d (8.5)	8.06 br d (8.0)
Fuc-1	4.84 d (7.0)	4.88 d (7.0)	4.90 d (7.0)	
-2	4.64 br t (9.0)	4.65 br t (9.0)	4.66 br t (9.0)	
-3	3.58 dd (9.0; 3.5)	3.57 dd (9.0; 3.5)	3.60 dd (9.0; 3.5)	
-4	3.76 m	3.75 m	3.76 m	
-5	3.79 m	3.81 m	3.81 m	
-6	1.29 d (6.5)	1.28 d (6.5)	1.30 d (6.5)	
Glc-1	4.94 d (7.5)			5.02
-2	3.66 br t (9.0)			3.61 br t (9.0)
-3	3.54 t (9.0)			3.50 t (9.0)
-4	3.40 t (9.0)			3.42 t (9.0)
-5	3.62 m			3.59 m
-6a	3.75 dd (12.0; 5.0)			3.72 dd (12.0; 5.0)
-6b	4.04 dd (12.0; 2.5)			4.02 dd (12.0; 3.0)
Rha-1				4.85 d (1.5)
-2				3.78 dd (3.0; 1.5)
-3				3.74 dd (9.5; 3.0)
-4				3.50 t (9.5)
-5				3.90 m
-6				1.27 d (6.5)

[a] Assignments were confirmed by 1D-TOCSY and DQF-COSY experiments. [b] Coupling constants (*J* values) are given in hertz in parentheses.

chrysin appear at about δ 6.50 and 6.60 in the ^{1}H NMR spectrum, and in the ^{13}C NMR spectrum C-6 appears at about 94.0 and C-8 at about 96.0 (in CD_3OD), very close to each other. It is therefore very difficult to determine the position of the substituent located at C-6 or C-8 in flavones by comparing their ^{1}H and ^{13}C NMR spectra with that of chrysin (*17*). However, unambiguous determination of the glycosidic linkages could be obtained from CH correlations (HMBC spectrum). The position of the β-D-fucopyranosyl unit in **1** was deduced from an HMBC experiment. Correlations were observed between H-1 of fucose and C-6, C-5, and C-7; between H-8 and C-7, C-9, C-6, C-10, and C-2; and between H-3 and C-2, C-1′, C-4, and C-10. A diagnostic long-range correlation was also observed between H-1_{Glc} (δ 4.94) and C-7 (164.8) of the aglycon. The ^{1}H and ^{13}C NMR data indicated a β configuration at the anomeric position for both the fucopyranosyl and glucopyranosyl units.

The structure chrysin 7-*O*-β-D-glucopyranosyl-6-*C*-fucopyranoside was therefore assigned to **1**.

The ESIMS spectrum of compound **2** showed the $[M + H]^+$ ion at *m/z* 401 and 423 $[M + Na]^+$.

The ^{13}C and ^{13}C-DEPT NMR data showed 21 signals of which 6 were assigned to the glycoside portion and 15 to the flavone moiety. Analysis of the NMR data of compound **2** and comparison with those of **1** showed **2** to differ only in the absence of the glucopyranosyl unit linked at C-7 of the aglycon (Tables 1 and 2). The structure chrysin-6-*C*-fucopyranoside was therefore assigned to **2**.

Compound **3** showed, in the ESIMS spectrum, an ion at *m/z* 417 $[M + H]^+$ and another at 439 $[M + Na]^+$, 16 mass units higher than that of **2**, and gave ^{13}C and ^{13}C-DEPT NMR data in accordance with a $C_{21}H_{20}O_9$ molecular formula. Comparison of ^{1}H and ^{13}C DEPT NMR data of both compounds indicated identical glycoside chains at C-6 and structural similarity in the aglycon moiety. The main differences were the downfield shift of C-4′ (155.0 ppm), the upfield shifts of C-2′, C-3′, C-5′, and C-6′ in the ^{13}C NMR spectrum, and the presence of two signals resonating as doublets (δ 7.87 and 6.97, both 2H $J = 8.5$ Hz) in the ^{1}H NMR spectrum, implying an additional hydroxyl group at C-4′. Thus, the aglycon of **3** was identified as apigenin (*19*), and compound **3** was defined as apigenin-6-*C*-fucopyranoside.

The ESIMS spectrum of **4** showed ions at *m/z* 563 $[M + H]^+$, 417 $[M + H - 146]^+$, due to the loss of a deoxyhexose unit, and 255 $[M + H - (146 + 162)]^+$, due to the subsequent loss of a hexose residue.

To compound **4** was assigned the molecular formula $C_{27}H_{30}O_{13}$. Mass spectrometry and ^{13}C and ^{13}C-DEPT NMR analyses indicated its flavonoid nature and, in particular, 15 carbon atoms ascribable to the aglycon and 12 to the sugar moiety. In the ^{1}H NMR spectrum the chemical shift and the coupling constants of the protons indicated a 5,7-dihydroxylated pattern for ring A and an unsubstituted ring B, establishing the aglycon as chrysin. Two anomeric protons were evident for compound **4**, and they resonated at δ 5.02 and 4.85 in the ^{1}H NMR spectrum and correlated in an HSQC experiment to carbons at 102.5 and 101.2 ppm, respectively. Analysis of the 1D-TOCSY and DQF-COSY spectra allowed complete assignments for all proton resonances of the glucose and rhamnose units starting from the anomeric proton signals (Table 2). An HSQC experiment correlated all proton resonances in **4** with those of the corresponding carbons (Tables 1 and 2). Data from the above experiments determined the position of the interglycosidic linkage by comparison of the carbon chemical shift observed with those of the corresponding methyl pyranoside and taking into account the known effects of glycosidation. The absence of any glycosidation shift for the rhamnopyranosyl residue suggested that rhamnose was a terminal unit, whereas a glycosidation shift for C-4 of the glucose unit linked at C-7 allowed the structure to be defined as glycoside chain linked to C-7. The ^{1}H and ^{13}C NMR data indicated an α configuration at the anomeric position for the

Table 3. Antioxidative Activity (AA) of Flavones 1−6 from *C. pedata*

compound	TEAC	% AA (1 h)	% AA (2 h)
BHT[a]		68.70	54.24
1	0.273 ± 0.01	47.16	3.29
2	0.790 ± 0.02	17.17	15.21
3	0.957 ± 0.01	21.34	11.56
4	0.616 ± 0.015	47.51	16.23
5	1.062± 0.04	39.07	37.01
6	1.09 ± 0.02	19.11	12.15
kaempferol	1.02 ± 0.02		
quercetin	2.770 ± 0.02		

[a] BHT (*tert*-butylhydroxytoluene) was used as standard.

rhamnopyranosyl and a β configuration for the glucopyranosyl units (*2*). The structure chrysin 7-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside was therefore assigned to **4**.

Antioxidant Activity. Trolox (a water soluble vitamin E analogue) equivalent antioxidant capacity (TEAC) has been used to determine the radical scavenging abilities of flavonoids as electron- or H-donating agents through their ability to scavenge $ABTS^{\bullet+}$. TEAC results for flavonoid compounds **1**−**6** and results for quercetin and kaempferol, used as reference compounds, are summarized in Table 3. All of the compounds (Table 3) had radical scavenging activity similar to the activity of kaempferol but lower than that of quercetin. The TEAC values indicate that the number of free phenolic OH groups (excluding OH groups on the sugar moiety) makes a statistically significant contribution to TEAC. These results are in agreement with data reported in the literature (*4*, *5*).

Membrane lipids are abundant in unsaturated fatty acids, which are most susceptible to oxidative processes, and linoleic acid especially is the target of lipidic peroxidation. The antioxidative effect of pure compounds **1**−**6** on the autoxidation of linoleic acid was also determined. The values of AA measured at $t = 60$ and 120 min, employing bleaching of β-carotene as a model system, are reported in Table 3. The data show that all tested compounds possess antioxidant activity, and, in particular, **1**, **4**, and **5** are the most active compounds and have antioxidant activities only slightly less than that of the standard phenolic antioxidant, BHT.

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