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Prenylated Flavanones with Anti-tyrosinase Activity from *Dalea boliviana*

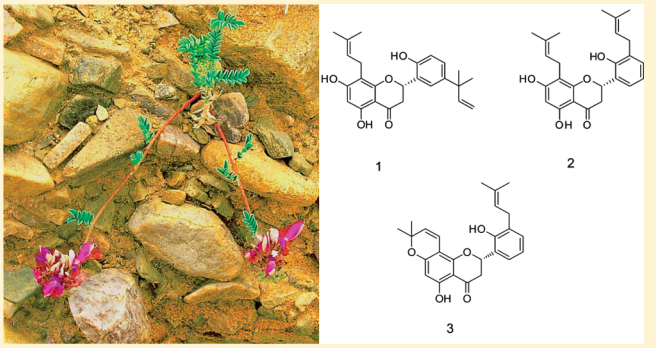
Mariana Andrea Peralta,[†,‡] María Gabriela Ortega,[†,‡] Alicia Mariel Agnese,[†,‡] and José Luis Cabrera*[,†,‡]

[†]Farmacognosia, Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, 5000 Córdoba, Argentina

[‡]Instituto Multidisciplinario de Biología Vegetal (IMBIV-CONICET), Ciudad Universitaria, 5000 Córdoba, Argentina

Supporting Information

ABSTRACT: Three new prenylated flavanones, (2*S*)-5,7,2′-trihydroxy-5′-(1‴,1‴-dimethylallyl)-8-prenylflavanone (**1**), (2*S*)-5,7,2′-trihydroxy-8,3′-diprenylflavanone (**2**), and (2*S*)-5,2′-dihydroxy-6″,6″-dimethylchromeno-(7,8:2″,3″)-3′-prenylflavanone (**3**), and a known chromeno (dimethylpyrano) flavanone, obovatin (**4**), were isolated from the *n*-hexane extract of *Dalea boliviana* roots. The compounds were evaluated *in vitro* in relation to their inhibitory effect on the tyrosinase activity by using a spectrophotometric method.



Dalea L. (Fabaceae) is an exclusively American genus with more than 250 species[1] distributed in arid regions of the southwestern United States to the central regions of Argentina and Chile.[2] There are four species described for Argentina, namely, *Dalea elegans* Gillies ex Hook. & Arn., *D. boliviana* Britton, *D. leporina* (Aiton) Bullock, and *D. elegans* var. *onobrychioides* (Griseb.) Barneby.[3]

The isolation of two prenylated flavanones from *D. elegans*, 6-prenylpinocembrin and 2′,4′-dihydroxy-5′-(1‴,1‴-dimethylallyl)-6-prenylpinocembrin, has been reported,[4] with the latter compound showing antibacterial and antifungal properties.[5,6] Its demonstrated antioxidant and antiradical DPPH activities, as well as enzymatic lipid peroxidation inhibition and toxic effects against human tumor cells,[7] prompted us to investigate this genus further. Thus, we investigated the phytochemical and biological activity of *D. boliviana*, a small shrub that grows in northwestern Argentina, Bolivia, and Perú at elevations of 2600 and 4000 m above sea level.

We herein report the isolation and structural elucidation of three new prenylated flavonoids (**1**−**3**) from the *n*-hexane extract of *D. boliviana* roots together with the known obovatin (**4**), previously isolated from the *Tephrosia* genus.[8,9] Their structures were established by 1D and 2D NMR spectroscopy as well as HRMS analysis. Furthermore, all the compounds were evaluated *in vitro* as tyrosinase inhibitors by spectrophotometric methods and using kojic acid as positive control.

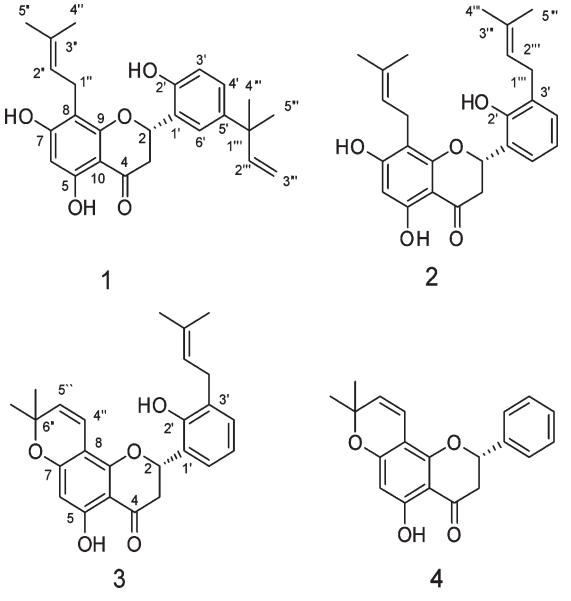


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Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data for 1 (acetone-d_6), 2, 3, and 4 ($CDCl_3$)

	1		2		3		4	
position	δ_C, mult.	δ_H mult. (J in Hz)	δ_C, mult.	δ_H mult. (J in Hz)	δ_C, mult.	δ_H mult. (J in Hz)	δ_C, mult.	δ_H mult. (J in Hz)
2	74.8, CH	5.78 dd (3.2, 12.5)	76.6, CH	5.67 dd (3.0, 13.0)	76.4, CH	5.70 dd (3.8, 12.2)	79.1, CH	5.43 dd (3.4, 12.7)
3a	41.8, CH_2	2.90 dd (3.2, 17.1)	41.9, CH_2	2.90 dd (3.0, 17.3)	42.0,CH_2	2.90 dd (3.8, 17.3)	43.3, CH_2	2.82 dd (3.4, 17.1)
3b		3.05 dd (12.5, 17.1)		3.19 dd (13.0, 17.3)		3.05 dd (12.2, 17.3)		3.05 dd (12.7, 17.1)
4	196.8, qC		196.7, qC		196.2, qC		195.6, qC	
5	164.0, qC		163.4, qC		163.9, qC		163.8, qC	
6	95.6, CH	6.07 s	97.1, CH	6.04 s	97.8, CH	6.00 s	97.7, CH	5.99 s
7	162.1, qC		162.3, qC		162.1, qC		162.3, qC	
8	107.5, qC		106.3, qC		102.0, qC		102.0, qC	
9	160.4, qC		159.5, qC		156.6, qC		156.8, qC	
10	102.4, qC		103.2, qC		102.9, qC		102.9, qC	
1′	125.2, qC		124.9, qC		125.0, qC		138.5, qC	
2′	151.7, qC		152.0, qC		151.9, qC		126.0, CH	7.40 m
3′	115.0, CH	6.89 d (8.3)	127.7, qC		127.5, qC		128.8, CH	7.40 m
4′	124.2, CH	7.20 dd (2.5, 8.3)	130.1, CH	7.12 dd (1.3, 7.5)	130.1, CH	7.14 dd (1.8, 7.6)	128.7, CH	7.40 m
5′	139.7, qC		120.6, CH	6.91 t (7.5)	120.7, CH	6.92 t (7.6)	128.8, CH	7.40 m
6′	126.8, CH	7.62 d (2.5)	124.7, CH	7.24 dd (1.3, 7.5)	124.7, CH	7.28 dd (1.8, 7.6)	126.0, CH	7.40 m
1″	21.6, CH_2	3.29 d (7.5)	21.7, CH_2	3.31 d (7.3)				
2″	122.9, CH	5.30 t (7.5)	121.5, CH	5.19 t (7.3)				
3″	130.4, qC		135.0, qC					
4″	25.0, CH_3	1.63 s	25.7, CH_3	1.72 s	115.5, CH	6.54 d (10.0)	115.6, CH	6.55 d (10.0)
5″	17.2, CH_3	1.65 s	17.8, CH_3	1.72 s	126.6, CH	5.47 d (10.0)	126.5, CH	5.46 d (10.0)
6″					78.1, qC		78.1, qC	
1‴	40.4, qC		29.8, CH_2	3.38 d (7.3)	29.9, CH_2	3.39 d (7.2)		
2‴	148.4, CH	6.04 dd (10.5, 17.3)	121.4, CH	5.32 t (7.3)	121.3, CH	5.33 m		
3‴	110.0, CH_2	5.04 *cis* d (17.3)	135.4, qC		135.6, qC			
3‴		5.01 *trans* d (10.5)						
4‴	28.0, CH_3	1.78 s	25.8, CH_3	1.78 s	25.8, CH_3	1.78 s		
5‴	28.0, CH_3	1.78 s	17.8, CH_3	1.78 s	17.9, CH_3	1.79 s		
5-OH		12.10 s		12.04 s		12.12 s		12.06 s
2′-OH		5.91 br s		5.90 br s		5.92 br s		
6″-Me					28.3, CH_3	1.42 s	28.3, CH_3	1.42 s
					28.5, CH_3	1.44 s	28.5, CH_3	1.44 s

RESULTS AND DISCUSSION

Compound **1** was isolated as an amorphous solid, and its molecular formula was deduced as $C_{25}H_{28}O_5$ from the analysis of its HRFABMS and NMR spectroscopic data. The UV absorption maxima at 293 and 339 (sh) nm were suggestive of a flavanone skeleton.[10] ^{1}H and ^{13}C NMR data allowed the complete structure elucidation of **1** (Table 1). The ^{1}H NMR spectrum exhibited a signal at δ_H 12.10 (1H, s) due to a hydrogen-bonded proton assigned to the C-5 hydroxy group. A one-proton aromatic singlet at δ_H 6.07 suggested a trisubstituted A-ring. The substitution of the B-ring was confirmed by three additional aromatic signals [δ_H 7.20 (1H, dd, J = 2.5 and 8.3 Hz), 6.89 (1H, d, J = 8.3 Hz), and 7.62 (1H, d, J = 2.5 Hz)], which were assigned as H-4′, H-3′, and H-6′, respectively. In addition, the spectrum showed signals for prenyl and α,α-dimethylallyl moieties, which were unambiguously established to be attached at C-8 and C-5′, respectively, by the detection of HMBC correlations (Figure 1). The absolute configuration at C-2 was determined to be *S* according to the positive Cotton effect at 334 nm and the negative Cotton effect at 293 nm in the CD spectrum[11] and the levorotatory optical rotation of **1**. Consequently, the structure of **1** was established as (−)-(2*S*)-5,7,2′-trihydroxy-5′-(1‴,1‴-dimethylallyl)-8-prenylflavanone (**1**).

Compound **2** was obtained as an amorphous solid. Its molecular formula was determined as $C_{25}H_{28}O_5$ according to the analysis of its HRFABMS and NMR spectroscopic data. The UV, IR, and ^{1}H and ^{13}C NMR spectra (Table 1) were consistent with a flavanone skeleton.[10] The ^{1}H NMR spectrum of **2** (Table 1) showed a characteristic flavanone ABX spin system, [δ_H 5.67 (1H, dd, J = 3.0 and 13.0 Hz, H-2), 3.19 (1H, dd, J = 13.0 and 17.3 Hz, H-3_{ax}), and 2.90 (1H, dd, J = 3.0 and 17.3 Hz, H-3_{eq})]. The C-5 hydroxy group was shifted downfield at δ_H 12.04 due to hydrogen bonding with the C-4 carbonyl. The singlet at δ_H 6.04 (1H, s, H-6) indicated a trisubstituted aromatic A-ring. The aromatic ^{1}H NMR signals of the B-ring [δ_H 7.12 (1H, dd, J = 1.3 and 7.5 Hz), 7.24 (1H, dd, J = 1.3 and 7.5 Hz), and 6.91 (1H, t, J = 7.5 Hz)] were assigned to H-4′, H-6′, and H-5′, respectively. The spectrum also displayed two sets of characteristic prenyl moiety signals at δ_H 3.31 (2H, d, J = 7.3 Hz, H-1″), 5.19 (1H, t, J = 7.3 Hz, H-2″), and 1.72 (6H, s, H-4″ and H-5″) and δ_H 3.38 (2H, d, J = 7.3 Hz, H-1‴), 5.32 (1H, t, J = 7.3 Hz, H-2‴), and 1.78 (6H, s, H-4‴ and H-5‴), which were

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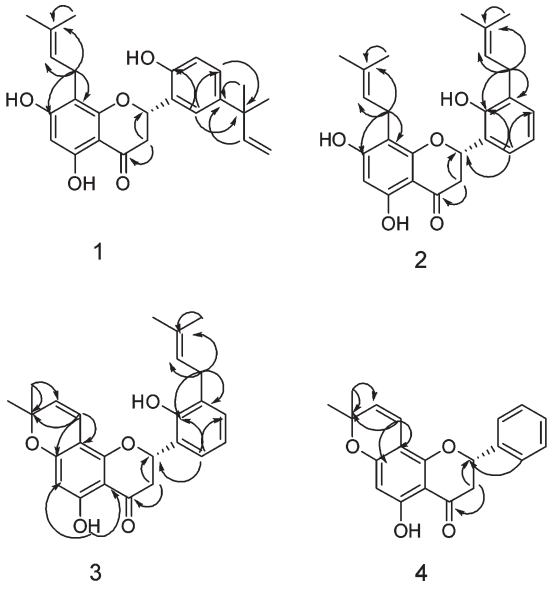

Figure 1. Key HMBC correlations of compounds **1**−**4**.

located at C-8 and C-3′, respectively, on the basis of HMBC correlations (Figure 1). The levorotatory optical rotation of **2** in addition to the positive Cotton effect at 340 nm and negative Cotton effect at 290 nm in its CD spectrum indicated a 2*S* absolute configuration for **2**.[11] Compound **2** was therefore identified as (−)-(2*S*)-5,7,2′-trihydroxy-8,3′-diprenylflavanone (**2**).

Compound **3** was obtained as an amorphous, pale yellow solid. Its molecular formula was established as $C_{25}H_{26}O_5$ on the basis of its HRFABMS data. The UV spectrum showed absorption maxima at 271 and 360 (sh) nm, typical for a flavanone skeleton.[10] The ^{1}H and ^{13}C NMR spectra were similar to those of **2** (Table 1), suggesting the same substitution pattern at the B-ring. The ^{1}H NMR spectrum showed a downfield signal at δ_H 12.12 (1H, s) due to the proton attached to the hydroxy group at C-5. Characteristic signals for a flavanone ABX spin system and an aromatic one-proton signal for the A-ring were observed. The ^{1}H NMR spectrum also revealed *cis*-coupled olefinic protons at δ_H 6.54 (1H, d, J = 10.0 Hz, H-4″), and 5.47 (1H, d, J = 10.0 Hz, H-5″) and *gem*-dimethyl singlets at δ_H 1.42 (1H, s) and 1.44 (1H, s), indicating the presence of a dimethylchromene ring.[12,13] The ^{1}H NMR spectrum also showed the characteristic signals for a prenyl moiety. The dimethylchromene and prenyl moieties were located at C-8 and C-3′, respectively, on the basis of HMBC correlations (Figure 1). The absolute configuration of **3** at C-2 was elucidated as *S* on the basis of CD data[11] and levorotatory optical rotation. The structure of **3** was, therefore, determined to be (−)-(2*S*)-5,2′-dihydroxy-6″,6″-dimethylchromeno-(7,8:2″,3″)-3′-prenylflavanone (**3**).

Compound **4** was obtained as a pale yellow, amorphous solid. The molecular formula $C_{20}H_{18}O_4$ was assigned from the HRFABMS measurement. The ^{1}H and ^{13}C NMR spectroscopic data of **4** (Table 1) were in agreement with those reported for obovatin.[9]

Tyrosinase acts as a rate-limiting enzyme in melanin biosynthesis. Therefore, tyrosinase inhibitors are important substances to treat abnormal pigmentation disorders, such as melasma, age spots, and sites of actinic damage, arising from accumulation of an excessive level of epidermal pigmentation.[14] Compounds **1**−**4** were tested for inhibitory activity against the tyrosinase enzyme. In addition, with the aim to highlight the structural

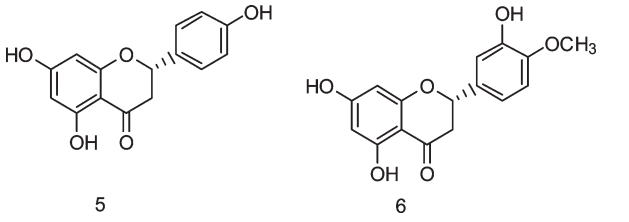

Figure 2. Structures of naringenin (**5**) and hesperetin (**6**).

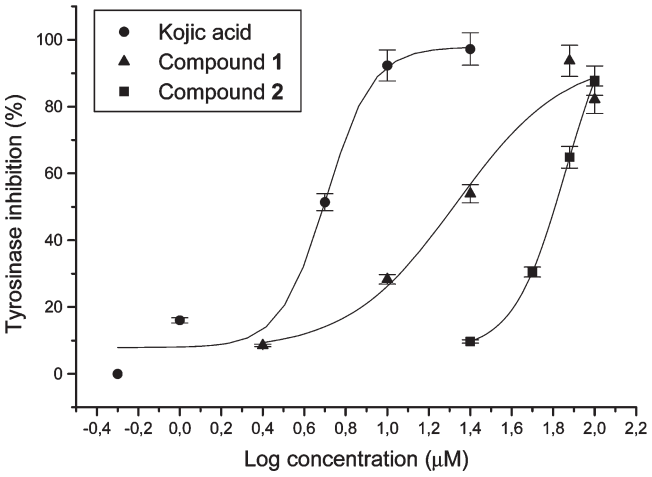

Figure 3. Dose-dependent inhibition of tyrosinase activity by the prenylated flavanones **1** and **2** and positive control kojic acid (N = 3).

Table 2. Anti-tyrosinase Activity of Flavanones 1−4, Naringenin, and Hesperetin[a]

compound	inhibition at 100 μM (%)	IC_{50} (μM)
1	82.1	27.5
2	87.8	68.5
3	6.4	
4	16.2	
5	2.8	
6	0.0	

[a] Positive control kojic acid. IC_{50} = 10.2 μM.

implication of the active compounds isolated of *D. boliviana* the antityrosinase activity of two flavanones, naringenin (**5**) and hesperetin (**6**) (Figure 2), was evaluated for comparative purposes.

The IC_{50} values for the compounds and the reference inhibitor were estimated using nonlinear fitting of concentration−response data. As a result, **1** and **2** showed inhibitory activity with IC_{50} values of 27.5 and 68.5 μM, respectively (Figure 3). Compounds **3**, **4**, **5**, and **6** demonstrated very low tyrosinase inhibition even at 100 μM (Table 2). Kojic acid was used as positive control (IC_{50} = 10.2 μM).

In conclusion, three new prenylated flavanones (**1**−**3**) were isolated from roots of *D. boliviana*, together with the previously known dimethylpyrano flavanone obovatin (**4**). This is the first report of dimethylchromene derivatives such as **3** and **4** in *Dalea* genus.

Compounds **1**−**4** were evaluated as tyrosinase enzyme inhibitors. They exhibited different capacities, **1** and **2** being better

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inhibitors than **3** and **4**. It is important to highlight that the first two compounds have in their structures a phloroglucinol A-ring with two free hydroxy groups. This moiety seems to be essential for inhibitory activity against the tyrosinase enzyme similar to the 4-substituted resorcinol moiety present in diverse structures such as stilbenes, flavonoids, and related compounds. Particularly, the relevance of the 4-substituted resorcinol moiety has been important when it is part of the B-ring in flavones and flavanones.[15,16] In the present paper the flavanones **1**−**4** present a substituted dihydroxyphloroglucinol A-ring moiety, and its importance is emphasized by the weak activity of compounds **3** and **4**, probably as a consequence of the formation of the chromene moiety.

In relation to this hypothesis Lee et al.[17] showed a similar behavior when the activity of two flavones isolated of *Morus alba* L. was compared. In effect, kuwanon C, with its 4-substituted phloroglucinol A-ring and a resorcinol B-ring, showed significant anti-tyrosinase activity, while for morusin, the activity was drastically reduced due to the cyclization of the isoprenyl group at C-8.

Additionally, in the present paper the activity of naringenin and hesperetin was evaluated with the aim to analyze their behavior in view that both are flavanones without a C-8 isoprenyl moiety. The significant difference in activity comparing to **1** and **2** can be seen in Table 2. Several studies show the influence of this moiety; however we consider that more studies about other additional structural factors are necessary to reveal inhibitory activity on tyrosinase.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Jasco P-1010 polarimeter. UV spectral data were recorded on a Shimadzu 160A spectrophotometer. CD spectra were obtained with a Jasco J-810 spectropolarimeter. ^{1}H and ^{13}C NMR spectra were acquired in $CDCl_3$ or acetone-d_6 on a Brüker Advance II 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer using TMS as internal standard. HRFABMS were obtained on a JEOL JMS-AX505 mass spectrometer. FTIR spectra were recorded as KBr disks, using a Nicolet 5SXC spectrophotometer. Column chromatography was performed on Merck silica gel. TLC was carried out using 20 × 20 cm plates with a 0.5 mm layer of silica gel GF_{254} (Merck). Spots were visualized by UV illumination (254 nm).

Plant Material. *D. boliviana* was collected in February 2007, during the flowering period, near Iturbe village in Humahuaca Department, Jujuy Province, Argentina. Plant material was identified by Prof. Dr. Gloria Barboza from the Botanical Museum−UNC. A voucher specimen is on deposit as CORD 1066.

Extraction and Isolation. *D. boliviana* was dried at room temperature, and the roots (60 g) were separated from the aerial parts, powdered, and extracted with *n*-hexane (250 mL) at room temperature for 24 h. The solvent was subsequently removed to yield crude extract (4 g), which was dissolved in acetone (100 mL). Filtration and removal of the solvent under reduced pressure gave a brown residue (3 g), which was subjected to column chromatography (CC) using silica gel as stationary phase and eluted successively with *n*-hexane/EtOAc (100:0 to 0:100) and EtOH. Fractions were combined on the basis of their TLC profiles (*n*-hexane/EtOAc, 7:3) into five fractions (F1 through F5). F1 (106 mg, R_f 0.75) was chromatographed by preparative TLC (trichloroethylene) to afford **3** (20 mg) and **4** (6 mg). F2 (29 mg, R_f 0.55) was subjected to preparative TLC ($CHCl_3$/EtOH, 99:1) to give **2** (14 mg). F3 (130 mg, R_f 0.45) was separated by preparative TLC ($CHCl_3$/MeOH, 97:3) to give **1** (25 mg).

*(−)-(2S)-5,7,2′-Trihydroxy-5′-(1‴,1‴-dimethylallyl)-8-prenylflavanone (**1**)*:. amorphous solid; $[\alpha]_D^{25}$ −105 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 293 (4.09), 339 (sh) (3.44) nm; CD (*c* 0.098, MeOH,) $[\theta]_{293}$ −36.68, $[\theta]_{334}$ +2.33; IR (KBr) ν_{max} 3389 (OH), 2962, 2921,1637 (C═O), 1601, 1506 (C═C), 1270 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) and ^{13}C NMR (acetone-d_6,100 MHz), see Table 1; EIMS *m*/*z* (rel int) 408 $[M]^+$ (6.9), 221 (9.4), 220 (13.3), 206 (22.5), 205 (30.6), 188 (3.5), 166 (50.4), 165 (100), 161 (4.8), 69 (52.2); HRFABMS *m*/*z* 409.2016 $[M + H]^+$ (calcd for $C_{25}H_{29}O_5$, 409.2015).

*(−)-(2S)-5,7,2′-Trihydroxy-8,3′-diprenylflavanone (**2**)*:. amorphous solid; $[\alpha]_D^{25}$ −75 (*c* 0.41, MeOH); UV (MeOH) λ_{max} (log ε) 274 (sh) (1.98), 293 (2.09), 339 (sh) (1.45) nm; CD (*c* 0.049, MeOH) $[\theta]_{290}$ −41.05, $[\theta]_{340}$ +2.47; IR (KBr) ν_{max} 3446 (OH), 1631 (C═O), 1542 (C═C), 1446 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; EIMS *m*/*z* (rel int) 408 $[M]^+$ (13.4), 221 (12.3), 220 (10.4), 206 (23.7), 205 (28.9), 188 (3.5), 166 (51.5), 165 (100), 161 (3.7) and 55 (36.4); HRFABMS *m*/*z* 409.2009 $[M + H]^+$ (calcd. for $C_{25}H_{29}O_5$, 409.2015).

*(−)-(2S)-5,2′-Dihydroxy-6″,6″-dimethylchromeno-(7,8:2″,3″)-3′-prenylflavanone (**3**)*:. pale yellow, amorphous solid; $[\alpha]_D^{25}$−17 (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ε) 271 (4.65), 297 (4.10), 360 (sh) (3.52) nm; CD (*c* 0.015, MeOH) $[\theta]_{315}$ +2.17, $[\theta]_{294}$ −5.20; IR (KBr) ν_{max} 3445 (OH), 1642 (C═O), 1543 (C═C), 1439 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$,100 MHz), see Table 1; EIMS *m*/*z* (rel int) 406 $[M]^+$ (28.0), 391 (31.2), 219 (10.7), 218 (2.7), 204 (12.2), 203 (100), 161 (3.4), 69 (52.2); HRFABMS *m*/*z* 407.1850 $[M + H]^+$ (calcd for $C_{25}H_{27}O_5$, 407.1858).

*(−)-(2S)-5-Hydroxy-6″,6″-dimethylchromeno-(7,8:2″,3″)-flavanone (obovatin) (**4**)*:. pale yellow solid; $[\alpha]_D^{25}$ −40 (*c* 0.09, MeOH); UV(MeOH) λ_{max} (log ε) 271 (3.95), 297 (3.41), 360 (sh) (2.89) nm; CD (*c* 0.020, MeOH) $[\theta]_{315}$ +1.89, $[\theta]_{294}$ −4.73; IR (KBr) ν_{max} 3446 (OH), 1637 (C═O), 1542 (C═C), 1446 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data are in agreement with those already published;[9] see Table 1; EIMS *m*/*z* (rel int) 322 $[M]^+$ (15.4), 307 (55.0), 219 (0.4), 218 (1.5), 204 (11.5), 203 (100), 104 (5.1), 77 (10.0); HRFABMS *m*/*z* 323.1288 $[M + H]^+$ (calcd for $C_{20}H_{19}O_4$, 323.1283).

Tyrosinase Inhibitory Activity Assay. The assay was performed according to the procedure of Rahman et al.[18] with minor modifications. The assay medium consisting of 0.25 mL of mushroom tyrosinase solution (250 U/mL, Sigma), 0.50 mL of Na_3PO_4 buffer (pH 6.8), and 0.75 mL of sample solution [prepared with compounds **1**−**4** and **5**, **6** (Sigma) dissolved in DMSO (final concentration 0.1 v/v) and subsequently diluted to the appropriate concentrations with the above buffer] was mixed and preincubated at 25 °C for 10 min. Then 0.50 mL of L-tyrosine (1.7 mM, Sigma) solution was added. The absorbance was measured at 475 nm after 20 min of incubation. The absorbance of the same mixture without the compounds was used as control. Kojic acid was used as positive control agent. Each treatment was replicated three times. The percent inhibition of tyrosinase activity was calculated as follows: % inhibition = $[(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$, where $Abs_{control}$ is the absorbance of the control and Abs_{sample} is the absorbance of the experimental sample. The IC_{50} values were estimated by using nonlinear fitting of concentration−response data.

ASSOCIATED CONTENT

Supporting Information. ^{1}H, ^{13}C, HMQC, and HMBC spectra of the new compounds (**1**, **2**, and **3**). This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: +54-351-4334163. Fax: +54-351-4334127. E-mail: jcabrera@fcq.unc.edu.ar.

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