Antihyperglycemic Activity and Chemical Constituents of *Eysenhardtia platycarpa*

José M. Narváez-Mastache,[†] María Luisa Garduño-Ramírez,[‡] Laura Alvarez,[‡] and Guillermo Delgado*[,†]

Instituto de Química de la Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, México D.F., and Centro de Investigaciones Químicas de la Universidad Autónoma del Estado de Morelos, Avenida Universidad 1001, Chamilpa 62210, Cuernavaca, Morelos, México

Received April 12, 2006

The methanolic extracts from branches (BEP) and leaves (LEP) of *Eysenhardtia platycarpa* significantly decreased the blood glucose levels in normal and streptozotocin (STZ)-induced diabetic rats. One new flavone, (1″*R*)-5,4′,1″-trihydroxy-6,7-(3″,3″-dimethylchroman)flavone (**1**), together with the known compounds 5,7-dihydroxy-6-methyl-8-prenylflavanone (**3**), 5,7-dihydroxy-8-methyl-6-prenylflavanone (**4**), 5,7-dihydroxy-6-prenylflavanone (**5**), 5,7-dihydroxy-8-prenylflavanone (**6**), 3-*O*-acetyloleanolic acid (**7**), oleanolic acid, 3β-acetoxy-11α,12α-epoxy-oleanan-28,13β-olide, lupeol, betulinic acid, β-sitosterol, β-sitosteryl β-D-glucopyranoside, β-sitosteryl palmitate, and 3-*O*-methyl-*myo*-inositol were isolated from BEP. Additionally, one new flavanone, (2*S*)-4′-*O*-methyl-6-methyl-8-prenylnaringenin (**2**), as well as the known compounds **3**, **4**, **6**, 4′-*O*-methyl-8-prenylnaringenin (**8**), and 5-hydroxy-7-methoxy-8-prenylflavanone (**9**) were isolated from LEP. 3-*O*-Acetyloleanolic acid (**7**), identified as the major constituent of BEP, showed a significant decrease (31 mg/kg of body weight, $P < 0.05$) in the glucose level of STZ-induced diabetic rats. The obtained results correlate with the traditional use of this species.

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by abnormalities in carbohydrate, lipid, and lipoprotein metabolism, which not only leads to hyperglycemia but also is associated with a high risk of atherosclerosis and renal, nervous system, and ocular damage.[1] Recent projections indicate that the prevalence of DM will rise in the world population ca. 4.4% by 2030.[2] Current treatment approaches include diet, exercise, and a variety of oral hypoglycemic agents. However, these compounds are often toxic with prolonged administration.[3] Herbal medicine has been used for many years in traditional medicine, for both the prevention and treatment of the complications associated with DM. The use of ca. 306 species from 235 genera and 93 families as hypoglycemic agents in Mexican traditional medicine has been documented;[4] however, the chemical and pharmacological knowledge about these herbs is relatively limited.[4−14]

The genus *Eysenhardtia* (Leguminosae), which comprises 14 species, is located in the northern and central parts of Mexico, and some of its members have been used in traditional medicine for their biological activities such as diuretic, antidiabetic,[11,13] and antiseptic[15] activity and for the treatment of kidney and bladder infections.[16] *E. platycarpa* is a small tree distributed in Southern Mexico, where it is known as "taray", "palo dulce" (sweet wood), and "palo azul" (blue wood). It is well-known in traditional herbal medicine and used for the treatment of kidney diseases, as well as bladder infections and as a diuretic. It is also used for the treatment of complications derived from DM.[16,17]

The hypoglycemic activity of the extracts of *E. polystachya* has been previously evaluated,[11] and chemical studies from *Eysenhardtia* species have resulted in the isolation of flavonoids[15,17−21] with cytotoxic[20] and antibacterial activity.[15]

As part of our continuous search for biologically active substances,[22] the present study was carried out to evaluate the hypoglycemic activity of the extracts obtained from leaves, branches, and bark of *E. platycarpa* in normal and STZ-induced diabetic rats, the hypoglycemic activity of 3-*O*-acetyloleanolic acid (**7**) in STZ-induced diabetic rats, and the isolation and structure elucidation of two new flavonoids (**1** and **2**), along with 15 known compounds.

Results and Discussion

The methanolic extracts of leaves (LEP), branches (BEP), and bark (REP) of *E. platycarpa* were evaluated for hypoglycemic activity in normal and STZ-induced diabetic rats. REP did not display a hypoglycemic effect. Figure 1 shows the effect over time of 30, 100, and 300 mg/kg doses of LEP and BEP in the variation of blood glucose levels of normal fasted rats. The oral administration of LEP at all the studied doses displayed an increase of the blood glucose level from its initial time that was maintained until 90 min after administration (Figure 1a). This initial hyperglycemic effect has been observed previously in the evaluation of some plants with hypoglycemic activity[23,24] and may probably be attributed to the increase of anaerobic glycolysis and/or decrease of gluconeogenesis, due to the increase of transfer rate of circulating glucose in other tissues of the rats; high concentrations of extract did not favor the increase of blood glucose level. In normal rats, a dose of 30 mg/kg resulted in a significant decrease in glucose level at 3 h (−16.1 ± 5.8%), 5 h (−21.4 ± 2.9%), and 7 h (−22.7 ± 3.3%). The maximum hypoglycemic effect for the doses of 100 and 300 mg/kg was observed after 5 h of administration (−22.6 ± 3.4% and −21.1 ± 3.1%, respectively). The maximum effect shown by all doses at this time probably was due to a saturation effect for doses of 100 and 300 mg/kg. Therefore the best effect was at 30 mg/kg, and this was maintained for 7 h. The initial glycemia level was recovered after 9 h with all tested doses. BEP (Figure 1b) did not display glycemia variation for 5 h, and then the blood glucose level decreased. These effects suggest that BEP did not act directly via insulin liberation (in contrast with glibenclamide), since the hypoglycemic effect was exhibited after 7 h of administration at all doses.

The administration of STZ (60 mg/kg) to fasted rats increased blood glucose levels compared with the control group. Figure 2 shows the effect of LEP and BEP in STZ-induced diabetic rats. The administration of LEP (Figure 2a) to this group did not show an antihyperglycemic effect at all tested doses. These results suggest that some pancreatic function or the presence of insulin is required for the hypoglycemic activity of the leaf extract, since this material displayed an effect in normal but not in diabetic rats. However, the administration of BEP at 30, 100, and 300 mg/kg significantly increased the blood glucose levels after 5 h of administration, and this increase was reverted with the three doses at 7 h. Only the 300 mg/kg doses showed a significant decrease in glycemia 7 h

* Corresponding author. Tel: (52-55)-5622-4446. Fax: (52-55)-5616-2217. E-mail: delgado@servidor.unam.mx.
[†] Instituto de Química de la UNAM.
[‡] Centro de Investigaciones Químicas de la UAEMor.

10.1021/np060166z CCC: $33.50
Published on Web 11/17/2006

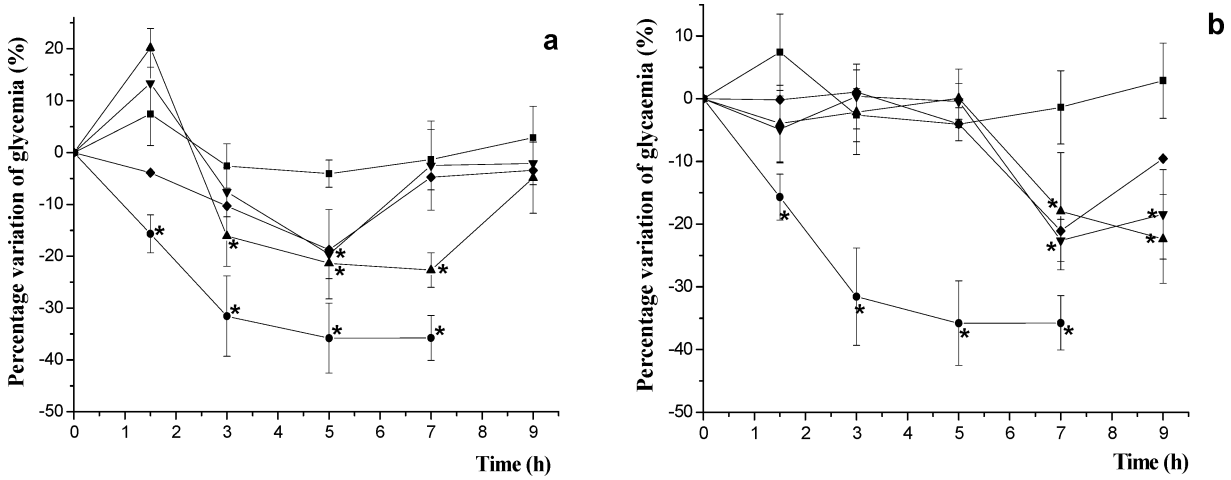


Figure 1. Acute effect of LEP (a) and BEP (b) in normal rats: (■) control, (●) glibenclamide (10 mg/kg), (▲) 30 mg/kg, (▼) 100 mg/kg, and (♦) 300 mg/kg. Values are expressed as mean of variation percent of glycemia + SEM; $n = 5$ and triplicates for each group; *statistically significant difference from the corresponding zero time value, $P < 0.05$.

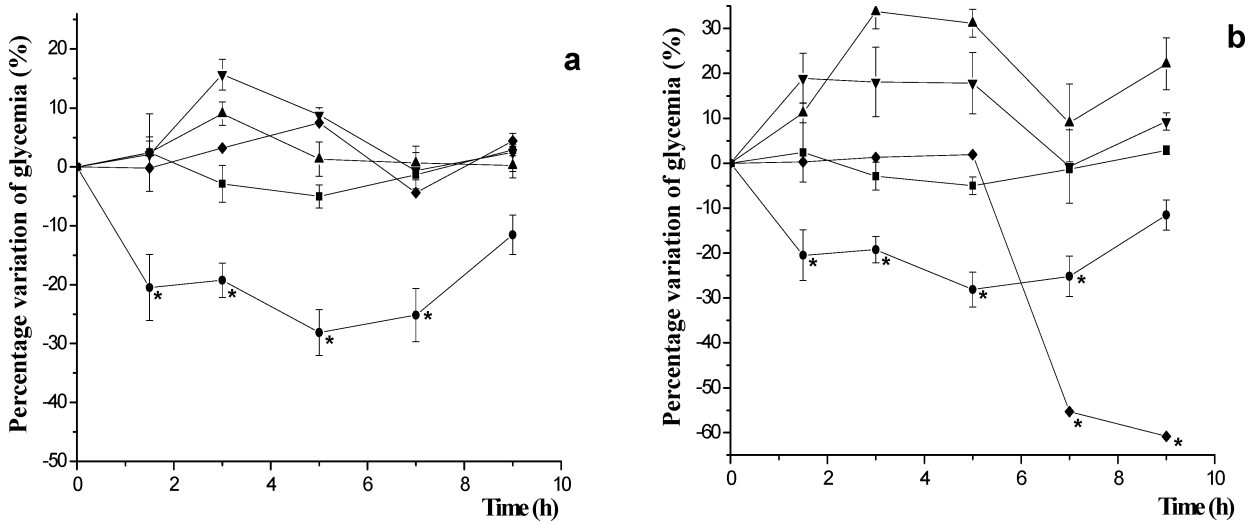
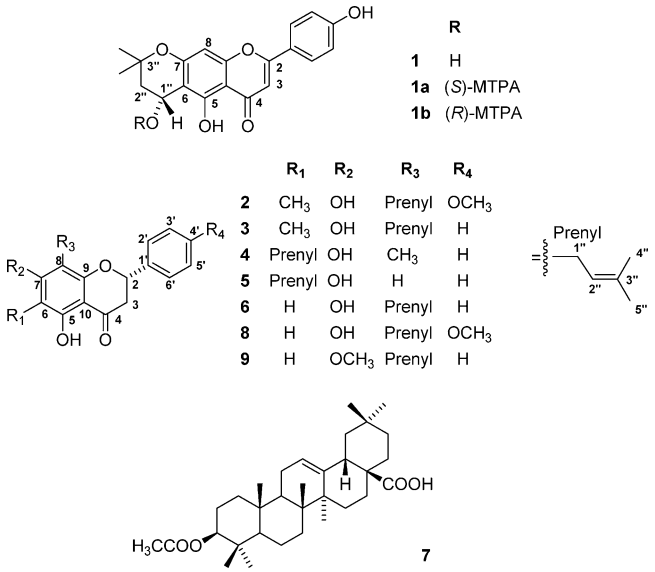


Figure 2. Acute effect of LEP (a) and BEP (b) in streptozotocin-induced diabetic rats: (■) control, (●) glibenclamide (10 mg/kg), (▲) 30 mg/kg, (▼) 100 mg/kg, and (♦) 300 mg/kg. Values are expressed as mean of variation percent of glycemia + SEM; $n = 5$ and triplicates for each group; *statistically significant difference from the corresponding zero time value, $P < 0.05$.

after administration (−55.3 ± 3.8%), which was maintained until 9 h (−60.8 ± 4.5%). These results indicate that BEP produced an extrapancreatic effect, such as the inhibition of enzymes,[25] and the increase of stimulation of reception of glucose by several tissues.[26] 3-*O*-Acetyloleanolic acid (**7**), the major constituent of BEP (1.01% yield from the extract), was evaluated as a hypoglycemic agent in STZ-induced diabetic rats. Oral administration of 31 mg/kg of **7** significantly reduced the hyperglycemia after 7 h of treatment (−26.3 ± 3.7%), compared with the respective zero time (Figure 3). It has been previously demonstrated that oleanolic acid and some of its derivatives inhibit α-glucosidase,[25] and the hypoglycemic effect of **7** in STZ-induced diabetic rats after 7 h of treatment is remarkable.

Fractionation of the active methanolic extracts of BEP and LEP led to the isolation of two new compounds (**1** and **2**), together with 15 known compounds. Compound **1** was obtained as an optically active yellow, amorphous solid. Its molecular formula was deduced to be $C_{20}H_{18}O_6$ from the EIMS and ^{13}C DEPT NMR analyses. The IR spectrum showed absorption bands due to hydroxyl (ν_{max} 3358, 3245 cm^{-1}) and carbonyl (ν_{max} 1669 cm^{-1}) groups. Its ^1H NMR spectrum showed a signal for a hydrogen-bonded hydroxyl proton at δ 13.18 (s, 1H), and two one-proton singlets at δ 6.78 and 6.58 were assigned to H-3 and H-8 or H-6 of a flavonoid structure.[27] The characteristic ^1H NMR chemical shifts at δ 7.91 and 6.91 (dd, $J = 2$, 9 Hz, 2H each) revealed the presence of a *p*-disubstituted B ring, which was confirmed by the signals at δ_C 128.4 and 115.9,

Chart 1. Structures of Compounds **1**−**9**



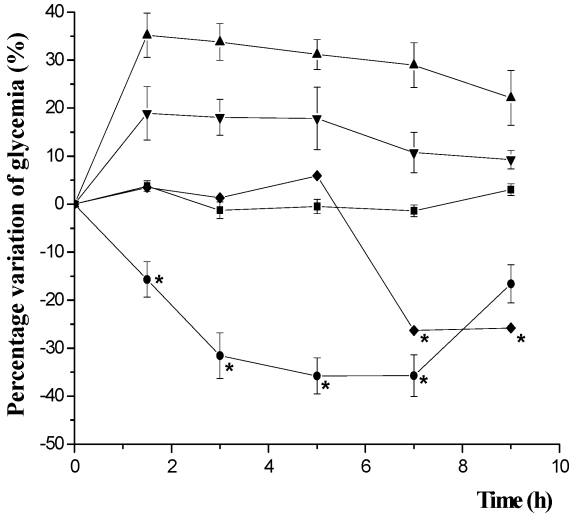


Figure 3. Acute effect of 3-*O*-acetyloleanolic acid in streptozotocin-induced diabetic rats: (■) control, (●) glibenclamide (10 mg/kg), (▲) 3.1 mg/kg, (▼) 10 mg/kg, and (♦) 31 mg/kg. Values are expressed as mean of variation percent of glycemia + SEM; n = 5 and triplicates for each group; *statistically significant difference from the corresponding zero time value, $P < 0.05$.

assignable to C-2′, C-6′ and C-3′, C-5′ of the B ring. The presence of one oxymethine [δ_C 91.4 (d)], one methylene [δ_C 25.9 (t)], a *gem*-dimethyl group [δ_C 25.7 (q) and 24.8 (q)], a quaternary carbon at δ_C 70.0, and the resonances at δ_H 3.04 (d, J = 8.7, 2H) and 4.73 (t, J = 8.7, 1H) established the hydroxydimethyldihydropyran fragment (see Table 1). The structure of **1** was confirmed by the observed NOESY and HMBC correlations shown in Figure 4. In particular, the NOESY correlation of H-1″ with H-2″a and H-2″b and with the hydrogen-bonded hydroxyl group and the HMBC correlations of H-1″ with C-5 and C-6 confirmed the spectroscopic assignments. The *R*-configuration at C-1″ was established via the Mosher method.[28] Separate samples of **1** were treated with (*R*)-(+)- and (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl) in anhydrous pyridine-d_5, to afford the (*S*)- and (*R*)-MTPA ester derivatives (**1a** and **1b**, respectively). The chemical shift values of the protons of the (*R*)-MTPA ester were subtracted from the protons of the (*S*)-MTPA ester [$\Delta\delta = \delta$ (*S*)-MTPA − (*R*)-MTPA]. The negative values from H-2″a (−0.47 ppm) and H-2″b (−0.53 ppm) indicated that C-1″ possesses the *R*-configuration.

Compound **2**, which was optically active ($[\alpha]^{25}_D$ −40 (*c* 0.1, $CHCl_3$), was obtained as a yellow solid from CH_2Cl_2−MeOH. Its FABMS and ^{13}C NMR data were in agreement with the molecular formula $C_{22}H_{24}O_5$. The IR exhibited the presence of hydroxyl (ν_{max} 3304 cm^{-1}) and carbonyl (ν_{max} 1633 cm^{-1}) functionalities, and the ^{13}C NMR spectrum showed signals for an oxymethine, a methylene, and a carbonyl group at δ_C 78.2 (d), 43.3 (t), and 196.6 (q), respectively. The ^{1}H NMR resonances for an ABX system [δ 3.00 (dd, J = 12.6, 17.2 Hz, 1H), 2.70 (dd, J = 3.2, 17.2 Hz, 1H), and 5.30 (dd, J = 3.2, 12.6 Hz, 1H)] are in agreement for H-2 and H-3 of the C ring of the flavanone. The NMR spectrum also showed resonances for an AA′BB′ system (δ_H 7.32, 6.89, and δ_C 127.5, 114.1), assignable to the B ring atoms (Table 1). The *O*-methyl group [δ_H 3.78 and δ_C 55.4] was attached to C-4′ according to NOESY correlations of H-3′ and H-5′ with *O*-CH_3 group. The presence of a prenyl group was evident by the resonances for two methyl [δ_H 1.77 and δ_C 17.9; δ_H 1.70 and δ_C 25.9], one methylene [δ_H 3.31 and δ_C 21.3], and a vinylic methine [δ_H 5.18 and δ_C 121.6] group, which could be attached at C-8 or C-6. The prenyl group was attached to C-8 by HMBC evidence that indicated a cross-peak for the methylene protons of the prenyl group [δ 3.31 (d, J = 7.2 Hz, 2H] and C-8. Therefore, the methyl group (δ_H 1.94; δ_C 7.6) was attached to C-6. The absolute configuration for **2** was determined to be 2*S* on the basis of the observation of a positive Cotton effect at 344 nm (θ +2.91) and the negative Cotton effect at 292 nm (θ −17.97) in its circular dichroism spectrum (CD).[29] Therefore, the structure of the new compound **2** was established to be (2*S*)-4′-*O*-methyl-6-methyl-8-prenylnaringenin. The demethyl derivative of **2** has been isolated from *E. texana.*[15] The fractionation of the active methanolic extracts from branches and leaves allowed the isolation of the known compounds 5,7-dihydroxy-6-methyl-8-prenylflavanone (**3**),[30] 5,7-dihydroxy-8-methyl-6-prenylflavanone (**4**),[31] 5,7-dihydroxy-6-prenylflavanone (**5**),[32] 5,7-dihydroxy-8-prenylflavanone (**6**),[33] 3-*O*-acetyloleanolic acid (**7**), oleanolic acid,[34] 3β-acetoxy-11α,12α-epoxyoleanan-28,13β-olide,[35] 4′-*O*-methyl-8-prenylnaringenin (**8**),[36] 5-hydroxy-7-methoxy-8-prenylflavanone (**9**),[33] lupeol,[37] betulinic acid,[38] β-sitosterol, β-sitosteryl β-D-glycopyranoside, β-sitosteryl palmitate,[39] and 3-*O*-methyl-*myo*-inositol.[40]

The observed hypoglycemic activity of the extracts of *E. platycarpa* and that of 3-*O*-acetyloleanolic acid as well as the isolation of some secondary metabolites, which have been previously demonstrated to display hypoglycemic activities[25,41,42] on in vitro and in vivo models, correlated with the traditional use of this species.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer 341 polarimeter. CD analysis was performed on a JASCO J-720 spectropolarimeter. Glibenclamide and streptozotocin were obtained from Sigma-Aldrich. Melting points were obtained on a Fisher-Johns apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Plus-500 and Varian INOVA-400 instruments, and the chemical shifts are expressed in parts per million (δ) relative to TMS. Infrared spectra were recorded with Nicolet Magna IR TM 750 and Perkin-Elmer 283B instruments. MS spectral data were recorded with a JEOL JMS-AX 505 HA mass spectrometer. EIMS were obtained at 70 eV ionization energy. Vacuum chromatography was performed on Merck Kiesel gel 60 (0.040−0.863 mm), and the eluent is specified in each experiment. TLC analyses were performed on Alugram Sil G/UV$_{254}$ silica gel plates. The chromatograms were examined under UV and by ceric ammonium sulfate/H_2SO_4 spray reagent.[43]

Plant Material. Leaves (300 g), branches (2500 g), and bark (500 g) of *E. platycarpa* were collected in Tetipac, Guerrero, in June 1998. A voucher specimen was deposited at the Herbarium of Facultad de Ciencias de la Universidad Nacional Autónoma de México (voucher specimen Ramiro Cruz 1325).

Extraction and Isolation. Air-dried leaves, branches, and bark were powdered and extracted with MeOH (3 × 3 L). The solvent was removed under reduced pressure to give the corresponding residues [49.9 g of leaves (LEP), 153.4 g of branches (BEP), and 38.5 g of bark (REP)]. BEP (150 g) was suspended in CH_2Cl_2 to yield soluble (20.5 g) and insoluble (123.4 g) fractions. The soluble fraction was fractionated using vacuum column chromatography and eluting with a gradient of *n*-hexane−EtOAc (from *n*-hexane 100% to EtOAc 100%, 350 mL each fraction) to obtain 10 main fractions (F-1 to F-10). F-1 (780 mg, *n*-hexane 100%) contained mainly fatty acids; F-2 (812 mg, *n*-hexane−EtOAc, 9:1) was rechromatographed using an *n*-hexane−EtOAc gradient (from *n*-hexane 100% to EtOAc 100%, 15 mL each fraction) as eluent, affording 5,7-dihydroxy-6-methyl-8-prenylflavanone (**3**, 80 mg), mp 183−184 °C [lit. 182−185 °C],[30] and 5,7-dihydroxy-8-methyl-6-prenylflavanone (**4**, 12 mg), mp 156−157 °C [lit. 156−158 °C];[31] F-3 (1.2 g, *n*-hexane−EtOAc, 8:2) was dissolved in 20 mL of EtOH and treated with activated charcoal (30 mg) for 30 min. The residue was filtered under vacuum over Celite. The filtrate was evaporated, and the residue was chromatographed using *n*-hexane−CH_2Cl_2 as gradient elution system (from *n*-hexane 100% to *n*-hexane−CH_2Cl_2, 7:3, 20 mL each fraction), yielding 3β-acetoxy-11α,12α-epoxyoleanan-28,13β-olide (205 mg), mp >300 °C.[35] Treatment of F-4 (1.98 g, *n*-hexane−EtOAc, 7:3) with *n*-hexane led to the isolation of 5,7-dihydroxy-6-prenylflavanone (**5**, 18 mg, as yellow solid)[30] and a soluble fraction. From the chromatography of the *n*-hexane-soluble fraction, eluted with *n*-hexane−EtOAc gradient, and subsequent crystal-

Table 1. ^{1}H and ^{13}C NMR Data for Compound **1** and **2**[a]

	1			**2**	
position	δ_C	δ_H (J in Hz)[b]	δ_H (J in Hz)[c]	δ_C[d]	δ_H (J in Hz)[e]
2	166.1			78.2	5.30 (dd, 3.2 12.6)
3	102.8	6.78 (s)	6.89 s	43.3	a 3.00 (dd, 12.6, 17.2) b 2.70 (dd, 3.2, 17.2)
4	181.9			196.6	
5	157.2			158.8	
6	109.0			102.8	
7	163.7			162.3	
8	88.8	6.58 (s)	6.81 s	106.0	
9	155.6			158.3	
10	104.6			103.3	
1′	121.1			131.0	
2′	128.4	7.91 (dd, 2.0, 9.0)	7.91 (dd, 2.0, 9.0)	127.5	7.32 (d, 8.8)
3′	115.9	6.91 (dd, 2.0, 9.0)	7.23 (dd, 2.0, 9.0)	114.1	6.89 (d, 8.8)
4′	161.2			159.8	
5′	115.9	6.91 (dd, 2.0, 9.0)	7.23 (dd, 2.0, 9.0)	114.1	6.89 (d, 8.8)
6′	128.4	7.91 (dd, 2.0, 9.0)	7.91 (dd, 2.0, 9.0)	127.5	7.32 (d, 8.8)
1″	91.4	4.73 (t, 8.7)	5.01 (dd, 5.1, 7.2)	21.3	3.31 (d, 7.2)
2″	25.9	3.04 (d, 8.7)	a 3. 56 (dd, 5.1, 13.8) b 3.48 (dd, 7.2, 13.8)	121.6	5.18 (t, 7)
3″	70.0			136.2	
4″	25.7	1.13 (s)	1.12 (s)	17.9	1.77 (s)
5″	24.8	1.14 (s)	1.13 (s)	25.9	1.70 (s)
CH_3-Ar				7.6	1.94 (s)
OCH_3-4′				55.4	3.78 (s)
OH-5		13.2 (s)	14.13 (s)		12.29 (s)
OH-7					6.20 (s)
OH-4′		10.31 (s)			
OH-1″		4.73 (s)	5.29 (s)		

[a] The assignment was based upon DEPT, COSY, HSQC, NOESY (mixing time: 1 s), and HMBC (delay: 1 s) experiments. [b]DMSO-d_6. [c]Pyridine-d_5. [d]$CDCl_3$. [e]^{1}H NMR (400 MHz).

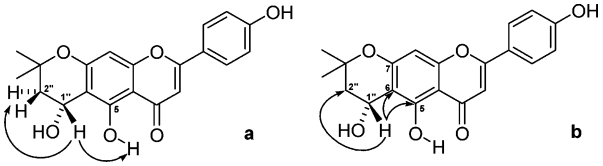


Figure 4. NOESY (a) and HMBC (b) correlations of **1**.

lization from CH_2Cl_2, 3-*O*-acetyloleanolic acid (**7**, 1.55 g), mp 260−262 °C [lit. 260−261 °C],[34] lupeol (150 mg), mp 215−216 °C,[37] and β-sitosterol (500 mg) were isolated. F-5, -6, and -7 contained one major spot and were pooled and chromatographed using CH_2Cl_2−MeOH as gradient elution system (from CH_2Cl_2 to CH_2Cl_2−MeOH, 9:1, 20 mL each fraction). After successive recrystallizations with CH_2Cl_2−MeOH, (1″*R*)-5,4′,1″-trihydroxy-6,7-(3″,3″-dimethylchroman)flavone (**1**, 223 mg), oleanolic acid (33 mg, mp >300 °C [lit. 306−308 °C]),[34] betulinic acid (320 mg, mp 277−279 °C [lit. 275−278 °C]),[38] and β-sitosteryl palmitate were obtained.[39] The CH_2Cl_2-insoluble fraction (8 g) was treated with Ac_2O (10 mL) and pyridine (1 mL) for 3 h. The product was purified using vacuum column chromatography (*n*-hexane−EtOAc, gradient from *n*-hexane 100% to *n*-hexane−EtOAc, 7:3, 75 mL each fraction) to yield β-sitosteryl β-D-glucopyranoside tetracetate (75 mg, *n*-hexane−EtOAc, 9:1), sucrose acetate (1.73 g, *n*-hexane−EtOAc, 8:2), and 5-*O*-methyl-*myo*-inositol pentacetate (5.7 g, *n*-hexane−EtOAc, 7:3).[40]

LEP (22 g) was fractionated using vacuum column chromatography on silica gel (66 g) eluting with *n*-hexane−EtOAc gradient (from *n*-hexane 100% to EtOAc 100%, 120 mL each fraction) and followed by MeOH to give 33 main fractions. The chromatography of F-3 (1.422 g, *n*-hexane 100%) on a silica gel column allowed the isolation of 4′-*O*-methyl-6-methyl-8-prenylnaringenin (**2**, 81.8 mg), mp 152−154 °C, and 5,7-dihydroxy-6-methyl-8-prenylflavanone (**3**, 180 mg), mp 183−184 °C [lit. 182−185 °C].[30] F-4−7 (0.329 g, *n*-hexane 100%) were subjected to successive recrystallizations with *n*-hexane−CH_2Cl_2 to yield 5,7-dihydroxy-6-prenylflavanone (**5**, 36.7 mg, mp 214−216 °C [lit. 212−214 °C]).[32] TLC of F-9 (0.378 g, *n*-hexane−EtOAc, 9:1) showed several spots, and it was subjected to preparative TLC with *n*-hexane−EtOAc (8:2, R_f 0.61) as developing solvent (one development), affording 5-hydroxy-7-methoxy-6-prenylflavanone (**9**, 31 mg), mp 95−96 °C [lit. 90−92].[33] F-11 (317 mg) was rechromatographed using *n*-hexane−EtOAc as gradient elution system to yield 4′-*O*-methyl-8-prenylnaringenin (**8**, 9.1 mg), mp 165−168 °C [lit. 165−167 °C].[36]

5,4′,1″-Trihydroxy-6,7-(3″,3″-dimethylchroman)flavone (1): yellow powder; mp 254−255 °C; $[\alpha]^{25}_D$ +0.3 (MeOH; *c* 0.1); UV (MeOH) λ_{max} (log ϵ) 218 (4.47), 301 (4.14) 334 (4.31) nm; IR (KBr) ν_{max} 3358, 1669, 1618, 1507, 1093, 1024 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz), ^{1}H NMR (Py-d_5, 300 MHz), and ^{13}C NMR (DMSO-d_6, 125 MHz) shifts, see Table 1; EIMS *m*/*z* 354 [M]$^+$ (65), 321 (31), 295 (100), 268 (13), 267 (9), 239 (2), 176 (6), 121 (5), 149 (4).

(*S*)- and (*R*)-MTPA Esters of 1. Compound **1** (7 mg) was dissolved in anhydrous pyridine-d_5 and placed in an NMR tube, and (*R*)-MTPA chloride was added (10 μL). In another NMR tube containing **1** as above, (*S*)-MTPA chloride was added (10 μL). In each case, the resulting mixture was stirred to afford the respective esters (**1a** and **1b**), and their ^{1}H NMR spectra were obtained (300 MHz).

(*S*)-MTPA ester of 1 (1a): ^{1}H NMR (Py-d_5, 300 MHz) δ 7.61 (2H, m, MTPA-ArH), 7.43 (3H, m, MTPA-ArH), 3.45 (3H, s, MTPA-OCH_3), 5.23 (1H, dd, J = 5.2, 7.0 Hz, H1″), 3.51 (1H, dd, J = 5.2, 13.2 Hz, H2″a), 3.43 (1H, dd, J = 7.3, 13.2 Hz, H2″b).

(*R*)-MTPA ester of 1 (1b): ^{1}H NMR (Py-d_5, 300 MHz) δ 7.63 (2H, m, MTPA-ArH), 7.47 (3H, m, MTPA-ArH), 3.47 (3H, s, MTPA-OCH_3), 5.28 (1H, dd, J = 5.1, 7.2 Hz, H1″), 3.98 (1H, dd, J = 7.2, 13.8 Hz, H2″a), 3.96 (1H, dd, J = 5.1, 13.8 Hz, H2″b).

4′-*O*-Methyl-6-methyl-8-prenylnaringenin (2): yellow solid; mp 152−154 °C (CH_2Cl_2); UV (MeOH) λ_{max} (log ϵ) 338 (1.86), 292 (8.51), 244 (6.01), 326 (1.80) 256 (3.17) nm; CD ($CHCl_3$, *c* 0.0125) $[\theta]_{292}$ −17.97, $[\theta]_{344}$ +2.91; IR (KBr) ν_{max} 3304, 2930, 2870, 1633, 1591, 1458, 1336, 1253, 1120, 1035; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; FABMS *m*/*z* 369 [M + H]$^+$ (100), 353 (18), 325 (18), 313 (25), 219 (72), 179 (66), 134 (32), 83 (14), 65 (5), 43 (5).

Hypoglycemic Activity Assay. Normal Rats. Male Wistar rats of 60−65 days old, weighing 200−250 g, fasted 18 h were divided randomly in six groups of five rats: group I served as control; group II received the vehicle (0.05% Tween 80 in saline solution); groups III, IV, and V received extracts by intragastrical route at the following doses: 30, 100, and 300 mg/kg, respectively; and group VI served as positive control (glibenclamide 10 mg/kg). Blood samples were collected, from caudal vein by means of a little incision at the end of the tail, at 0, 1.5, 3, 5, 7, and 9 h after the administration of extracts.

Blood glucose concentration was determined by enzymatic glucose oxidase method using a commercial glucometer (One Touch Basic I, Johnson−Johnson). Percentage variation of glycemia for each group was calculated with respect to initial (0 h) level according to the following: %variation = $(G_t - G_i)/G_i \times 100$ where G_i was the initial glycemia values and G_t the value at each time of sampling.[22]

Hypoglycemic Activity Assay in Streptozotocin-Induced Diabetic Rats. Male Wistar rats (60−65 days old, 200−250 g/weight) were used. Diabetes was induced through intraperitoneal administration of 60 mg/kg of streptozotocin before experimentation.[44] Six groups of five rats were randomly formed: group I (control); II (vehicle, 0.05% Tween 80 in saline solution); III (doses 30 mg/kg); IV (doses 100 mg/kg); V (doses 300 mg/kg); and VI (positive control, glibenclamide 10 mg/kg). Blood samples were collected at 0, 1.5, 3, 5, 7, and 9 h, and the glucose concentration was determined by enzymatic glucose oxidase method using a commercial glucometer (One Touch Basic I, Johnson−Johnson). The detailed protocol is published.[22] 3-*O*-Acetyloleanolic acid was evaluated at doses of 3.1, 10, and 31 mg/kg.

Statistical Analysis. Data were expressed as mean ± SEM. Statistical evaluation was done using one-way analysis of variance (ANOVA), followed by Dunnett's *t* test, $P < 0.05$. Differences were considered to be significant at $P < 0.05$.

Acknowledgment. We thank B. Quiroz, Ma. I. Chávez, Ma. T. Ramírez-Apan, A. Nieto, L. Velasco, and J. Pérez-Flores (Instituto de Química de la UNAM) for technical assistance. We also thank R. C. Durán (Herbario de la Facultad de Ciencias, UNAM) for collection and identification of the plant material. This work was supported in part by UNAM and CONACyT (Grant No. 181608).

References and Notes

(1) Brownlee, M. *Nature* **2001**, *414*, 813−820.
(2) Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. *Diabetes Care* **2004**, *27*, 1047−1053.
(3) Villarreal-Ríos, E.; Salinas-Martínez, A. M.; Medina-Jauregui, A.; Garza-Elizondo, M. E.; Núñez-Rocha, G.; Chuy-Díaz, E. R. *Arch. Med. Res.* **2000**, *31*, 511−514.
(4) Andrade-Cetto, A.; Heinrich, M. *J. Ethnopharmacol.* **2005**, *99*, 325−348.
(5) Garduño-Ramírez, M. L.; Trejo, A.; Navarro, V.; Bye, R.; Linares, E.; Delgado, G. *J. Nat. Prod.* **2001**, *64*, 432−435.
(6) Aguilar, C. A.; Xolalpa, M. S. *Ciencia* (Méx.) **2002**, *53*, 24−35.
(7) Román-Ramos, R.; Lara-Lemus, A.; Alarcón-Aguilar, F. J.; Flores-Saenz, J. L. *Arch. Med. Res.* **1992**, *23*, 105−109.
(8) Román-Ramos, R.; Alarcón-Aguilar, F. J.; Lara-Lemus, A.; Flores-Saenz, J. L. *Arch. Med. Res.* **1992**, *23*, 59−64.
(9) Román-Ramos, R.; Flores-Saenz, J. L.; Alarcón-Aguilar, F. J. *J. Ethnopharmacol.* **1995**, *48*, 25−32.
(10) Alarcón-Aguilar, F.; Román-Ramos, R.; Jiménez-Estrada, M.; Reyes-Chilpa, R.; González-Paredes, B.; Flores-Sáenz, J. L. *J. Ethnopharmacol.* **1997**, *55*, 171−177.
(11) Alarcón-Aguilar, F. J.; Román-Ramos, R.; Pérez-Gutiérrez, S.; Aguilar-Contreras, A.; Contreras-Weber, C. C.; Flores-Saenz, J. L. *J. Ethnopharmacol.* **1998**, *61*, 101−110.
(12) Alarcón-Aguilar, F. J.; Jiménez-Estrada, M.; Reyes-Chilpa, R.; Román-Ramos, R. *J. Ethnopharmacol.* **2000**, *72*, 21−27.
(13) Alarcón-Aguilar, F. J.; Jiménez-Estrada, M.; Reyes-Chilpa, R.; González-Paredes, B.; Contreras-Weber, C. C.; Román-Ramos, R. *J. Ethnopharmacol.* **2000**, *68*, 205−207.
(14) Ankli, A.; Heinrich, M.; Bork, P.; Wolfram, L.; Bauerfeind, P.; Brun, R.; Schmid, C.; Weiss, C.; Bruggisser, R.; Gertsch, J.; Wasescha, M.; Sticher, O. *J. Ethnopharmacol.* **2002**, *79*, 43−52.
(15) Wächter, G. A.; Hoffman, J. J.; Furbacher, T.; Blake, M. E.; Timmermann, B. N. *Phytochemistry* **1999**, *52*, 1469−1471.
(16) McVaugh, R. *Flora Novo-Galiciana: A Descriptive Account of the Vascular Plants of Western Mexico*; The University of Michigan, 1987; Vol. 5, Leguminosae, pp 1−786.
(17) Garduño-Ramírez, M. L.; Delgado, G.; Alvarez, L.; Narváez-Mastache, J. M. *Rev. Soc. Quím. Méx.* **2002**, *46*, Special Issue, 107.
(18) Burns, D. T.; Dalgarno, B. G.; Gargan, P. E.; Grimshaw, J. *Phytochemistry* **1984**, *23*, 167−169.
(19) Beltrami, E.; De Bernardi, M.; Fronza, G.; Mellerio, G.; Vidari, G.; Vita-Finzi, P. *Phytochemistry* **1980**, *21*, 2931−2933.
(20) Alvarez, L.; Rios, M. Y.; Esquivel, C.; Chávez, M. I.; Delgado, G.; Aguilar, M. I.; Villarreal, M. L.; Navarro, V. *J. Nat. Prod.* **1998**, *61*, 767−770.
(21) Alvarez, L.; Delgado, G. *Phytochemistry* **1999**, *50*, 681−687.
(22) Garduño-Ramírez, M. L.; Delgado, G. *Rev. Soc. Quím. Méx.* **2003**, *47*, 160−166.
(23) Akhtar, M. S.; Iqbal, J. *J. Ethnopharmacol.* **1991**, *31*, 49−57.
(24) El-Shabrawy, O. A.; Nada, S. A. *Fitoterapia* **1996**, *67*, 99−102.
(25) Shaiq, A. M.; Jahangir, M.; Shazad ul Hussan, S.; Choudhary, M. I. *Phytochemistry* **2002**, *60*, 295−299.
(26) Enigbokan, M. A.; Felder, T. B.; Thompson, J. O.; Kuti, J. O.; Ekpenyong, K. I. *Phytother. Res.* **1996**, *10*, 379−382.
(27) Mabry, T. J. K.; Marham, R.; Thomas, M. B. *The Systematic Identification of Flavonoids*; Springer-Verlag: Berlin, 1970.
(28) Nelly, D. R. *Tetrahedron: Asymmetry* **1999**, *10*, 2927−2934.
(29) Slade, D.; Ferreira, D.; Marais, J. P. J. *Phytochemistry* **2005**, *66*, 2177−2215.
(30) Ahluwalia, V. K.; Sharma, N. D.; Mittal, B.; Gupta, S. R. *Ind. J. Chem.* **1988**, *27B*, 238−241.
(31) Kaouadji, M.; Ravanel, P.; Marionett, A. M. *J. Nat. Prod.* **1986**, *49*, 153−155.
(32) Filho, R. B.; Gottlieb, O. R.; Mourao, A. P. *Phytochemistry* **1975**, *14*, 261−263.
(33) Mitscher, L. A.; Rao, G. S. R.; Khanna, I.; Veysoglu, T.; Drake, S. *Phytochemistry* **1983**, *22*, 573−576.
(34) Alves, H. M.; Arndt, V. H. W. D.; Ollis, W. B.; Eyton, O. R.; Magalhães, G. T. *Phytochemistry* **1966**, *5*, 1327−1330.
(35) Delgado, G.; Cárdenas, X.; Alvarez, L.; Romo de Vivar, A.; Pereda-Miranda, R. *J. Chem. Res.* **1986**, *286*, (S) 286−287; (M) 2565−2579.
(36) Parsons, L. C.; Gray, A. I.; Waterman, P. G. *J. Nat. Prod.* **1993**, *56*, 46−53.
(37) Arthur, H. R.; Ko, P. D. S. *Aust. J. Chem.* **1969**, *22*, 597−600.
(38) Aplin, R. T.; Halsall, T. G.; Norin, T. *J. Chem. Soc., Abstr.* **1963**, 3269−3273.
(39) Sengupta, P.; Manju, S.; Manjusree, B.; Pakrashi, S. C.; Esahak. A. *Ind. J. Chem. B* **1976**, *14B*, 817−818.
(40) Angyal, S. J.; Odier, L. *Carbohydr. Res.* **1983**, *123*, 23−29.
(41) Mustafa, G; Anis, E.; Ahmed, S.; Anis, I.; Ahmed, H.; Malik, A.; Shahzad-ul-Hassan, S.; Choudhary, M. I. *J. Nat. Prod.* **2000,** *63*, 881−883.
(42) Chen, J.; Liu, J.; Zhang, L.; Wu, G.; Hua, W.; Wu, X.; Sun, H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2915−2919.
(43) Farnsworth, N. R.; Blomster, R. N.; Damratoski, D.; Meer, W. A.; Cammarato, L. V. *Lloydia* **1964**, *27*, 302−314.
(44) Merzouk, H.; Madani, S.; Chabane, D.; Prost, J.; Bouchenak, M.; Belleville, J. *Clin. Sci.* **2000**, *98*, 21−30.

NP060166Z