Hypoglycemic Activity of Extracts and Compounds from the Leaves of *Hintonia standleyana* and *H. latiflora*: Potential Alternatives to the Use of the Stem Bark of These Species[§,1]

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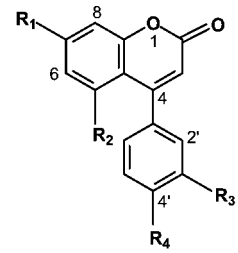
The CH_2Cl_2−MeOH (1:1) extract of the leaves of *Hintonia standleyana* and *H. latiflora* caused significant decrease in blood glucose levels in both normal and streptozotozin (STZ)-induced diabetic rats when compared with vehicle-treated groups ($p < 0.05$). These extracts were not toxic to mice according to the Lorke criteria. From the hypoglycemic extract of *H. standleyana*, two new 4-phenylcoumarins, namely, 6″-*O*-acetyl-5-*O*-β-D-galactopyranosyl-7,4′-dihydroxy-4-phenylcoumarin (**1**) and 6″-*O*-acetyl-5-*O*-β-D-galactopyranosyl-7,3′,4′-trihydroxy-4-phenylcoumarin (**2**), were obtained. The analogous extract of *H. latiflora* yielded the new 5-*O*-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl]-7,4′-dimethoxy-4-phenylcoumarin (**3**) along with several known compounds, including ursolic acid and desoxycordifolinic acid. Phenylcoumarins **1** and **2** showed hypoglycemic activity. HPLC profiles of the leaf extracts of both plants revealed the presence of known hypoglycemic phenylcoumarins as well as chlorogenic acid. The overall results have indicated that the leaves of *H. standleyana* and *H. latiflora* possess similar antidiabetic potential to their stem bark. Therefore, the leaves from these species could represent an alternative to the use of their stem bark, which, in turn, would contribute to the conservation of these Mexican medicinal plants.

The extremely bitter stem bark of some Mexican Rubiaceae species, commonly known as "copalchis", is highly valued in folk medicine for treating type 2 diabetes.[3−5] *Hintonia standleyana* Bullock, *H. latiflora* (Sesse et Mociño ex DC.) Bullock (syn. *Coutarea latiflora* Sesse et Mociño ex DC.), *Exostema caribaeum* (Jacq.) Roem. et Schult., and *E. mexicanum* Gray are the most widely used species. The chemical composition[6−11] as well as the antidiabetic,[3,4,11−15] antimalarial,[10,16] and antimicrobial[17] properties of the stem bark of *H. latiflora* have been the subject of several investigations. More recently, we have described the antidiabetic and antinociceptive properties as well as some chemical constituents of *H. standleyana* stem bark.[18,19] In the last 50 years, the stem bark of both these *Hintonia* species has been exploited extensively and commercialized, locally and outside of Mexico, without any official regulation. Furthermore, the crude drugs are harvested from wild plants, thereby causing irreversible damage to these resources. Accordingly, the "copalchis" are now scarce and in danger of extinction.[20] For this reason, it is important to protect these valuable resources by regulating their commercialization and finding similar therapeutic alternatives. With this scenario, the present investigation was undertaken as a preliminary step to determine if the aerial parts of *H. standleyana* and *H. latiflora* possess the same antidiabetic properties as their stem bark in animal models. The final goal would be to recommend the use of the leaves rather than the stem bark, thus contributing to the conservation of both species. Consequently, herein, we report the potential toxicity and short-term hypoglycemic and antihyperglycemic effects of crude organic extracts obtained from the leaves of *H. standleyana* and *H. latiflora*. In addition, the isolation and identification of three new analogues of known 4-phenylcoumarins (**1**−**3**) from these two species is described.



	R_1	R_2	R_3	R_4
1	OH	6"-*O*-acetyl-5-*O*-β-D-galactopyranosyl	H	OH
2	OH	6"-*O*-acetyl-5-*O*-β-D-galactopyranosyl	OH	OH
3	OCH_3	5-*O*-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl]	H	OCH_3
4	OCH_3	5-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl]	OH	OH
5	OCH_3	5-*O*-[β-D-xylopyranosyl- (1→6)-β-D-glucopyranosyl]	OH	OH
6	OCH_3	5-*O*-β-D-galactopyranosyl	OH	OH
7	OCH_3	5-*O*-β-D-glucopyranosyl	OH	OH
8	OH	5-*O*-β-D-glucopyranosyl	OH	OH

Results and Discussion

The potential toxic effects of the leaves of *H. latiflora* (HLE) and *H. standleyana* (HSE) were assessed preliminarily in mice according to the Lorke procedure.[21] For this, mice were treated orally with increasing doses (10−5000 mg/kg) of their crude extracts prepared with CH_2Cl_2−MeOH (1:1). In the case of *H. latiflora*, the calculated LD_{50} of the extract was 1.67 g/kg; this figure was slightly higher than the LD_{50} previously reported for the stem bark extract (2.85 g/kg).[22] On the other hand, the LD_{50} of HSE was higher than 5 g/kg. In either case, the treatments did not provoke behavioral alterations, lesions, or bleeding of the internal tissues and organs of the animals throughout the experiments. Therefore, according to the Lorke criteria, neither of the extracts was regarded as toxic.

Next, the hypoglycemic activity of the crude extracts (HSE and HLE) in normoglycemic and streptozotocin (STZ)-induced diabetic

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Table 1. Effects of the Crude Extracts of the Leaves of *Hintonia standleyana* and *H. latiflora* on Blood Glucose Levels in Normal Rats[a]

test samples	dose (mg/kg)	initial glycemia (mg dL^{-1})	% variation of glycemia				
		0 h	1.5 h	3 h	5 h	7 h	9 h
control (vehicle)		96.7 ± 1.9	2.3 ± 5.1	0.2 ± 5.7	−15.7 ± 3.4	−16.6 ± 4.0	−22.3 ± 3.9
H. standleyana							
glibenclamide	10	101.2 ± 3.1	−12.1 ± 3.9*	−22.5 ± 6.1*	−38.2 ± 5.3*	−49.1 ± 5.1*	−43.4 ± 4.4*
H. standleyana							
HSE	100	97.3 ± 2.2	−2.2 ± 5.8	−17.2 ± 4.5*	−36.3 ± 1.2*	−39.2 ± 4.7*	−36.1 ± 6.7*
HSE	300	99.5 ± 5.6	−2.2 ± 7.4	−18.5 ± 3.9*	−26.0 ± 6.9*	−48.2 ± 4.4*	−44.6 ± 3.2*
control (vehicle)		93.5 ± 3.3	−0.2 ± 4.1	−10.4 ± 3.6	−23.5 ± 3.3	−16.9 ± 1.9	−13.0 ± 2.3
H. latiflora							
glibenclamide	10	104 ± 3.4	−14.4 ± 5.1*	−31.6 ± 6.2*	−43.2 ± 4.5*	−45.1 ± 4.4*	−48.5 ± 4.4*
H. latiflora							
HLE	100	94.6 ± 2.3	−1.7 ± 2.3	−17.6 ± 2.2	−24.5 ± 1.8	−17.1 ± 3.1	−22.5 ± 0.8*
HLE	300	96.3 ± 3.0	−10.0 ± 4.1	−29.6 ± 3.6*	−33.6 ± 2.6*	−33.3 ± 3.0*	−37.6 ± 2.1*

[a] Each value is the mean ± SEM for 6 rats in each group. * $p < 0.05$ significantly different ANOVA followed by Dunnett's *t*-test for comparison with respect to negative control values at the same time.

Table 2. Effect of the Crude Extract of the Leaves of *Hintonia standleyana* and *H. latiflora* on Blood Glucose Levels in STZ-Induced Diabetic Rats[a]

test samples	dose (mg/kg)	initial glycemia (mgdL^{-1})	% variation of glycemia				
		0 h	1.5 h	3 h	5 h	7 h	9 h
control (vehicle)		279.3 ± 6.3	8.7 ± 3.8	−3.9 ± 3.7	−7.9 ± 6.7	−9.8 ± 4.9	−17.6 ± 3.3
H. standleyana							
glibenclamide	10	286.2 ± 3.1	−17.8 ± 7.7*	−23.5 ± 7.1*	−34.6 ± 6.8*	−35.4 ± 5.9*	−33.9 ± 5.7*
H. standleyana							
HSE	100	287.8 ± 2.2	−6.6 ± 5.5	−22.0 ± 5.2*	−31.6 ± 7.6*	−32.2 ± 6.5*	−30.9 ± 9.1*
HSE	300	289.0 ± 5.6*	−15.7 ± 2.1	−25.4 ± 4.1*	−34.2 ± 3.8*	−34.1 ± 4.7*	−39.6 ± 4.8*
control (vehicle)		253 ± 32.3	13.8 ± 4.0	19.9 ± 15.0	6.7 ± 8.7	−6.3 ± 7.9	−12.8 ± 10.8
H. latiflora							
glibenclamide	10	245.5 ± 51.8	−9.3 ± 8.6	−24.8 ± 11.1	−40.2 ± 12.7*	−52.5 ± 13.3*	−59.7 ± 11.4*
H. latiflora							
HLE	100	273.8 ± 46.5	3.56 ± 6.9	−12.7 ± 11.3	−44.2 ± 14.4*	−57.0 ± 17.3*	−62.6 ± 13.5*
HLE	300	272.3 ± 19.5	−3.9 ± 10.5	−25.5 ± 10.4*	−50.8 ± 14.5*	−64.4 ± 14.0*	−68.8 ± 11.7*

[a] Each value is the mean ± SEM for 6 rats in each group. * $p < 0.05$ significantly different ANOVA followed by Dunnett's *t*-test for comparison with respect to negative control values at the same time.

Table 3. Effect of Compounds **1** and **2** from the Leaves of *Hintonia standleyana* on Blood Glucose Levels in Normal Rats[a]

test samples	dose (mg/kg)	initial glycemia >(mg dL^{-1})	% variation of glycemia				
		0 h	1.5 h	3 h	5 h	7 h	9 h
control (vehicle)		86.6 ± 2.2	3.1 ± 5.7	−2.5 ± 3.8	−18.7 ± 1.6	−20.2 ± 4.0	−18.2 ± 3.5
glibenclamide	10	96.4 ± 3.4	−8.4 ± 4.4*	−30.1 ± 5.1*	−47.6 ± 1.9*	−41.8 ± 2.8*	−37.1 ± 2.9*
compound **1**	10	91.0 ± 3.0	−10.3 ± 3.8*	−17.8 ± 3.9*	−20.9 ± 3.6*	−27.2 ± 1.4*	−24.0 ± 6.7*
compound **2**	10	96.8 ± 2.2	−9.2 ± 4.1	−29.9 ± 3.2*	−28.6 ± 3.2*	−25.0 ± 2.2*	−30.6 ± 3.2*

[a] Each value is the mean ± SEM for 6 rats in each group. * $p < 0.05$ significantly different ANOVA followed by Dunnett's *t*-test for comparison with respect to negative control values at the same time.

rats was determined using standard protocols.[11,18] As shown in Tables 1 and 2, HSE and HLE treatments (100 and 300 mg/kg) caused a significant decrease in blood glucose levels in both normal and STZ-diabetic rats when compared with vehicle-treated groups ($p < 0.05$). In normoglycemic animals, the highest hypoglycemic effect of both extracts was observed at a dose of 300 mg/kg. In the case of HSE the activity (−48.2%) was observed with the dose of 300 mg/kg after 7 h and persisted throughout the experiment; for HLE, an effect (−37.6%) was observed at 300 mg/kg after 9 h (Table 1). In diabetic animals, the maximum hypoglycemic effect of both extracts was observed at the 300 mg/kg dose, but the effect of HLE was significantly higher than the HSE effect (HSE: −39.6% at 9 h; HLE: −68.8% at 9 h) (Table 2).

The active extracts were subjected to chromatographic fractionation. This process led to the isolation of the new 4-phenylcoumarin analogues 6″-*O*-acetyl-5-*O*-β-D-galactopyranosyl-4′,7-dihydroxy-4-phenylcoumarin (**1**) and 6″-*O*-acetyl-5-*O*-β-D-galactopyranosyl-3′,4′,7-trihydroxy-4-phenylcoumarin (**2**) from *H. standleyana*. In the case of *H. latiflora*, the new 4-phenylcoumarin 5-*O*-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl]-7,4′-dimethoxy-4-phenylcoumarin (**3**) was isolated. The structures of **1**−**3** were elucidated using spectroscopic methods including one- and two-dimensional NMR techniques.

The HRFABMS (positive mode) of **1**−**3** were very similar, showing quasimolecular ions $[M + H]^+$ at *m/z* 475.1159 ($C_{23}H_{22}O_{11}$), 491.1109 ($C_{23}H_{22}O_{12}$), and 593.1799 ($C_{28}H_{32}O_{14}$), respectively. The NMR spectra (CD_3OD, Table 5) were very similar to other 4-phenylcoumarin glycosides isolated from the bark of *H. standleyana*, *H. latiflora*, and *E. caribaeum*.[11,18,23,24] The aromatic region of the ^{1}H NMR spectrum of **1**−**3** (Table 5) displayed signals

Table 4. Effect of Compounds **1** and **2** from the Leaves of *Hintonia standleyana* on Blood Glucose Levels in STZ-Induced Diabetic Rats[a]

		initial glycemia (mg dL^{-1})	% variation of glycemia				
test simples	dose (mg/kg)	0 h	1.5 h	3 h	5 h	7 h	9 h
control (vehicle)		282.8 ± 3.6	0.3 ± 3.5	−15.6 ± 1.7	−20.7 ± 2.1	−23.0 ± 1.5	−19.3 ± 1.4
glibenclamide	10	280.2 ± 5.1	−13.7 ± 3.6*	−35.5 ± 1.4*	−35.9 ± 2.6*	−35.2 ± 1.6*	−36.7 ± 2.5*
compound **1**	10	277.4 ± 4.4	−11.0 ± 1.8*	−24.9 ± 2.1*	−22.7 ± 3.6	−25.2 ± 2.5	−27.1 ± 4.5
compound **2**	10	276.4 ± 4.2	−5.0 ± 2.8	−20.7 ± 4.6*	−29.5 ± 4.8*	−34.7 ± 5.2*	−37.2 ± 5.5*

[a] Each value is the mean ± SEM for 6 rats in each group. * $p < 0.05$ significantly different ANOVA followed by Dunnett's *t*-test for comparison with respect to negative control values at the same time.

Table 5. NMR Spectroscopic Data (100 MHz ^{13}C, 400 MHz ^{1}H, CD_3OD) for the 4-Phenylcoumarins **1**−**3**

	1		**2**		**3**	
position	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
2	163.2		163.2		162.9	
3	112.5	5.87, s	112.5	5.87, s	113.4	5.93, s
4	158.3		158.0		157.9	
4a	104.3		104.2		105.7	
5	159.0		158.2		158.0	
6	98.2	6.52, d (2.2)	98.2	6.52, d (2.4)	96.4	6.69, d (2.5)
7	163.3		163.3		165.0	
8	100.8	6.48, d (2.2)	100.8	6.47, d (2.4)	100.5	6.70, d (2.0)
8a	158.0		157.4		157.1	
1′	132.4		132.9		133.3	
2′	130.4	6.85, d (8.4)	117.0	6.85, d (2.1)	130.3	7.30, d (9.0)
3′	115.5	7.24, d (8.8)	145.3		114.1	6.97, d (9.0)
4′	157.4		147.1		161.4	
5′	115.5	6.85, d (8.4)	115.8	6.83, d (7.8)	114.1	6.97, d (9.0)
6′	130.4	7.24, d (8.8)	120.8	6.73, dd (8.1, 2.1)	130.3	7.30, d (9.0)
1″	101.9	4.67, d (7.8)	101.8	4.69, d (7.8)	101.3	4.80, d (8.0)
2″	71.8	3.08, dd (7.8)	71.8	3.13, dd (7.8)	74.3	2.55, dd (9.2, 8.0)
3″	74.4	3.42, dd (9.8, 3.4)	74.4	3.44, dd, (9.6,3.3)	77.7	3.26, dd (9.0)
4″	69.8	3.78, brd (2.4)	69.9	3.78, da (3.3)	71.2	3.16, dd (9.5)
5″	74.4	3.83, m	74.3	3.84, m	77.7	3.25, dd (9.5)
6″	64.5	4.36, dd (11.6, 8.4) 4.16, dd (11.6, 4.0)	64.5	4.36, dd (11.4, 8.2) 4.15, dd (11.4, 4.2)	70.2	4.00, dd (11.7, 2.5) 3.73, dd (12.0, 6.5)
1‴					105.4	4.24, d (7.5)
2‴					74.9	3.18, dd (9.0, 7.5)
3‴					77.2	3.55, dd (9.5)
4‴					71.1	3.47, m
5‴					66.8	3.08 dd (11.5, 10.0) 3.82 dd (11.5, 5.0)
OCH_3-7					56.5	3.90, s
OCH_3-4′					55.9	3.85, s
$-\underline{OCO}CH_3$	169		169			
$-OCO\underline{CH_3}$	20	2.10, s	20	2.10, s		

due to (i) two *meta*-coupled hydrogens (H-6 and H-8) and (ii) an ABX system formed by H-2′, H-5′, and H-6′ in the case of **2**, or an AA′BB′ system produced by H-2′, H-6′, H-3′, and H-5′ for **1** and **3**. (iii) Finally, the typical singlet (~δ_H 5.87) signal for the hydrogen of the α-pyrone ring system (H-3) was observed. The NMR spectra of **1** and **2** displayed also the resonances for a sugar moiety, and those of **3** for a disaccharide unit. The carbon resonances (Table 5) of the 4-phenylcoumarin portion were assigned through the analysis of the HSQC and HMBC experiments and by comparison with suitable models.[11,18,23,24] The ^{13}C NMR chemical shifts (Table 5) observed in the sugar region of **1** and **2** confirmed the presence of one β-D-acetylgalactopyranosyl moiety in these molecules. The presence of fragment ions at *m/z* 415 $[M + H - CH_3CO_2H]^+$ and 313 $[M + H - C_6O_5H_{11}]^+$ or *m/z* 431 $[M + H - CH_3CO_2H]^+$ and 329 $[M + H - C_6O_5H_{11}]^+$ in the FABMS of **1** and **2**, respectively, indicated consecutive losses of one hexose unity and an acetoxy moiety from the molecular ions. Furthermore, the HMBC correlations between C-5 and the anomeric hydrogen of galactose (H-1″) clearly indicated that the acetylgalactosyl unit is attached to the C-5 hydroxyl of the aglycon in both cases. The upfield shift of H-2″, due to the protection exerted by the phenyl group at C-4 of the phenylcoumarin core, was consistent with this proposal. In the case of compound **3**, the nature of the disaccharide unit was initially determined by detailed analysis of the carbon chemical shifts of the sugar region, which were identical to those of 5-*O*-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl]-7-methoxy-3′,4′-dihydroxy-4-phenylcoumarin previously isolated from *H. latiflora*[12] and *Coutarea hexandra*.[26] This observation was consistent with the existence of ion fragments at *m/z* 460 $[M + H - C_5H_9O_4]^+$ and 299 $[M + H - C_{11}O_{18}H_9]^+$, indicating consecutive losses of one xylose unit and one glucose moiety from the molecular ion. The HMBC correlations of the anomeric hydrogen of glucose (H-1″; δ_H 4.8) to C-5 (δ_C158.0) of the aglycon unit and the anomeric hydrogen of xylose (H-1‴; δ_H 4.24) to C-6″ (δ_C 70.2) were in agreement with the 1→6 linkage and with the attachment of the glucose moiety at the C-5 hydroxyl. The NOESY interaction H-6 (δ_H 6.69)/H-1″ (δ_H 4.8) was in accord with the disaccharide linkage site. The nature of the sugars was confirmed by methanolysis with MeOH and 1 M HCl.[25] Thus, compounds **1** and **2** led to the isolation of the β-D-methylgalactopyranoside with a positive optical rotation. Compound **3**, under the same conditions, afforded β-D-

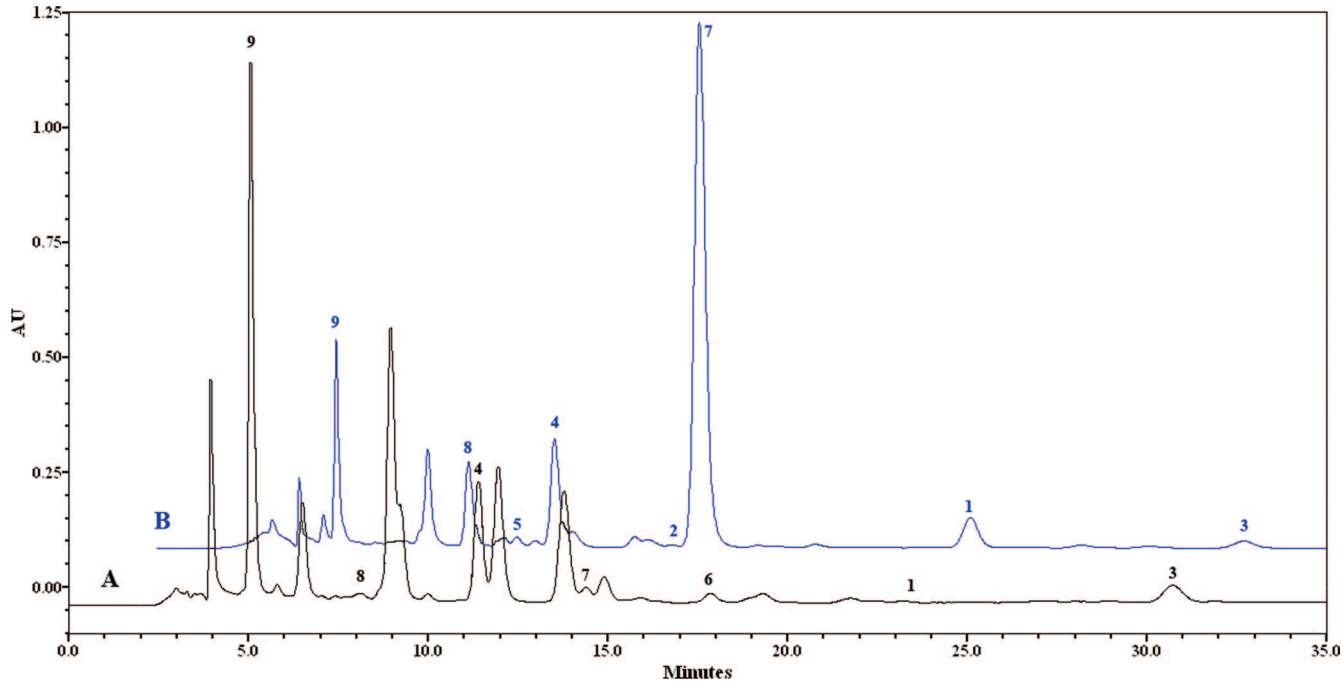

Figure 1. Analytical HPLC chromatogram recorded at 327 nm of the components of the leaf infusion from *H. latiflora* (A) and *H. standleyana* (B). (A) Peak identification (t_R, min): **1** (23.41); **3** (30.82); **4** (11.33); **6** (17.94); **7** (14.36); **8** (8.30); and **9** (5.04). (B) Peak identification (t_R, min): **1** (23.54); **2** (14.44); **3** (30.35); **4** (11.17); **5** (10.11); **7** (14.2); **8** (8.77); and **9** (5.09). For chromatographic conditions, see the Experimental Section.

methylglucopyranoside and β-D-methylxylopyranoside with positive and negative optical rotations, respectively.

The active principles of the vernacular preparations (infusions and decoctions) obtained from the stem barks of "copalchis" are mainly 4-phenylcoumarins glycosides.[27] Therefore the infusions of the leaves of both *Hintonia* species were analyzed by HPLC in order to establish their 4-phenylcoumarin profiles. The results are summarized in Figure 1. In each case, most of the components were identified by spiking the infusion with the appropriate standards. In the case of *H. standleyana*, the main component was 5-*O*-β-D-glucopyranosyl-7-methoxy-3′,4′-dihydroxy-4-phenylcoumarin (**7**), which possesses the noted hypoglycemic effect. However, in the case of *H. latiflora*, the main component was not a phenylcoumarin but chlorogenic acid (**9**). The antidiabetic[28] and antioxidant[29] properties of chlorogenic acid have been demonstrated. Compound **9** promotes a significant reduction in the plasma glucose peak during the oral glucose tolerance test, which suggests its potential as a glycemic index lowering agent and for reducing the risk of developing type 2 diabetes.[30,31] The remaining constituents in the infusions were mostly 4-phenylcoumarins with known hypoglycemic activity.[11,18]

Coumarins **1** and **2**, newly isolated in this study, were evaluated in the same assays as the extracts. As shown in Tables 3 and 4, compound **2** (10 mg/kg) did cause a significant decrease in blood glucose levels in both normal and STZ-diabetic rats when compared with vehicle-treated groups ($p < 0.05$). In normoglycemic animals, the hypoglycemic effect of **2** was around −30.6% at 9 h. In diabetic animals, the highest antihyperglycemic effect of **2** was observed at 7 and 9 h (−34.7% and −37.2%, respectively). Compound **3** was not evaluated due to the scarcity of the sample. As previously observed, the results found in the present investigation showed that the nature of the sugar moiety at C-5 is not relevant for the biological action since **2** was more active than **1**. On the other hand, the presence of a catechol unit in **2** revealed its relevance for the hypoglycemic effect.

The presence of ursolic acid, with known hypoglycemic and antioxidant[32,33] properties as well as its stimulating glucose uptake and enhancing insulin receptor phosporylation activities,[34] could partially account for the observed hypoglycemic effect of HLE.

In conclusion, the leaves of *H. standleyana* and *H. latiflora* are effective as hypoglycemic agents in diabetic conditions using experimental animal models. The antidiabetic properties demonstrated in this study for both *Hintonia* species are due mainly to the presence of 4-phenylcoumarins. Since the leaves of both species are devoid of toxic effects and displayed discernible hypoglycemic effects, they could be an excellent alternative to the use of their stem bark. The use of the leaves as renewable resources might also contribute to the conservation of these Mexican plants, which have been recently overexploited.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 digital polarimeter. IR spectra were recorded as KBr pellets or film on a Perkin-Elmer 59913 spectrophotometer. NMR spectra were recorded on a Varian VXR-300S spectrometer, at either 400 MHz (^{1}H) or 100 (^{13}C) MHz, in CD_3OD using tetramethylsilane (TMS) as an internal standard. EIMS were obtained on a JMS-AX505HA mass spectrometer. Positive FABMS data were obtained with a JEOL SX 102 mass spectrometer using an NBA matrix. Open column chromatography was carried out on silica gel 60 (70−230 mesh, Merck). HPLC analysis was performed on a Waters HPLC instrument equipped with a UV/visible detector model 2487 set at 327 nm, a pump (Waters 600), a low-pressure solvent mixing valve, a manual sampler injector, and a degasser unit. Control of the equipment, data acquisition, processing, and management of chromatographic information were performed by the Millennium 32 software program (Waters). TLC analysis was performed on silica gel 60 F_{254} plates (Merck), and visualization of the plates was carried out using ceric sulfate (10%) solution in H_2SO_4 or anisaldehyde-H_2SO_4 reagent (for sugars).

Plant Material. The leaves (500 g) of *H. standleyana* were collected in Atenango del Rio, Guerrero, in May 2005 and identified by Raymundo Rufino and Francisco Ramos from the Mexican National Herbarium (MEXU); a voucher specimen (P. Hersch No 423) was deposited at the ASFM-INAH Herbarium, Cuernavaca. The leaves (370 g) of *H. latiflora* were collected in Batopilas, Chihuahua, in November

2006 and identified by Robert Bye from the UNAM Botanical Garden; a voucher specimen (R. Bye 34880) was deposited at MEXU, Mexico.

Extraction and Isolation. Dried and shredded leaves of *H. standleyana* (500 g) and *H. latiflora* (370 g) were macerated with CH_2Cl_2−MeOH (1:1) (2 L × 3) for 6 days at room temperature. The extracts were evaporated in vacuo to yield 80 g of a green residue from *H. standleyana* and 60 g of a green residue from *H. latiflora*. The dried extract of *H. standleyana* (50 g) was chromatographed in a glass column packed with silica gel (1 kg) eluting with hexane−EtOAc (3:7 → 0:1) and EtOAc−MeOH (1:0 → 0:1) to yield eight primary fractions (FH1−FH8). Fraction FH3 (1.2 g), eluted with hexane−EtOAc (5:95), was further chromatographed over a silica gel column using hexane−EtOAc (1.5:8.5 → 0:1) and EtOAc−MeOH (1:0 → 8:2) as eluents to give seven fractions (FH3I−FH3VII). From fraction FH3V (700 mg), eluted with EtOAc−MeOH (99:1), spontaneously crystallized 400 mg of **2**. Fraction FH5 (4 g), eluted with EtOAc−MeOH (9:1), was further chromatographed over a silica gel column, using EtOAc−MeOH (1:0 → 5:5) as eluents, to render 10 fractions (FH5I−FH5X). From fraction FH5VII eluted with EtOAc−MeOH (8:2) spontaneously crystallized 650 mg of **1**.

The dried extract of *H. latiflora* (40 g) was chromatographed in a glass column packed with silica gel (1.5 kg) eluting with hexane−EtOAc (1:0 → 0:1) and EtOAc−MeOH (1:0 → 1:1) to yield six primary fractions (F1−F6). From fraction F2 (500 mg), eluted with EtOAc−MeOH (9:1), spontaneously crystallized 371 mg of ursolic acid. Fraction F6 (5 g), eluted with EtOAc−MeOH (1:1), was further chromatographed over a silica gel column with $CHCl_3$−MeOH (1:0 → 1:1) to obtain 13 fractions (F6I−F6XIII). Fraction F6XI (24.1 mg), eluted with $CHCl_3$−MeOH (1:1), was purified by using preparative TLC [silica gel; $CHCl_3$−MeOH-H_2O (5:1:1)] in order to isolate 5 mg of desoxycordifolinic acid. Fraction F4 (5 g), eluted with EtOAc−MeOH (1:1), was additionally chromatographed over a silica gel column with CH_2Cl_2−MeOH (1:0 → 1:1) to afford 13 fractions (F4I−F4XIII). HPLC chromatographic separation of fraction F4VII (200 mg) on a Merck-Purospher Star RP-18e (5 μm; 250 mm × 4.6 mm) column, eluting with CH_3CN−H_2O (3:7), 0.4 mL/min flow rate, yielded 12 mg of compound **3**.

6″-*O*-Acetyl-5-*O*-β-D-galactopyranosyl-4′,7-dihydroxy-4-phenylcoumarin (1): yellow solid; mp 153−156 °C; $[\alpha]_D$ −27 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 325 (3.95), 329 (4.05) nm; IR (KBr) λ_{max} 3450, 1705, 1620, 1510,1430, 1360, 1265, 1090 cm^{-1}; ^{13}C and ^1H NMR, see Table 5; FABMS *m/z* 475 $[M + H]^+$, 415 $[M + H - CH_3CO_2H]^+$, 313 $[M + H - C_6O_5H_{11}]^+$; HRFABMS *m/z* 475.1159 $[M + H]^+$ (calcd for $C_{23}H_{23}O_{11}$, 475.1162).

6″-*O*-Acetyl-5-*O*-β-D-galactopyranosyl-3′,4′,7-trihydroxy-4-phenylcoumarin (2): beige solid; mp 155−158 °C; $[\alpha]_D$ −29 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 328 (4.05), 332 (4.11) nm; IR (KBr) λ_{max} 3430, 1705, 1615, 1515,1435, 1365, 1265, 1080 cm^{-1}; ^{13}C and ^1H NMR, see Table 5; FABMS *m/z* 491 $[M + H]^+$, 431 $[M + H - CH_3CO_2H]^+$, 329 $[M + H - C_6O_5H_{11}]^+$; HRFABMS *m/z* 491.1109 $[M + H]^+$ (calcd for $C_{23}H_{23}O_{12}$, 491.1111).

5-*O*-[β-D-Xylopyranosyl-(1→6)-β-D-glucopyranosyl]-7,4′-dimethoxy-4-phenylcoumarin (3): yellow solid; mp >320 °C; $[\alpha]_D$ −26 (*c* 0.01, MeOH); UV (MeOH) λ_{max} 327 (4.00), 330 (4.10) nm; IR (KBr) ν_{max} 3403, 1702, 1653, 1612, 1512, 1367, 1074 cm^{-1}; ^{13}C and ^1H NMR, see Table 5; FABMS *m/z* 593 $[M + H]^+$, 460 $[M + H - C_5H_9O_4]^+$, 299 $[M + H - C_{11}O_{18}H_9]^+$; HRFABMS *m/z* 593.1799 $[M + H]^+$ (calcd for $C_{28}H_{33}O_{14}$, 593.1792).

Analysis of Sugar Moieties of Compounds 1−3. A 10 mg quantity of each compound (**1**−**3**), dissolved in 5 mL of MeOH, was refluxed separately with 10 mL of HCl (1 M) for 2 h at 90 °C. The reaction mixture was diluted with H_2O and extracted twice with EtOAc. The H_2O layer was passed through an Amberlite MB-3 column. The eluates were then concentrated in vacuo to dryness to yield 2 mg of methyl-β-D-galactopyranoside as a glassy solid, $[\alpha]_D$ +44.5 (*c* 0.01, H_2O), in the case of **1** and **2**. For compound **3** the solid residue was subjected to HPLC separation using the same Merck-Purospher Star RP-18e column (eluting with H_2O and 0.4 mL/min flow rate) to afford 1 mg of methyl-β-D-glucopyranoside, $[\alpha]_D$ +120 (*c* 0.01, H_2O), and methyl β-D-xylopyranoside, $[\alpha]_D$ −55 (*c* 0.01, H_2O).

HPLC Profiles. The standards 5-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl]-7-methoxy-3′,4′-dihydroxy-4-phenylcoumarin (**4**), 5-*O*-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl]-7-methoxy-3′,4′-dihydroxy-4-phenylcoumarin (**5**), 5-*O*-β-D-galactopyranosyl]-7-methoxy-3′,4′-dihydroxy-4-phenylcoumarin (**6**), 5-*O*-β-D-glucopyranosyl-7-methoxy-3′,4′-dihydroxy-4-phenylcoumarin (**7**), and 5-*O*-β-D-glucopyranosyl]-7,3′,4′-trihydroxy-4-phenylcoumarin (**8**) were isolated from the stem bark of *H. standleyana* and *H. latiflora*, respectively, as previously described.[8,9,11,18] Their purity was verified by HPLC and NMR. Chlorogenic acid (**9**) was purchased from Sigma-Aldrich (St. Louis, MO). HPLC grade CH_3CN and MeOH were obtained from Honeywell Burdick & Jackson (Morristown, NJ); trifluoroacetic acid was purchased from Sigma-Aldrich. The stock standard solutions were prepared separately by accurately weighing 10 mg, pouring into 10 mL volumetric flasks, and dissolving in CH_3CN−H_2O (1:3). The solutions for analysis were prepared using 1.5 g of milled (particle size < 2000 μm, mesh size 2 mm) *H. standleyana* or *H. latiflora* extracted in 100 mL of hot water for 30 min and then filtered through Whatman No. 1 filter paper and poured into a volumetric flask and made up to 250 mL with distilled water. For the HPLC profile of each aqueous extract, a Symmetry C_8 (series WO3251R012) column (5 μm particle size, 3.9 × 150 mm i.d.) was used. The elution system consisted of CH_3CN−H_2O 0.1% trifluoroacetic acid (19:81) at a flow rate of 0.4 mL/min, and the injection volume was 20 μL in all cases. Identification of compounds in the chromatograms was performed from their retention times and by spiking with standards (10 μL of standard stock solution) separated under the same conditions.

Test Animals. The toxicity studies were performed on male mice ICR (body weight range, 25−30 g); the antidiabetic tests were accomplished with Wistar rats (body weight range 180−220 g). Both types of animal were purchased from Centro UNAM-Harlan (Harlan México, SA de CV). Procedures involving animals and their care were conducted in conformity with the Mexican Official Norm for Animal Care and Handing (NOM-062-ZOO-1999) and in compliance with international rules on care and use of laboratory animals. The animals were housed under standard laboratory conditions and maintained on standard pellet diet and water ad libitum.

Acute Toxicity Study in Mice. The experiments were carried out in two phases as previously described.[22] In the first, intragastric doses of 10, 100, and 1000 mg/kg of crude extract were administered. On the second phase, doses of 1600, 2900, and 5000 mg/kg were administered to mice following the Lorke method criteria. In both phases, mice were observed daily during a period of 14 days for mortality, toxic effects, and/or changes in behavioral pattern. At the end of the experiments, the animals were sacrificed in a CO_2 chamber.

Preparation of the Test Samples and Collection of Blood Samples. The samples were suspended in 0.05% Tween 80 in saline solution. Glibenclamide (10 mg/kg bw, Sigma-Aldrich Co.) was used as a hypoglycemic model drug.[18] Control rats received only the vehicle (0.05% Tween 80 in saline solution) in the same volume (0.5 mL of vehicle/100 g bw) by the same route. Blood samples were collected from caudal vein by means of a small incision in the end of the tail. Blood glucose levels (mg/dL) were estimated by enzymatic glucose oxidase method using a commercial glucometer (One Touch Ultra, Milpitas, CA). Percentage variation of glycemia for each group was calculated with respect to initial (0 h) level according to the following: % variation of glycemia = $[(G_i - G_t)/G_i] \times 100\%$ where G_i is initial glycemia values and G_t is the glycemia value after sample administration.

Acute Hypoglycemic Assay. The assays were carried out as previously reported.[11,18] HSE and HLE treatments (100 and 300 mg/kg bw), compounds **1** and **2** (10 mg/kg bw), or glibenclamide was administered by intragastrical route. Blood samples were collected at 0, 1.5, 3, 5, 7, and 9 h after drug administration.

Induction of Experimental Diabetes and Antihyperglycemic Assay. Diabetes mellitus was induced in rats by a single intraperitoneal injection of freshly prepared STZ (50 mg/kg; Sigma-Aldrich Co.) dissolved in 0.1 M citrate buffer, pH 4.5, in a volume of 1 mL/kg bw. After 7 days of STZ administration, blood glucose levels of each rat were determined. Rats with blood glucose levels higher than 250 mg/dL were considered diabetic and included in the study.[11] Diabetic rats were treated as previously described.[11,18] HSE and HLE (100 and 300 mg/kg bw), pure **1** and **2** (10 mg/kg bw), or glibenclamide (10 mg/kg bw) was administrated by intragastric route. Blood samples were collected at 0, 1.5, 3, 5, 7, and 9 h after drug administration.

Statistical Analysis. Data are expressed as the means ± SEM for the number ($n = 6$) of animals in each group. Repeated measurement analysis of variance (ANOVA) was used to analyze the changes in blood glucose and other parameters. Dunnett range post hoc compari-

sons were used to determine the source of significant differences where appropriate; $p < 0.05$ was considered statistically significant. Prisma Graph-Pad (version 4.0) software was used for statistics.

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