Antioxidant Phenylethanoid Glycosides and a Neolignan from *Jacaranda caucana*

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Extracts from several plants of the family Bignoniaceae from Panama were submitted to a rapid DPPH TLC test for the detection of radical-scavenging activity. The MeOH extract of the stems of *Jacaranda caucana*, a tree that grows from Costa Rica to Colombia, was selected due to its interesting activity and the lack of phytochemical studies on the polar extract. This extract was partitioned between ethyl acetate, butanol, and water. The EtOAc fraction afforded two new phenylethanoid glycosides (**1**, **2**), along with protocatechuic acid, acteoside, and jionoside D. Further purifications yielded isoacteoside and martynoside. The BuOH fraction afforded a new rhamnosyl derivative of sisymbrifolin (**8**), a neolignan. The structures were determined by means of spectrometric methods, including 1D and 2D NMR experiments and MS analysis.

Several plant extracts were screened as part of our continuing investigations on Panamanian Bignoniaceae species,[1,2] with the aim of discovering potential antioxidant drug candidates. The Bignoniaceae family comprises about 120 genera and 800 species, growing mainly in Africa and Central and South America.[3] Species of this family are used for many purposes, such as horticulture, ornamentals, timber, food, handicrafts, dyes, and medicine.[4] The best-known medicinal use comes from the inner bark preparations from various species of *Tabebuia*, mainly *T. impetigosa*, called *pau d'arco* in Brazil, as an analgesic, anti-inflammatory, antineoplasic, and diuretic.[5] Members of the family have not been extensively chemically investigated.[6] The plants from the genus *Jacaranda* are mainly trees, used as ornamentals around the world because of their spectacular flowers. Previous phytochemical studies indicate that the genus is a source of various secondary metabolites, such as phenylethanoid glycosides, flavonoids, quinones, phytosterols, and anthocyanidins.[7−9]

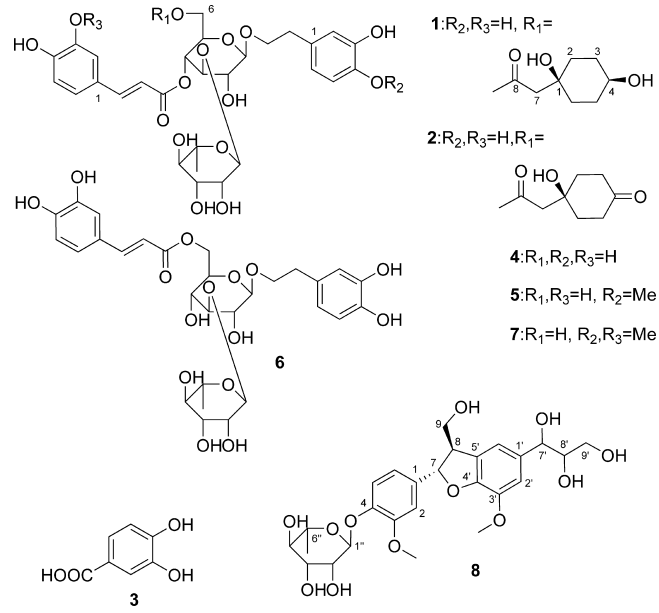
In this work, 18 extracts (CH_2Cl_2 and MeOH) from seven Panamanian plants of the family Bignoniaceae were submitted to a rapid TLC 1,1-diphenyl-2-picrylhydrazyl (DPPH) test.[10] The MeOH extract from the stems of *Jacaranda caucana* Pittier subsp. *sandwithiana* A.H.Gentry was selected due to its good radical-scavenging activity.

J. caucana was first described in 1917 by Pittier. It was found in the Cauca Department of Colombia, hence its name. Further studies by Gentry divided the species into four subspecies: *calycina*, *caucana*, *glabrata*, and *sandwithiana*.[11] In Panama, only the former has been described.[12] The only phytochemical studies were done by Ogura et al.,[13,14] who reported cytotoxic activities of *J. caucana* due to the presence of jacaranone, a quinonid compound. They also isolated and characterized, from a total MeOH extract of the twigs and leaves, several triterpenes including betulinic and jacarandic acids. The subspecies were not mentioned. A recent study showed a moderate activity of the MeOH extract of the leaves against *Plasmodium falciparum* and reported the use of this species for the treatment of leishmaniasis by the local population in an area of Southwestern Colombia.[15]

This paper describes the isolation and characterization of two new phenylethanoid glycosides (**1**, **2**), a new glycoside of the neolignan sisymbrifolin (**8**), and five known compounds, as well as their antioxidant and radical-scavenging activities.

Results and Discussion

A liquid−liquid extraction of the MeOH extract using H_2O, EtOAc, and H_2O-saturated BuOH afforded three fractions. They were tested against DPPH. The two organic fractions were active. The EtOAc fraction was then chromatographed on RP-18 by MPLC. This afforded directly several pure compounds: the new phenylethanoid glycosides **1** and **2**, protocatechuic acid **3**,[16] acteoside **4**,[17] and jionoside D **5**.[18] Further separation by semipreparative LC yielded isoacteoside **6**[17] and martynoside **7**.[19] This is the first report of protocatechuic acid and martynoside in the genus *Jacaranda*. The new neolignan **8** was isolated from the BuOH fraction by reversed-phase flash chromatography.



Compound **1** had a UV spectrum quite similar to that of acteoside (λ_{max} of acteoside: 218, 290, and 330 nm, λ_{max} of **1** in Experimental Section). Moreover, the ^1H and ^{13}C NMR spectra (Table 1) were

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1** and **2** (500 and 125 MHz, in CD_3OD)

	1		**2**	
	^{1}H	^{13}C	^{1}H	^{13}C
		acetylcyclohexyl		
1		70.4		70.0
2−6	1.78 (2H, m)	35.9	2.62 (2H, m)[a]	37.5[b]
	1.51 (2H, m)		2.16 (2H, m)[a]	37.6[b]
3−5	1.67 (4H, m)	31.4	2.06 (2H, m)[a] 1.93 (2H, m)[a]	
4	3.50 (1H, m)	70.6		214.3
7	2.48 (2H, s)	48.3	2.68 (2H, s)	47.4
8		172.6		172.3
		glycosyl		
1′	4.42 (1H, d, J = 8.3 Hz)	104.4	4.40 (1H, d, J = 8.3 Hz)	104.4
2′	3.41 (1H, m)	76.3	3.39 (1H, m)	76.3
3′	3.84 (1H, t, J = 9.0 Hz)	81.5	3.84 (1H, t, J = 9.0 Hz)	81.5
4′	5.01 (1H, t, J = 9.5 Hz)	73.2	5.01 (1H, t, J = 9.5 Hz)	72.6
5′	3.76 (1H, m)	73.9	3.78 (1H, m)	73.2
6′	4.22 (1H, dd, J = 11.7, 2.7 Hz)	63.9	4.23 (1H, dd, J = 12.0, 2.7 Hz)	63.9
	4.12 (1H, dd, J = 11.7, 5.1 Hz)		4.14 (1H, dd, J = 12.0, 5.1 Hz)	
		aglycone		
1″		131.5		131.5
2″	6.70 (1H, d, J = 2.4 Hz)	117.2	6.69 (1H, d, J = 2.4 Hz)	117.3
3″		146.3		147
4″		144.9		144.9
5″	6.69 (1H, d, J = 8.3 Hz)	116.5	6.67 (1H, d, J = 8.3 Hz)	116.5
6″	6.58 (1H, dd, J = 8.3, 2.0 Hz)	121.4	6.56 (1H, dd, J = 8.3, 1.9 Hz)	121.4
α(8″)	3.98 (1H, m)	72.6	3.98 (1H, m)	72.3
	3.72 (1H, m)		3.72 (1H, m)	
β(7″)	2.81 (2H, *m*)	36.9	2.78 (2H, *m*)	36.8
		caffeoyl		
1‴		127.8		127.8
2‴	7.07 (1H, d, J = 1.9 Hz)	115.4	7.05 (1H, d, J = 1.9 Hz)	115.4
3‴		147.0		146.3
4‴		150.0		150.0
5‴	6.80 (1H, d, J = 8.3 Hz)	116.7	6.80 (1H, d, J = 8.3 Hz)	116.5
6‴	6.97 (1H, dd, J = 8.3, 1.9 Hz)	123.4	6.97 (1H, dd, J = 8.3, 1.9 Hz)	123.4
α(8‴)	6.29 (1H, d, J = 16.1 Hz)	114.7	6.27 (1H, d, J = 16.1 Hz)	114.7
β(7‴)	7.60 (1H, d, J = 15.6 Hz)	148.3	7.59 (1H, d, J = 15.6 Hz)	148.4
CO		168.2		168.2
		rhamnosyl		
1⁗	5.20 (1H, d, J = 1.5 Hz)	103.2	5.19 (1H, d, J = 1.5 Hz)	103.2
2⁗	3.93 (1H, m)	72.5	3.92 (1H, m)	72.5
3⁗	3.57 (1H, m)	70.6	3.54 (1H, m)	70.4
4⁗	3.31 (1H, m)	73.9	3.28 (1H, m)	73.9
5⁗	3.60 (1H, d, J = 2.9 Hz)	72.2	3.57 (1H, m)	72.6
6⁗	1.11 (3H, d, J = 6.4 Hz)	18.6	1.09 (3H, d, J = 6.4 Hz)	18.6

[a] Interchangeable signals. [b] Interchangeable signals.

very similar, particularly for the caffeoyl, hydroxytyrosyl, rhamnosyl, and glucosyl moieties. Notable differences were observed between 2 and 3 ppm, where there was no signal in the ^{1}H NMR spectrum of **4**, but signals were present in the spectrum of **1**. This suggested that **1** could be acteoside with an additional substituent. Compound **1** was shown to have the molecular formula $C_{37}H_{48}O_{18}$ ([M − H]$^-$: m/z 779.2759 by HRMS). Thus, the new substituent could have the formula $C_8H_{12}O_3$. Downfield chemical shifts of the glucosyl signals H-6′ (δ 3.62 and 3.52 in **4**, vs 4.22 and 4.12 in **1**) and C-6′ (δ 62.5 in **4**, vs 63.9 in **1**) suggested that this position was esterified. Furthermore, in the long-range ^{1}H−^{13}C HMBC spectrum, a correlation between the H-6 protons of the glucosyl group and a new carbon (δ 172.6) confirmed this hypothesis. Singlet protons (H-7, δ 2.48, 2H) also correlated with this carbon, suggesting a substituted acetyl group. These protons in the HMBC spectrum had correlations with two more carbons, one at δ 70.4, typical of a hydroxy group, and one at δ 35.9. The carbon with a signal at δ 70.4 was quaternary, as it did not show any correlation in the short-range ^{1}H−^{13}C HSQC spectrum. According to HSQC and ^{1}H spectra, carbons with signals at δ 35.9 and 31.4 were respectively linked to protons with δ 1.78, 1.51 (H-2, H-6, 2H each) and 1.67 (H-3, H-5, 4H). The 2D COSY ^{1}H−^{1}H short-range correlations spectrum helped to establish the connectivity between the protons. Finally, the proton at δ 3.50, characteristic of a methine linked to an OH group, had a 2D-COSY ^{1}H−^{1}H correlation with H-3 and H-5. These elements pointed us to the presence of a saturated symmetric C-6 ring, with OH groups in positions 1 and 4 and an acetyl group in position 1. Zhang et al.[20] isolated the 1,4-dihydroxycyclohexanacetic acid, with chemical shifts equivalent to those observed for this substituent. No information was given concerning the relative or absolute configuration. Nevertheless, Endo et al.[21] isolated a natural product with a closely related structure, rengyol (*cis* isomer), and synthesized isorengyol (*trans* isomer). They confirmed both positions of the OH group in the two molecules. The tertiary OH group was in an axial position in the two molecules, and the secondary OH group of rengyol adopted an equatorial orientation, and an axial orientation in isorengyol. Both structures are displayed in Figure 1. A comparison of the ^{13}C NMR shifts is presented in Table 2. The chemical shifts of the cyclohexanacetyl substituent in **1** were much closer to those of rengyol. Moreover, a NOE correlation was observed between H-4 and H-2/6 (for the signal at δ 1.51), indicating that these protons

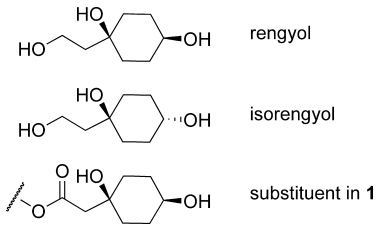

Figure 1. Structures of Rengyol, Isorengyol, and the Substituent in **1**.

Table 2. Comparison of the ^{13}C NMR Shifts for *cis*/*trans* Rengyol (both in $CDCl_3$) and the Acetylcyclohexanyl Substituent in **1** (in CD_3OD)

	trans-rengyol	*cis*-rengyol	subs. in **1**
1	71.2	69.9	70.4
2	34.4	36.1	35.9
3	30.9	31.6	31.4
4	67.4	69.8	70.6
5	30.9	31.6	31.4
6	34.4	36.1	35.9
7	42.9	45.1	48.3
8	58.9	58.7	172.6

were in an axial orientation, and thus the OH group in position 4 was in an equatorial position. These elements demonstrate the *cis* orientation. Compound **1** is thus 6′-*O*-(*cis*-1,4-dihydroxycyclohexanacetyl)acteoside, a new structure.

The UV spectrum of **2** was very similar to that of **1**, and the molecular formula of **2** was $C_{37}H_{46}O_{18}$ ($[M - H]^-$: *m*/*z* 777.2575 by HRMS), which differed from **1** by the lack of two hydrogens. Compound **2** could thus be the oxidized form of **1**. The ^1H and ^{13}C NMR spectra confirmed this. The signals for the acteoside moiety were not modified, but those for the cyclohexanacetyl substituent showed major changes. Indeed, in **2**, one hydroxylated carbon was missing, whereas one carbon with a chemical shift typical of a ketone (δ 214.3) was observed. The substituent was thus a (1-hydroxy-4-oxo)cyclohexanacetyl group. Comparison of NMR data with the literature[22] confirmed this hypothesis. In this case, quercetin-3-*O*-rutinoside substituted with the same cyclohexanacetyl group was isolated, and the NMR shifts were in accordance with those of **2**. Compound **2** is thus 6′-*O*-(1-hydroxy-4-oxo-cyclohexanacetyl)acteoside, also an original structure.

Compound **8** showed a $[M - H]^-$ ion peak at *m*/*z* 537.2037 in the HRMS, indicating the molecular formula $C_{26}H_{34}O_{12}$. As shown in the Experimental Section, the UV spectrum of **8** differed from those of **1** and **2**, indicating that the structure did not belong to the same class of compounds. The ^1H NMR spectrum (Table 3) showed the presence of an aromatic ABX system: δ 7.03 (1H, d, $J = 2.0$ Hz, H-2), 7.08 (1H, d, $J = 8.3$ Hz, H-5), and 6.91 (1H, dd, $J =$ 8.3, 2.0 Hz, H-6). The long-range $^1H-^{13}C$ HMBC spectrum helped to identify the groups that occupied positions 3 and 4 of the benzene ring. The latter appeared to be substituted by an *O*-rhamnosyl group. The anomeric proton (δ 5.34, 1H, d, $J = 1.5$ Hz, H-1″) correlated with an oxygenated aromatic carbon (δ 146.6, C-4), and the rest of the sugar signals were in accordance with those of *O*-rhamnosyl in **1** and **2**, for example. Moreover, a correlation between a methoxy group (δ 3.80, 3H, s) and another oxygenated aromatic carbon at δ 152.2 (C-3) was observed. Furthermore, in support of the position of the OCH_3 group, a NOE was observed between the OCH_3 protons and H-2. Finally, position 1 of this ring was a quaternary carbon (δ 138.8), as it did not show any correlation in the short-range $^1H-^{13}C$ HSQC spectrum. The 2D $^1H-^1H$ TOCSY spectrum suggested a sequence of methine−methine−methylene successively coupled in this order: δ 5.60 (1H, d, $J = 5.9$ Hz, H-7), 3.46 (1H, m, H-8), 3.86 (1H, m, H-9a), and 3.77 (1H, m, H-9b). Moreover, the HMBC spectrum revealed a correlation between H-8 and C-1.

Table 3. ^1H and ^{13}C NMR Data of Compound **8** (500 and 125 MHz, in CD_3OD)

	^1H	^{13}C
1		138.8
2	7.03 (1H, d, $J = 2.0$ Hz)	111.4
3		152.2
4		146.6
5	7.08 (1H, d, $J = 8.3$ Hz)	119.7
6	6.91 (1H, dd, $J = 8.3, 2.0$)	119.2
7	5.60 (1H, d, $J = 5.9$ Hz)	88.9
8	3.46 (1H, m)	55.7
9	a 3.86 (1H, m)	65.1
	b 3.77 (1H, m)	
3-OMe	3.80 (3H, s)	56.6
1′		137.2
2′	6.95 (1H, br s)	112.8
3′		145.4
4′		149.0
5′		129.6
6′	6.90 (1H, br s)	116.8
7′	4.57 (1H, d, $J = 5.9$ Hz)	75.7
8′	3.68 (1H, m)	77.6
9′	a 3.53 (1H, dd, $J = 11.5, 4.2$ Hz)	64.4
	b 3.39 (1H, dd, $J = 11.2, 6.4$ Hz)	
3′-OMe	3.89 (3H. s)	56.9
1″	5.34 (1H, d, $J = 1.5$ Hz)	101.5
2″	4.06 (1H, dd, $J = 3.2, 1.7$ Hz)	72.2
3″	3.86 (1H, m)	72.3
4″	3.46 (1H, m)	74.0
5″	3.77 (1H, m)	70.9
6″	1.22 (3H, d, $J = 6.4$ Hz)	18.1

Another long-range correlation between C-8 and an aromatic proton (δ 6.90, 1H, br s, H-6′) showed the presence of a second aromatic ring. Another aromatic proton (δ 6.95, 1H, br s, H-2′) was observed on this ring, as well as two oxygenated aromatic carbons (δ 145.4, C-3′ and 149.0, C-4′) and a quaternary nonoxygenated carbon C-1′ (δ 137.2). Furthermore, the HMBC data showed a correlation between a methoxy group (δ 3.89, 3H, s) and C-3′. The TOCSY spectrum showed another methine−methine−methylene system: δ 4.57 (1H, d, $J = 5.9$ Hz, H-7′) 3.68 (1H, m, H-8′) 3.53 (1H, dd, $J = 11.5, 4.2$ Hz, H-9′a), and 3.39 (1H, dd, $J = 11.2, 6.4$ Hz, H-9′b), which was connected at C-1′, according to the HMBC spectrum.

The chemical shifts of the protons and carbons of this side chain pointed to a trihydroxypropanoyl group. All these elements revealed a 8−5′ lignan, often called neolignan.[23] The aglycone, dihydroxydehydrodiconiferyl alcohol or sisymbrifolin, has already been isolated from *Eucommia ulmoides* Oliv., Eucommiaceae.[24] The relative configurations of H-7 and H-8 were *trans*. García-Muñoz et al.[25] concluded in their work on the synthesis of dihydrodehydrodiconiferyl alcohol and derivatives that the coupling constant of H-7 could not be used to determine the relative configuration. However, a NOE correlation was observed between H-7 and H-9 a/b and another one between H-8 and both H-2 and H-6. These correlations indicated that the relative configuration of H-7 and H-8 was *trans*. Moreover, the CD spectrum of **8** aided the determination of the absolute configuration of C-7 and C-8. Indeed, it showed a negative Cotton effect at 222 nm and a positive one at 292 nm. Nakanishi et al.[26] observed a negative Cotton effect around 220 nm for junipercomnoside A, also a *trans* neolignan. They concluded that the absolute configuration was 7*S*,8*R*. In the same way, Antus et al.[27] observed a positive Cotton effect around 290 nm for some compounds of a set of *trans* neolignans and assigned a 7*S*,8*R* absolute configuration. Thus, the same absolute configuration could be deduced for C-7 and C-8 in **8**. While the absolute configuration of C-7′ and C-8′ could not be established from the available data, the relative configuration was determined from the coupling constant of H-7′. Indeed, Deyama et al.[24] isolated both *erythro*- and *threo*-dihydroxydehydrodiconiferyl alcohol. They observed a coupling constant of 5.7 Hz for the *erythro* isomer and 7.5 Hz for the *threo* one. As in **8**, the coupling constant was 5.9 Hz, and it could be

Table 4. Activities of Compounds in DPPH and ALP Assays

compound	ER_{50} (DPPH)[a]	EC_{50} (ALP) (μM)[b]
isoacteoside (**6**)	0.070 ± 0.004	1.20 ± 0.60
acteoside (**4**)	0.078 ± 0.002	1.04 ± 0.39
2	0.079 ± 0.001	1.27 ± 0.17
1	0.098 ± 0.001	1.27 ± 0.07
jionoside D (**5**)	0.17 ± 0.02	1.10 ± 0.13
protocatechuic acid (**3**)	0.23 ± 0.03	2.56 ± 0.67
martynoside (**7**)	0.38 ± 0.15	2.42 ± 0.98
8	1.84 ± 0.70	9.07 ± 2.18
quercetin (positive control)	0.090 ± 0.01	1.00 ± 0.07

[a] ER_{50} is the ratio of antioxidant concentration to DPPH• concentration producing a 50% decrease in DPPH• at steady state. [b] EC_{50} is the antioxidant concentration that protects ALP to 50% from peroxyl radical-induced activity loss.

reasonably concluded that the relative configuration of the trihydroxypropanoyl chain was *erythro*. Compound **8** is thus 4-*O*-rhamnosyl-7*S*, 8*R*-7′,8′-*erythro*-sisymbrifolin, a new compound.

The radical-scavenging effects for compounds **1**−**8**, measured with DPPH, as well as the ALP test are shown in Table 4. The phenylethanoid glycosides **1**, **2**, acteoside, and isoacteoside presented good activity, with the same order of magnitude as the positive control, quercetin.[28] Both jionoside D (**5**), and martynoside (**7**), with respectively one and no remaining catechol functions, presented weaker activities. Compound **3**, with one catechol function, showed activity similar to that of jionoside D. Compound **8**, with no catechol function, presented poor activity.

The alkaline phosphatase test (ALP) is a simple fluorimetric test[29] to assess the antioxidant capacity of chemical entities to protect proteins from loss of activity caused by peroxyl radicals. As in the DPPH test, phenylethanoid glycosides **1**, **2**, acteoside, and isoacteoside presented interesting activities, similar to quercetine. Jionoside D, in the ALP test, showed comparable activity, differing from the results obtained with the DPPH test. The activity in the DPPH test seemed to be linked with the presence of catechol function(s). The ALP test, on the other hand, was associated with phenol function(s). In the case of jionoside D, since lacking one phenol, a slightly weaker activity to acteoside and isoacteoside was expected. It was not the case, but the loss was only one on four phenol moieties. Furthermore, protocatechuic acid and martynoside, both with two phenol functions, showed the same activity, only slightly lower than that of the compounds discussed above. Compound **8** displayed poor activity in the ALP test in accordance with the DPPH test.

Experimental Section

General Experimental Procedures. Specific rotations were measured on a Perkin-Elmer-241 polarimeter (MeOH, *c* in g/100 mL). UV spectra were recorded in MeOH on a Perkin-Elmer-Lambda-20 UV−vis spectrophotometer. UV spectra were recorded in MeOH. The circular dichroism (CD) spectrum was recorded with a Jasco J-810 spectrometer. The MeOH solution was thermostated at 20.0 °C using a Jasco PFD-425S system. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Inova NMR instrument. ^{1}H and ^{13}C NMR spectra were recorded in CD_3OD or DMSO-d_6 at 500 and 125 MHz, respectively. HRMS spectra were obtained on a Micromass LCT Premier (Waters) using electrospray as the ion source, negative mode, capillary voltage 2.8 kV, cone voltage 40 V, MCP detector voltage 2650 V, source temperature 120 °C, desolvation temperature 250 °C, cone gas flow 10 L/h, desolvation gas flow 550 L/h. TLC was performed on silica gel 60 F_{254} Al sheets (Merck). MPLC was performed with a Büchi 681 pump equipped with a Knauer UV detector using a RP-18 Lichroprep (40−63 μm; 230 × 50 mm i.d.; Merck) column. Flash chromatography was performed on a Spot System (ARMEN) with a precolumn SVF D26 (RP-18, 40−63 μm, Merck) and a column of RP-18 Lichroprep (15−25 μm; 400 × 30 mm i.d.). Semipreparative HPLC was performed with a LC-8 pump equipped with a SPD-10A VP (Shimadzu) detector using a XTerra Prep-MS C18 ODB column (5 μm, 19 × 150 mm; Waters), with detection at 254 nm using CH_3CN/H_2O gradients. HPLC-UV-DAD analyses were carried out on a HP1100 system equipped with a photodiode array detector (Agilent Technologies) with a Nova-Pak RP-18 column (5 μm; 150 × 3.9 mm i.d.; Waters) using a CH_3CN + 0.05% TFA/H_2O + 0.05% TFA gradient (2:98 to 40:60 in 40 min). The detection was performed at 210, 254, 280, and 360 nm. UPLC was performed prior to HRMS on an Acquity UPLC System (Waters) with an Acquity BEH C_{18} column (1.7 μm; 50 × 2.1 mm i.d.; Waters) using a CH_3CN + 0.1% FA/H_2O + 0.1% FA gradient (5:95 to 98:2 in 3 min).

Plant Material. The stems of *J. caucana* subsp. *sandwithiana* were collected in December 2005 in the Parque Nacional Soberania, Panama, and identified by Prof. Mireya Correa, director of the Herbarium of the University of Panama. Vouchers are deposited at the University of Panama (FLORPAN 6840) and at the Laboratory of Phamacognosy and Phytochemistry, Geneva, Switzerland (No. 2005007).

Extraction and Isolation. The air-dried powdered stems of *J. caucana* subsp. *sandwithiana* were first extracted at room temperature with CH_2Cl_2, then with MeOH, affording respectively 2.2 and 14.7 g of extracts. The MeOH extract (12.0 g) was partitioned by LLE between EtOAc and H_2O (500 mL of each). The aqueous fraction was then partitioned with H_2O-saturated n-BuOH (500 mL). This yielded 2.1 g of EtOAc, 3.4 g of n-BuOH, and 7.0 g of H_2O phases. The EtOAc phase was separated by medium-pressure liquid chromatography (MPLC) with a MeCN/H_2O step gradient (2:98 to 35:65 in 5% steps) to afford 85 fractions. This separation yielded 27 mg of protocatechuic acid (fraction 7, **3**), 33 mg of acteoside (fraction 33, **4**), 3 mg of jionoside D (fraction 34, **5**), 29 mg of **1** (fraction 44), and 8 mg of **2** (fraction 47).

Fractions 37 and 54 were purified by semipreparative LC with the eluent MeCN/H_2O, affording respectively 3 mg of isoacteoside (**6**) and 3 mg of martynoside (**7**).

The BuOH phase (1.5 g) was separated by flash chromatography with a MeCN/H_2O gradient (2:98 to 30:60) to afford 49 fractions. This separation yielded 10 mg of **8** (fraction 16).

The separations were monitored using HPLC.

Radical-Scavenging Activity (DPPH) TLC Assays. A TLC autographic assay of radical-scavenging activity using the stable DPPH radical was applied for extract screening. After application of 100 μg of the samples on silica gel 60 F_{254} Al plates (Merck), development was with hexane/EtOAc (1:1) for the CH_2Cl_2 extracts or CH_2Cl_2/MeOH/H_2O (13:7:1) for the MeOH extracts. Plates were thoroughly dried for complete removal of solvents. A solution of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•, 2 mg/mL in MeOH) was then sprayed. Inhibitors appeared as yellow spots against a purple background.[10]

ALP and DPPH Microplate Assay. The microplate ALP oxidation protection assay was used to determine the EC_{50} (the antioxidant concentration that protects ALP by 50% from peroxyl radical-induced activity loss) of the pure compounds as described before.[28] In the same approach, the determination of the ER_{50} (the ratio of antioxidant concentration to DPPH• concentration producing a 50% decrease in DPPH• at steady state) of pure compounds for the radical-scavenging activity of the stable DPPH• radical was done in a microplate assay, based on the technique described by Ancerewicz et al.[29]

6′-*O*-(*cis*-1,4-Dihydroxycyclohexanacetyl)acteoside (1): yellow, amorphous solid; $[\alpha]^{25}_D$ −39 (MeOH, *c* 1.0); UV (MeOH) λ_{max} (log ε) 218 (4.25), 289 (3.94), 332 (4.04) nm; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 779.2759 ($C_{37}H_{47}O_{18}$ [M − H]$^-$, requires 779.2762).

6′-*O*-(1-Hydroxy-4-oxo-cyclohexanacetyl)acteoside (2): yellow, amorphous solid; $[\alpha]^{25}_D$ −45 (MeOH, *c* 1.0); UV (MeOH) λ_{max} (log ε) 220 (4.29), 289 (4.02), 333 (4.14) nm; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 777.2575 ($C_{37}H_{45}O_{18}$ [M − H]$^-$, requires 777.2506).

4-*O*-Rhamnosyl-7*S*,8*R*-7′,8′-*erythro*-sisymbrifolin (8): yellow-orange, amorphous solid; $[\alpha]^{25}_D$ −45 (MeOH, *c* 1.0); UV (MeOH) λ_{max} (log ε) 229 (4.03), 281 (3.60), 328 (2.83) nm; CD (MeOH, *c* 0.05) λ_{max} ($\Delta\varepsilon$) 222 (−3.23), 244 (1.11), 292 (1.29); ^{1}H and ^{13}C NMR, see Table 3; HRESIMS *m*/*z* 537.2037 ($C_{26}H_{33}O_{12}$ [M − H]$^-$, requires 537.1972).

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1**, **2**, and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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