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ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · AUGUST 2004

Impact Factor: 3.8 · DOI: 10.1021/np049879c · Source: PubMed

New Lignans from the Roots of *Valeriana prionophylla* with Antioxidative and Vasorelaxant Activities

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Received April 5, 2004

Two new 7,9′:7′,9-diepoxylignan glycosides have been isolated from the roots of *Valeriana prionophylla*. Their structures have been established on the basis of 1D and 2D NMR experiments as prinsepiol-4-*O*-*β*-D-glucopyranoside (**1**) and fraxiresinol-4′-*O*-*β*-D-glucopyranoside (**2**). In addition, 8-hydroxypinoresinol-4′-*O*-*β*-D-glucopyranoside (**3**), 8-hydroxypinoresinol (**4**), prinsepiol (**5**), and chlorogenic acid were isolated. Compounds **1, 3, 4**, and **5** were evaluated for their antioxidative properties in Trolox equivalent antioxidant activity (TEAC) and chemiluminescence (CL) assays. The same compounds were tested for their vascular activity in rat aorta rings. The aglycones **4** and **5** displayed powerful antioxidant activity; in addition, aglycone **4** showed a higher vasorelaxant activity than compounds **1**, **3**, and **5**.

The genus *Valeriana*, with about 200 species, belongs to the family Valerianaceae and has a distribution throughout the world. The roots of *Valeriana* species comprise the drug valerian, which has been used as a sedative since it was described by ancient Greeks and Roman.[1]

Although the mild CNS-depressant effects of valerian have been demonstrated in mice,[2] as well as in some clinical studies,[3,4] the molecular mechanism of the sedative action is still unclear. It has been found that constituents other than the valepotriates, the major constituents of valerian, probably contribute to the pharmacological activity of valerian.[4,5] Recently, several lignans were isolated from polar extracts of *V. officinalis*: 8-hydroxypinoresinol was found to exhibit affinity for 5-HT_{1A} receptors in low micromolar concentrations,[6] and 4′-*O*-*β*-D-glucopyranosyl-9-*O*-(6″-deoxysaccharosyl)olivil was found to be a potent partial agonist at A_1 adenosine receptors.[7]

Valeriana prionophylla Standl. is a plant species spread in Guatemala, Mexico, and Costa Rica that is widely used in traditional medicines in the form of aqueous infusions as a sedative and antispasmodic. It is found in local commerce as capsules, powders, dried leaves, or aqueous or aqueous-alcoholic preparations. Although *V. prionophylla* is empirically used in folk medicine, there are no data in the literature concerning the possible pharmacological effects and the chemical constituents of this species.

In a continuing search for new bioactive metabolites from Central American species, we describe here the isolation and identification of 7,9′:7′,9-diepoxylignans from the roots of *V. prionophylla* and the evaluation of their antioxidant and vasorelaxant activities.

Results and Discussion

The dried roots of *V. prionophylla* were extracted with EtOH. Part of the extract was partitioned between *n*-BuOH and H_2O, and the *n*-BuOH-soluble portion fractionated on Sephadex LH-20 and purified by RP-HPLC to give six compounds. In addition to chlorogenic acid, five 7,9′:7′,9-diepoxylignans were identified. Three of these were 8-hydroxypinoresinol-4′-*O*-*β*-D-glucopyranoside (**3**), 8-hydroxypinoresinol (**4**), and prinsepiol (**5**), which have already been isolated from the roots of *V. officinalis*.[6,7] The compounds were identified by comparison of their spectroscopic data, especially NMR, with those in the literature.[8−10] The last two compounds were identified as new 7,9′:7′,9-diepoxylignan glycosides, prinsepiol-4-*O*-*β*-D-glucopyranoside (**1**) and fraxiresinol-4′-*O*-*β*-D-glucopyranoside (**2**), on the basis of the evidence outlined below (Figure 1).

The molecular formula $C_{26}H_{32}O_{13}$ of compound **1** was deduced using ESIMS and ^{13}C and DEPT NMR analyses. The ESIMS spectrum exhibited a peak at *m*/*z* 575, corresponding to the sodium adduct $[M + Na]^+$ and $[M - H]^-$ at *m*/*z* 551, thus indicating an M_r of 552.

The ^{13}C (Table 2) and DEPT NMR spectra showed 26 signals including 12 carbons for two aromatic rings, two methylenes (*δ* 76.8 and 76.7, C-9 and C-9′), two methines (*δ* 88.8, C-7, and 89.1, C-7′), two tertiary alcoholic carbons (*δ* 89.3, C-8 and C-8′), two methoxy carbons (*δ* 56.7 and 56.4), and six carbon resonances corresponding to a sugar moiety. The ^1H NMR spectrum (Table 1) showed two ABX-type coupling patterns, thus indicating the presence of two 1,3,4-trisubstituted phenyl groups [*δ* 7.16 (1H, d, $J = 1.5$ Hz, H-2), 7.20 (1H, d, $J = 8.0$ Hz, H-5), and 6.99 (1H, dd, $J = 1.5$ and 8.0 Hz, H-6); and at *δ* 7.09 (1H, d, $J = 1.5$ Hz, H-2′), 6.83 (1H, d, $J = 8.0$ Hz, H-5′), and 6.89 (1H, dd, $J = 1.5$ and 8.0 Hz, H-6′)], two benzylic oxymethine protons at *δ* 5.05 (1H, s, H-7) and 5.01 (1H, s, H-7′), two methylenes bearing an oxygen function at *δ* 4.02 (2H, d, $J = 9.4$ Hz, H-9a and H-9′a), 4.17 (1H, d, $J = 9.4$ Hz, H-9b), and 4.16 (1H, d, $J = 9.4$, H-9′b), two O-methyl singlets at *δ* 3.92 (3H, s) and 3.91 (3H, s), an anomeric proton at *δ* 4.94 (1H, d, $J = 7.5$ Hz), and signals in the *δ* 3.40−4.94 region attributable to a sugar moiety. Assignment of all ^1H and ^{13}C NMR signals were based on HMBC, HSQC, and DQF-COSY experiments. The COSY spectrum confirmed the presence of two 1,3,4-trisubstituted phenyl groups showing connectivities between the hydrogens H-6, H-2, and H-5 of ring A and the hydrogens H-6′, H-2′, and H-5′ of ring B. The aromatic carbon shifts suggested that the aryl groups

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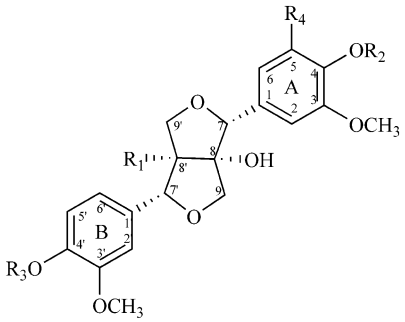
10.1021/np049879c CCC: $27.50
Published on Web 07/07/2004



	R_1	R_2	R_3	R_4
1 Prinsepiol-4-O-β-D-glucoside	OH	β-D-glucosyl	H	H
2 Fraxiresinol-4'-O-β-D-glucoside	H	H	β-D-glucosyl	OCH_3
3 8-Hydroxypinoresinol-4'-O-β-D-glucoside	H	H	β-D-glucosyl	H
4 8-Hydroxypinoresinol	H	H	H	H
5 Prinsepiol	OH	H	H	H

Figure 1. Compounds **1**−**5** isolated from *V. prionophylla* roots.

Table 1. ^{1}H NMR Spectroscopic Data of Compounds **1**, **2**, and **5** in CD_3OD at 600 MHz[a]

	1	**2**	**5**
position	δ_H (J_{H-H} in Hz)	δ_H (J_{H-H} in Hz)	δ_H (J_{H-H} in Hz)
1			
2	7.16 (d, 1.5)	6.75 (s)	7.09 (d, 1.5)
3			
4			
5	7.20 (d, 8.0)		6.83 (d, 8.0)
6	6.99 (dd, 1.5, 8.0)	6.75 (s)	6.89 (dd, 1.5, 8.0)
7	5.05 (s)	4.72 (s)	5.01 (s)
8			
9a	4.02 (d, 9.4)	3.90 (d, 9.2)	4.02 (d, 9.4)
9b	4.17 (d, 9.4)	4.13 (d, 9.2)	4.15 (d, 9.4)
3-OCH_3	3.92 (s)	3.91 (s)	3.91 (s)
5-OCH_3		3.91 (s)	
1′			
2′	7.09 (d, 1.5)	7.16 (d, 1.7)	7.09 (d, 1.5)
3′			
4′			
5′	6.83 (d, 8.0)	7.19 (dd, 1.7, 8.7)	6.83 (d, 8.0)
6′	6.89 (dd, 1.5, 8.0)	7.00 (d, 8.7)	6.89 (dd, 1.5, 8.0)
7′	5.01 (s)	4.93 (d, 5.7)	5.01 (s)
8′		3.07 (m)	
9′a	4.02 (d, 9.4)	3.82 (dd, 8.7, 6.6)	4.02 (d, 9.4)
9′b	4.16 (d, 9.4)	4.52 (t, 8.7)	4.15 (d, 9.4)
3′-OCH_3	3.91 (s)	3.93 (s)	3.91 (s)
Glc-1″	4.94 (d, 7.5)	4.92 (d, 7.5)	
Glc-2″	3.55 (dd, 9.5, 7.5)	3.53 (dd, 9.5, 7.5)	
Glc-3″	3.51 (t, 9.5)	3.50 (t, 9.5)	
Glc-4″	3.44 (t, 9.5)	3.42 (t, 9.5)	
Glc-5″	3.45 (m)	3.43 (m)	
Glc-6″	3.74 (dd, 12.1, 4.5)	3.72 (dd, 12.1, 4.5)	
	3.91 (dd, 12.1, 3.5)	3.90 (dd, 12.1, 3.5)	

[a] Chemical shifts are in ppm from TMS, and *J* values in Hz are presented in parentheses. All signals were assigned by DQF-COSY, HSQC, and HMBC experiments.

Table 2. ^{13}C NMR Data of Compounds **1**, **2**, and **5** in CD_3OD[a]

position	**1**	**2**	**5**
1	133.3	128.2	129.5
2	113.6	106.2	112.8
3	150.4	149.1	148.7
4	147.7	136.7	147.5
5	117.5	149.1	115.6
6	121.4	106.2	121.6
7	88.8	89.3	89.1
8	89.3	92.8	89.1
9	76.8	76.1	76.7
3-OCH_3	56.7	56.8	56.4
5-OCH_3	-	56.8	-
1′	129.5	137.2	129.5
2′	112.9	111.9	112.8
3′	148.7	151.1	148.7
4′	147.5	147.9	147.5
5′	115.6	117.8	115.6
6′	121.6	120.1	121.6
7′	89.1	87.1	89.1
8′	89.3	62.5	89.1
9′	76.7	72.1	76.7
3′-OCH_3	56.4	56.8	56.4
Glc-1″	102.9	102.9	
Glc-2″	74.9	74.9	
Glc-3″	77.8	77.9	
Glc-4″	71.4	71.2	
Glc-5″	78.2	78.2	
Glc-6″	62.5	62.5	

[a] All signals were assigned by DQF-COSY, HSQC, and HMBC experiments.

of compound **1** are 4-hydroxy-3-methoxyphenyl units and that one is linked to a glycosyl moiety. These spectroscopic data suggested that **1** is a glycosyl derivate of prinsepiol (**5**).

The 7,7′-diaryl-8,8′-dihydroxy-7,9′:7′,9-diepoxylignan aglycone structure was unambiguously confirmed by HMBC data. Correlations were observed between signals at δ 5.05 (H-7), 5.01 (H-7′), C-9 (δ 76.7), and C-9′ (δ 76.8) and between H_2-9, H_2-9′, C-8, and C-8′ (δ 89.3), confirming an 8,8′-dihydroxy-7,9′:7′,9-diepoxylignan structure. Cross-peaks were also observed between H-7 and C-1 (δ 133.3), C-2 (δ 113.6), and C-6 (δ 121.4), establishing that C-7 was attached to ring A. Correlations between H-7′ and C-1′ (δ 129.5), C-2′ (δ 112.9), and C-6′ (δ 121.6) established that the benzyl group B was at the C-7′ position. Furthermore, the HMBC spectrum allowed us to confirm the position of the methoxyl groups, showing a correlation between ^{1}H signals at δ 3.92 and C-3 (δ 150.4) and between δ 3.91 and C-3′ (δ 148.7).

The glycosidic linkage of **1** was determined by comparative studies of ^{1}H and ^{13}C NMR data of glycoside **1** and aglycon **5** (Tables 1 and 2). The observed downfield shifts of the *ortho*-correlated C-5 (+1.9 ppm) and C-3 (+1.8 ppm), the *para*-correlated C-1 (+3.7 ppm), and H-5 (+0.4 ppm), in comparison with those observed in **5**, were indicative of glycosidation at C-4. These shifts were almost superimposable on those observed in pinoresinol and 8-hydroxypinoresinol in comparison with pinoresinol-4-*O*-*β*-D-glucopyranoside and 8-hydroxypinoresinol-4-*O*-*β*-D-glucopyranoside and 8-hydroxypinoresinol-4′-*O*-*β*-D-glucopyrano-

side.[8,11] The sugar substituent was identified as β-D-glucopyranosyl from 1D TOCSY, DQF-COSY, and HSQC experiments and in comparison with literature data.[12] The configuration of the β-glucopyranosyl moieties was assigned after hydrolysis of **1** with 1 N HCl. The hydrolysate was trimethylsilylated, and GC retention times of each sugar were compared with those of authentic samples prepared in the same manner. In this way, the sugar unit of **1** were determined to be D-glucose. Consequently, the structure of **1** was defined as prinsepiol-4-*O*-β-D-glucopyranoside. The similarity of the Cotton effects of **1** {$[\theta] \times 10^{-3}$, (nm): −2.23 (239)} and prinsepiol (**5**) {$[\theta] \times 10^{-3}$, (nm): −1.16 (235)} indicated that they have the same absolute configuration; moreover comparison of the optical properties suggested that compounds **1** and prinsepiol (**5**) have the same configuration assigned unambiguously to wodeshiol.[10,13]

Compound **2** was assigned a molecular formula of $C_{27}H_{34}O_{13}$ as deduced by MS, ^{13}C NMR, and DEPT analysis. Its ESIMS showed a peak at *m*/*z* 589, corresponding to the sodium adduct $[M + Na]^+$ and $[M - H]^-$ at *m*/*z* 565, thus indicating an M_r of 566. The ^{13}C (Table 2) and DEPT NMR spectra showed 27 signals corresponding to the signals of 12 carbons for two aromatic rings, two methylenes (δ 76.1 and 72.1, C-9 and C-9′), three methines (δ 62.5, C-8′, 88.8, C-7, and 89.1, C-7′), a tertiary alcoholic carbon (δ 92.8, C-8), three methoxy carbons (δ 56.8), and six carbon resonances corresponding to a sugar moiety. The ^1H NMR spectrum of **2** (Table 1) exhibited signals at δ 7.16 (1H, d, $J = 1.7$ Hz, H-2′), 7.19 (1H, d, $J = 8.7$ Hz, H-5′), and 7.00 (1H, dd, $J = 1.7$ and 8.8 Hz, H-6′), indicating the presence of a 1,3,4-trisubstituted phenyl group), a singlet at δ 6.75, integrating for two protons (H-2 and H-6), a methine proton at δ 3.07 (1H, m H-8′), three O-methyl groups at δ 3.91 (6H, s) and 3.93 (3H, s), and the signals of glycosidic protons resembling those of **1**. Analysis of 1D and 2D NMR spectra with homo- and heteronuclear direct and long-range correlations allowed the assignments of ^1H and ^{13}C NMR signals as listed in Tables 1 and 2. The $^1H-^1H$ DQF-COSY spectrum showed correlations between H_2-9′, H-8′, and H-7′ typical of an 8-hydroxy-7,9′:7′,9-diepoxylignan. Comparison of the NMR data of **2** with those of **1** indicated the presence in **2** of a further methoxyl group (δ_H 3.91 and δ_C 56.8). The substitution at the C-5 position by this methoxy group was consistent with the ^1H NMR aromatic signals at δ 6.75 (2H, s) and is in full agreement with the observed carbon resonances of C-4, C-5, and C-6 (Table 2). These data indicated that the aryl groups of **2** consisted of guaiacyl and syringyl units and that compound **2** is a glycosyl derivate of fraxiresinol.

Multiple-bond heteronuclear correlations (HMBC) were observed between H-7 and C-9, between H-7′ and C-9, H_2-9, H_2-9′, C-7, C-7′, C-8, and C-8′, and between H-8′ and C-8, confirming for the aglycone the structure 8-hydroxy-7,9′:7′,9-diepoxylignan unambiguously. Cross-peaks were also observed between H-7, C-1, C-2, and C-6, establishing that C-7 was attached to ring A, while correlations between H-7′, C-1′, C-2′, and C-6′ established that the benzyl group B was at the C-7′ position. Moreover, the HMBC spectrum confirmed the position of the methoxyl groups, showing a correlation between proton signals at δ 3.91 ($2 \times OCH_3$) and C-3 and C-5 and at 3.93 (OCH_3) and C-3′. The glycosidic linkage of **2** was determined to be at the C-4′ position on the basis of the cross-peaks due to ^3J long-range coupling between anomeric-H (δ 4.92, H-1″) and C-4′ (δ 147.9) in the HMBC spectrum and by comparison with known hydroxypinoresinol-4′-*O*-β-D-glucopyranoside (**3**). The sugar substituent was identified as β-glucopyranosyl from 1D-TOCSY, DQF-COSY, and HSQC experiments, and also in this case, the D configuration of the glucose unit was determined by acid hydrolysis of **2** followed by GC analysis. The small coupling constant between H-8′ and the adjacent benzylic oxymethine H-7′ ($J = 5.7$) indicated a *trans*-disposition of these protons. From the ROESY spectrum correlations were observed between H-8′ and H-2′ and H-6′, which indicated that the orientation of the aromatic ring B was a pseudoequatorial type. The CD curve of **2** was compared with those of stereochemically established hydroxypinoresinol-4′-*O*-β-D-glucopyranoside (**3**),[11] and the similarity of the Cotton effects indicated that they have the same absolute configuration. Consequently, the structure of **2** was defined as (−)-7*R*,8*S*,7′*S*,8′*R*-fraxiresinol-4′-*O*-β-D-glucopyranoside.

Table 3. Antioxidant Activity of Compounds **1**−**5** in the TEAC and CL Assays[a]

compound	TEAC assay (mM) ± SD[b]	CL assay (mM) ± SD[b]
1	2.92 ± 0.11	1.84 ± 0.06
2	n.d.	n.d.
3	2.51 ± 0.09	1.44 ± 0.05
4	5.10 ± 0.12	6.31 ± 0.27
5	5.33 ± 0.18	6.70 ± 0.25
verbascoside	5.30 ± 0.36	8.20 ± 1.05

[a] For protocols used, see Experimental Section. Results are expressed in terms of mM Trolox equivalent. [b] $n = 3$.

The quantitative analysis of lignans from *V. prionophylla* extract was performed by HPLC with a diode array detector (DAD). The concentrations of each compound in the extracts, calculated from the experimental peak areas by interpolation to standard calibration curves, were 0.89% for compound **1**, 0.02% for compound **2**, 0.50% for **3**, 0.20% for **5**, and 0.19% for **4**. Relative standard deviations were in the range 3.76−4.12%, calculated as the mean of five replications, while for retention times it was less than 1%.

The free-radical scavenging activities of compounds **1**, **3**, **4**, and **5** were evaluated in the antioxidant (TEAC) and chemiluminescence (CL) assays. The first measures the relative ability of antioxidant substances to scavenge the radical cation 2,2′-azinobis(3-ethylbenzothiozoline-6-sulfonate) ($ABTS^{\bullet+}$) as compared to a standard amount of the synthetic antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid).[14] The CL assay measures the inhibition of iodophenol-enhanced chemiluminescence by a horseradish peroxidase/perborate/luminol system.[15] Trolox was used as the reference antioxidant. The results (Table 3) showed that aglycones **4** and **5** exhibited free-radical scavenging activity at potency levels comparative to the reference antioxidant compound verbascoside, while **1** and **3** had more moderate activities.

Compounds **1**, **3**, **4**, and **5** and the EtOH extract of *V. prionophylla* were also tested for their biological vasorelaxant activity. A pharmacological study was performed to evaluate the vasoactive effect on aorta rings of these compounds. It is well known that in the presence of NO, an endothelial-derived factor, free radicals generate the potent oxidant peroxynitrite; this reaction is prevented by antioxidants improving NO-induced relaxation.[16] In this regard we evaluated the compounds' ability to relax aorta rings in the presence or absence of endothelium in order to confirm the antioxidant properties demonstrated by the TEAC and chemiluminescence assays. Table 4 indicates the relaxation effect, observed at the maximal concentration tested, in the presence or absence of endothelium. Our pharmacological results are summarized in Figure 2. The *V. prionophylla* extract caused a concentration-dependent

Table 4. Vasorelaxant Activity of EtOH Extract (1 mg mL^{-1}) and Compounds **1**−**5** (100 μM) on Rat Aorta Rings Precontracted by Phenylephrine[a]

compound	with endothelium	without endothelium
1	20.4 ± 3.7[b]	0 ± 0
2	n.d.	n.d.
3	2.5 ± 0.6	1.7 ± 1.7
4	24.4 ± 7.0	29.0 ± 6.4
5	29.3 ± 10.0[b]	5.3 ± 3.0
extract	36.9 ± 11.0	36.7 ± 11.0

[a] Data are expressed as mean ± SEM at the maximal concentration tested ($n = 5$). [b] $p < 0.05$ versus effect without endothelium (% of relaxation).

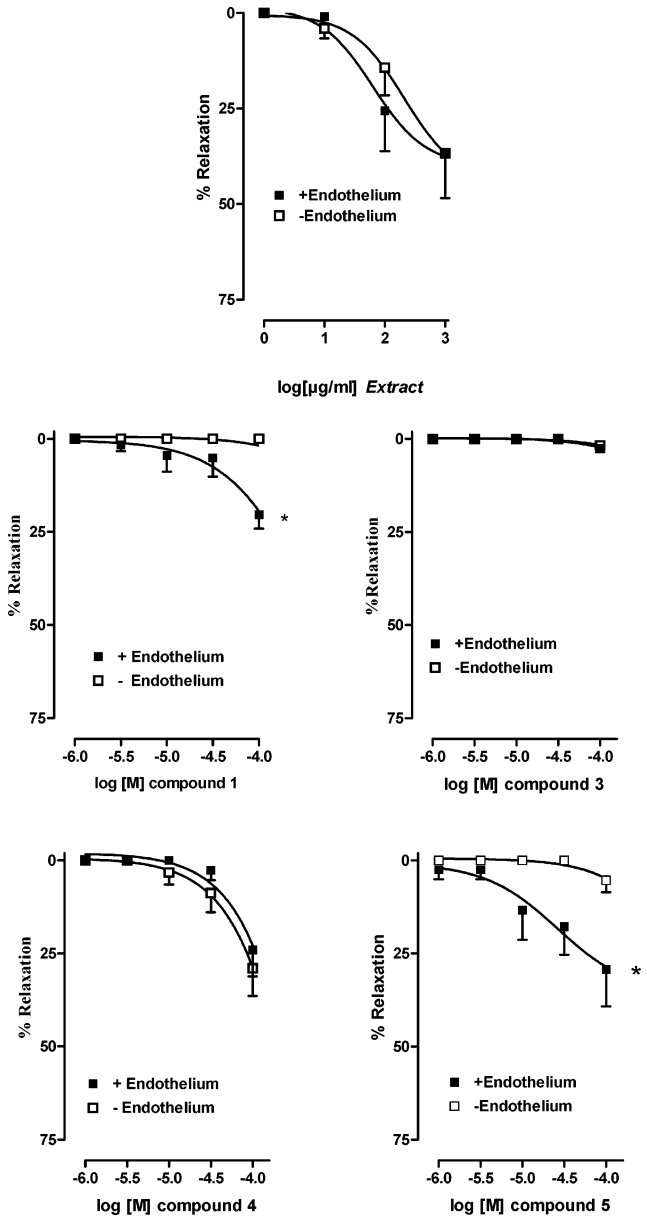


Figure 2. Concentration−response curve of the extract (1−1000 μg mL^{-1}) and compounds **1**−**5** (1−100 μM) on rat aorta rings precontracted with phenylephrine. Data are expressed as mean ± SEM ($n = 5$). *$p < 0.05$.

relaxation of rat aorta rings precontracted with phenylephrine (PE; 1 μM) in endothelium-independent fashion. All compounds were less potent compared to the extract; analyzing the endothelium-independent relaxation effect, compound **4** showed an appreciable activity compared to the others ($p < 0.05$ and $p < 0.01$ versus compounds **1** and **5**, respectively). On the other hand compound **3**, which is structurally related to the previous compound, was totally unaffected, suggesting that the presence of a glycoside causes the loss of the observed effect. Conversely compounds **1** and **5** showed a comparable ($p = 0.9$) endothelium-dependent relaxation (Figure 2), indicating an improvement of NO bioavailability due probably to the antioxidant properties, but we cannot exclude an action through estrogen receptors.

Compound **2** present in small amounts in the extract was not tested for its antioxidant and vasorelaxant activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and a 1 cm microcell. CD measurements were carried out on a Jasco J-710 dicograph. UV spectra were obtained with a Beckman DU 670 spectrophotometer and IR spectra with a Bruker IFS-48 spectophotometer. A Bruker DRX-600 spectrometer, operating at 599.19 MHz for ^{1}H and 150.858 for ^{13}C, using the UXNMR software package was used for NMR experiments in CD_3OD. $^1H-^1H$ DQF-COSY (double quantum filtered COSY), $^1H-^{13}C$ HSQC, HMBC, and ROESY experiments were obtained using conventional pulse sequences. The selective excitation spectra, 1D TOCSY,[13] were acquired using waveform generator-based GAUSS-shaped pulses, mixing times ranging from 100 to 120 ms, and MLEV-17 spin-lock field of 10 kHz preceded by a 2.5 ms trim pulse; chemical shifts are expressed in δ (ppm) referring to solvent peaks: δ_H 3.34 and δ_C 40.0 for CD_3OD. Electrospray ionization mass spectrometry (ESIMS) in the positive and negative ion mode was performed using a Finnigan LC-Q Deca instrument from Thermoquest (San Jose, CA) equipped with Excalibur software. Samples were dissolved in MeOH and infused in the ESI source by using a syringe pump; the flow rate was 3 μL/min. The capillary voltage was 5 V, the spray voltage was 5 kV, and the tube lens offset was 35 V. The capillary temperature was 220 °C. Data were acquired in the MS1 scanning mode (m/z 150−700). Exact masses were measured by a Q-Star Pulsar (Applied Biosystems) triple-quadrupole orthogonal time-of-flight (TOF) instrument. Electrospray ionization was used in TOF mode at 8.500 resolving power. Samples were dissolved in pure MeOH, mixed with the internal calibrant, and introduced directly into the ion source by direct infusion. Calibration was performed on the peaks of CsI and synthetic peptide (TOF positive ion calibration solution, Bachem) at m/z 132.9054 and 829.5398, respectively. Sodium-containing molecular ions of analytes were measured. HPLC separations were performed on a Waters 590 series pumping system equipped with a Waters R401 refractive index detector and a Waters μ-Bondapak C_{18} 10 μm (300 × 7.8 mm) column. Quantitative HPLC analyses were performed on a Agilent 1100 series system consisting of a G-1312 binary pump, a G-1328A Rheodyne injector (20 μL loop), a G-1322A degasser, and a G-1315A photodiode array detector, equipped with a 5 μm HyPurity Aquastar (150 × 4.6 mm) column. GC analyses were performed using a Chrompack (Middelburg, The Netherlands) model 9001 gas chromatograph with a data-handling system and FID.

Biological Material. The roots of *V. prionophylla* were collected in Cabricán, Quetzaltenango, Guatemala, in February 2000 and identified by Dr. Elfriede de Pöll, Universidad del Valle de Guatemala. A specimen of the plant (Voucher No. 119) used in this study has been deposited at the Herbarium of Laboratorio Farmaya, Guatemala.

Extraction and Isolation. Roots were extracted with EtOH (80%). Part of the EtOH extract (15 g) was partitioned between *n*-BuOH and H_2O to afford an *n*-BuOH-soluble portion (3 g), which was chromatographed over a Sephadex LH-20 column (100 × 5 cm) using MeOH as eluent. Fractions (8 mL) were collected and checked by TLC [Si gel, *n*-BuOH−HOAc−H_2O (60:15:25) and $CHCl_3$−MeOH−H_2O (80:18:2)]. Fractions

32−38 (170 mg), containing the crude lignan glycoside mixture, were submitted to RP-HPLC on a C_{18} μ-Bondapack column (30 cm × 7.8 mm, flow rate 2.5 mL min^{-1}) using MeOH−H_2O (25:75) as the eluent to yield compounds **1** (20.4 mg, t_R 21.6 min), **2** (1.3 mg, t_R, 29.6 min), and **3** (14.8 mg, t_R 35 min). Fractions 39−44 (25.6 mg), containing the crude lignan mixture, were separated by RP-HPLC using MeOH−H_2O (35:65) as the eluent at a flow rate of 2 mL/min, giving compounds **4** (6.9 mg, t_R 15.3 min) and **5** (8.7 mg, t_R 20.6 min). Fractions 49−59 contained chlorogenic acid.

Quantitative Analysis. Quantitative HPLC analyses were carried out on a Hypurity Aquastar 5 μm (150 × 4.6 mm) column. The elution solvents used were A (H_2O/0.2% TFA) and B (acetonitrile). The flow rate was 1 mL/min, and the wavelength of detection was λ = 285 nm. The gradients were as follows: (i) a linear gradient where solvent B increased from 5 to 20% over a 10 min period; (ii) an isocratic elution for 10 min to 20% of solvent B; (iii) a linear gradient where solvent B was increased from 20 to 30% over a 5 min period. Compound **3** was used as an external standard. A linear relationship between peak area and concentration (0.05−1 mg mL^{-1}) was observed with a correlation coefficient r = 0.9809. The relationship between peak areas (y) and concentrations in mg mL^{-1} (x) was y = 25765x − 2804. The minimum detection limit was 0.2 ng, which resulted in a signal-to-noise ratio of 3:1. Reproducibility was verified with five extracts of an identical sample. Relative standard deviations (%) were less than 5%, while for retention times they were less than 1%.

Prinsepiol-4-*O*-*β*-D-glucopyranoside (1): amorphous yellow residue; $[\alpha]^{25}_D$ −25.3 (*c* 1, MeOH); UV (MeOH) λ_{max} 230, 280 nm; IR (KBr) ν_{max} 3415, 2937, 2856, 1605, 1524 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m*/*z* 575 [M + Na]$^+$, *m*/*z* 551 [M − H]$^-$; HRESIMS (negative) *m*/*z* 551.3242 (calcd for $C_{26}H_{32}O_{13}$, 521.1843, [M − H]$^-$).

Fraxiresinol-4′-*O*-*β*-D-glucopyranoside (2): amorphous yellow residue; $[\alpha]^{25}_D$ −15.3 (*c* 1, MeOH); UV (MeOH) λ_{max} 232, 280; IR (KBr) ν_{max} 3405, 2929, 2863, 1648, 1566 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables 1 and 2; ESIMS *m*/*z* 589 [M + Na]$^+$, *m*/*z* 565 [M − H]$^-$; HRESIMS (negative) *m*/*z* 565.2052 (calcd for $C_{27}H_{34}O_{12}$, 565.3517, [M − H]$^-$).

Acid Hydrolysis of Compounds 1 and 2. A solution of compounds **1** and **2** (0.8 mg each) in 1 N HCl (0.25 mL) was stirred at 80 °C for 4 h. After cooling, the solution was concentrated by blowing with N_2. The residue was dissolved in 1-(trimethylsilyl)imidazole and pyridine (0.1 mL), and the solution was stirred at 60 °C for 5 min. After drying the solution with a stream of N_2, the residue was separated by water and CH_2Cl_2 (1 mL, 1:1 v/v). The CH_2Cl_2 layer was analyzed by GC using a l-Chirasil-Val column (0.32 mm × 25 m). Temperatures of the injector and detector were 200 °C for both. A temperature gradient system was used for the oven, starting at 100 °C for 1 min and increasing to 180 °C at a rate of 5 °C/min. The peak of the hydrolysate of **1** and **2** was detected by comparison with retention times of an authentic sample of D-glucose (Sigma Aldrich, St. Louis, MO) after being treated with 1-(trimethylsilyl)imidazole in pyridine.

ABTS Radical Cation Decolorization Assay. Evaluation of free-radical scavenging activity was performed with the TEAC assay. The TEAC value is based on the ability of the antioxidant to scavenge ABTS$^+$, the preformed radical monocation of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), with spectrophotometric analysis, according to Re et al.[14] Samples were diluted with MeOH to produce 0.3, 0.5, 1.0, 1.5, and 2.0 mM solutions. The reaction was enhanced by the addiction of 1.0 mL of diluted ABTS to 10 μL of each solution of sample or Trolox (standard) or 10 μL of MeOH (control). The determination was repeated three times for each sample solution. The percentage inhibition of absorbance at 734 nm was calculated for each concentration as a function of the control's absorbance, 1 min after initial mixing. The antioxidant activity was expressed as TEAC (Trolox equivalent antioxidant activity), which is the concentration of standard Trolox solution with equivalent percentage inhibition to a 1 mM solution of the tested compounds.

Chemiluminescence Assay. Total antioxidant capacity was assayed by chemiluminescence according to Whitehead et al.[15] Enhanced chemiluminescent signal reagent (Amersham, UK) comprising assay buffer and tablets A and B (containing luminol, *p*-iodophenol enhancer, and perborate oxidant) was prepared by adding tablets A and B to the buffer solution. Signal reagent (0.4 mL) was added to distilled H_2O (1 mL) in a glass cuvette containing a magnetic stirrer. The cuvette was placed in a Perkin-Elmer Wallac Victor 2 chemiluminometer, and the reaction commenced by addition of 25 μL of horseradish peroxidase (4 μg mL^{-1} in H_2O). Compounds (100 μL of 0.5 mg mL^{-1} dissolved in PBS, pH 7.4) were added to the cuvette, and the time for which light output was suppressed was determined. Comparison was made with a standard curve generated using Trolox (20 μg mL^{-1} in H_2O).

Vasorelaxant Activity: Organ Bath Experiments. Male Wistar rats (Charles River, Italy) were housed in an environment with controlled temperature (21−24 °C) and lighting (12: 12 h light−darkness cycle). Standard laboratory chow and drinking water were provided ad libitum. A period of 7 days was allowed for acclimatization of rats before any experimental manipulation was undertaken. At the time of the experiments, the body weight ranged from 200 to 300 g. All the experiments were conducted according to guidelines established by the U. B. C. Animal Care Committee. Rats were sacrificed by cervical dislocation after exposure to isoflurane, and their thoracic aorta were excised and carefully cleaned of connective tissue, cut in rings (2−3 mm). The vasorelaxant activity of compounds was evaluated either in the presence or in the absence of endothelium. The rings were then hooked in 2.5 mL water-jacketed organ baths filled with thermostated (37 °C) and gassed (95% O_2 and 5% CO_2) Krebs solution with the following composition (mM): NaCl, 115.3; KCl, 4.9; $CaCl_2$, 1.46; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0, and glucose, 11.1. Rings were connected to isometric force transducers (model 7002, Basile, Comerio, Italy), and change in tension was recorded continuously using a polygraph linearcorder (WR3310, Graphtec, Japan). Tissues were preloaded with 0.5 g and allowed to equilibrate for at least 90 min, during which time Krebs solution was changed about every 15 min. After equilibration, a concentration-dependent curve by acethylcholine (Ach) was performed on a stable tone of phenylephrine (PE; 1 μM), to evaluate the presence of endothelium. In another set of experiments the endothelium was mechanically removed. The extract (1−1000 μg mL^{-1}) or compound **1**, **3**, **4**, or **5** (1−100 μM) was added to the organ bath to evaluate the relaxant activity on rat aorta rings precontracted with PE (1 μM) both in the presence and in the absence of endothelium. The relaxing response results are expressed in percent of relaxation as mean ± SE mean. All curves were compared by two-way analysis of variance (ANOVA), and a values of $p < 0.05$ were taken as significant. All salts for Krebs solution were purchased from Carlo Erba (Milan, Italy). PE and Ach were purchased from Sigma (Milan, Italy). The extract and compound **1** were dissolved in distilled H_2O, while the other compounds were dissolved in poly(ethylene glycol) (1:1 ratio).

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NP049879C