Spasmolytic Effects, Mode of Action, and Structure−Activity Relationships of Stilbenoids from *Nidema boothii*[†,⊥]

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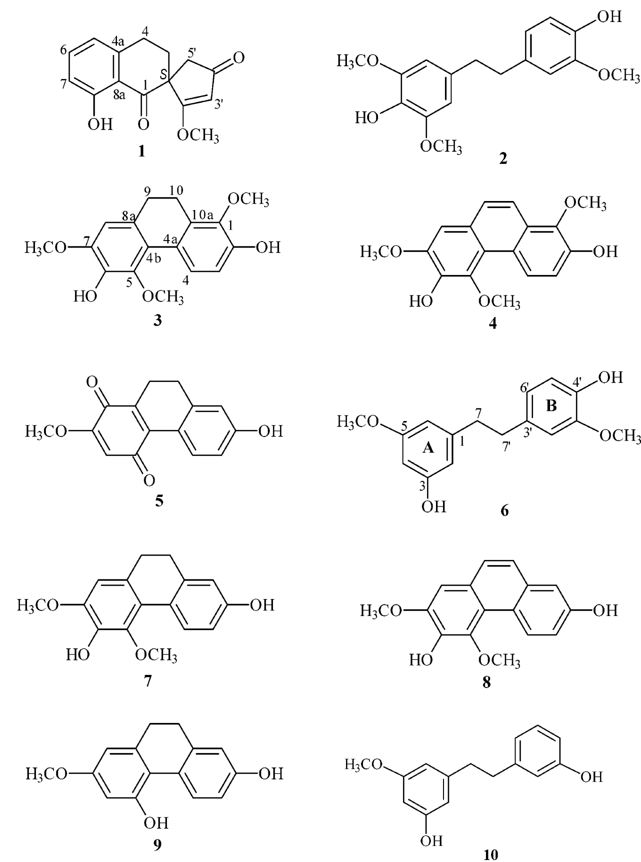
A CH_2Cl_2−MeOH (1:1) extract prepared from the whole plant of *Nidema boothii* inhibited spontaneous contractions ($IC_{50} = 6.26 \pm 2.5$ μg/mL) of the guinea-pig ileum. Bioassay-guided fractionation of the active extract led to the isolation of the novel spiro compound **1**, which was given the trivial name nidemone, and the new dihydrophenanthrene **3**, characterized as 1,5,7-trimethoxy-9,10-dihydrophenanthrene-2,6-diol. In addition, the known stilbenoids aloifol II (**2**), 1,5,7-trimethoxyphenanthrene-2,6-diol (**4**), ephemeranthoquinone (**5**), gigantol (**6**), ephemeranthol B (**7**), 2,4-dimethoxyphenanthrene-3,7-diol (**8**), lusianthridin (**9**), and batatasin III (**10**) were obtained. The isolates were characterized structurally by spectroscopic data interpretation. Compounds **2**−**6**, **9**, and **10** induced notable concentration-dependent inhibition of the spontaneous contractions of the guinea-pig ileum with IC_{50} values that ranged between 0.14 and 2.36 μM. Bibenzyl analogues **23**−**35** were synthesized and tested pharmacologically. The results indicated that for maximum spasmolytic activity the bibenzyls should have oxygenated substituents on both aromatic rings; on the other hand, methylation of free hydroxyl groups as well as the increment of oxygenated groups in relation to compounds **6** and **10** decreased the smooth muscle relaxant activity. It was also demonstrated that bibenzyls **6** and **10** might exert their spasmolytic action not only by a nitrergic mechanism but also by inhibiting CaM-mediated processes.

Nidema boothii (Lindl.) Schltr. (Orchidaceae), also known as *Epidendrum boothii* (Lindl.) L.O. Williams, is an orchid found from Mexico to Panama, Cuba, and Surinam, and its habitat is in tropical moist forests up to 1500 m elevation. A previous phytochemical study on this species resulted in the isolation and characterization of a novel rearranged tetracyclic triterpene named nidemin after the genus of the plant.[1] *N. boothii* is not used as a traditional or alternative remedy; however, the related species *Scaphyglottis livida* (Lindl.) Schltr. and *Maxillaria densa* Lindl. are employed for the treatment of stomachache in the State of Veracruz, Mexico.[2,3] Furthermore, bioactivity-guided fractionation of the spasmolytic extracts prepared from these orchids resulted in the isolation and characterization of several stilbenoids, including 1,5,7-trimethoxyphenanthrene-2,6-diol (**4**), gigantol (**6**), 2,4-dimethoxyphenanthrene-3,7-diol (**8**), lusianthridin (**9**), and batatasin III (**10**) (Chart 1), among others.[2,3] These natural products induce a concentration-dependent inhibition of the spontaneous contractions of the rat ileum with a potency higher than or comparable to that of papaverine.[2] It was also demonstrated that the spasmolytic effect of **6** was mediated by the nitric oxide (NO)/cyclic guanosine monophosphate (*c*GMP) system.[2]

In the present investigation *N. boothii* was selected initially as potential source of spasmolytic compounds following a chemotaxonomic approach. Thereafter, a pharmacological test, using the guinea-pig ileum system,[2] of a CH_2Cl_2−MeOH (1:1) extract of the plant confirmed this hypothesis. Thus, the first aim of the present study was to isolate the spasmolytic principles of *N. boothii*. In addition, this investigation was designed to provide more information about the mode of action of bibenzyls **6** and **10**, also isolated from *N. boothii*, by evaluating (i) their in vitro activity on the regulatory protein calmodulin (CaM) and

Chart 1. Compounds Isolated from *Nidema boothii*



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[⊥] Dedicated to the late Dr. Monroe E. Wall and to Dr. Mansukh C. Wani of Research Triangle Institute for their pioneering work on bioactive natural products.

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Table 1. NMR Spectral Data for Nidemone (**1**) in $CDCl_3$

position	δ_H[a] (J in Hz)	δ_C[b]	HMBC[c]	NOESY
1		202.7	3, 5	
2		59.2	3, 4, 3′, 5	
3A	2.47 ddd (14, 7.4, 4.0)	25.6	4, 5	3B, 4A, 4B, 5′
3B	2.20 ddd (13.8, 9.3, 4.8)			3A, 4A, 4B
4B	3.48 brddd (16.7, 9.4, 5.0)	32.1	3, 5	3A, 3B, 4A, 5
4A	2.87 ddd (16.8, 5.8, 5.8)			3A, 3B, 4B, 5′A, 5′
4a		145.4	3, 4, 6	
5	6.72 brdd (7.5, 1)	118.9	3, 4, 7	4A, 4B
6	7.39 dd (7.5, 7.5)	137.0	5, 7	5, 7
7	6.80 dd (7.5, 1)	115.8	5, 6	6
8		163.4	6	
8a		116.4	5, 7	
2′		189.6	3, 3′, 5′, $-OCH_3$	
3′	5.27 t (1.2)	101.9	5′	$-OCH_3$
4′		202.3	3′, 5′	
5′A	3.29 dd (17.5, 1.5)	39.7	3, 3′	3A, 4A, 5′B
5′B	2.48 dd (17.5, 1.5)			4A, 5′A
$-OCH_3$	3.91 s	59.1		3′
−OH-8	12.23 d (0.6)			

[a] Recorded at 300 MHz. [b] Recorded at 75 MHz. [c] Protons showing long-range correlation with indicated carbon.

(ii) their effects on the spontaneous contraction of the guinea-pig ileum in the presence of 1*H*-[1,2,4]oxadiazolo-[4,3-α]quinoxalin-1-one (ODQ) and N^G-nitro-L-arginine methyl ester (L-NAME), inhibitors of the enzymes guanylate cyclase and nitric oxide synthase (NOS), respectively, and finally to evaluate the spasmolytic effect of several natural and synthetic analogues of bibenzyls **6** and **10**, in an attempt to establish the influence of the oxygenated substituents on the pharmacological effects of both these lead compounds.

Results and Discussion

A CH_2Cl_2−MeOH (1:1) extract prepared from the whole plant of *N. boothii* inhibited spontaneous contractions ($IC_{50} = 6.26 \pm 2.5$ μg/mL) of the guinea-pig ileum when tested in vitro. Accordingly, this spasmolytic extract was selected for bioassay-guided fractionation. This process led to the isolation of the novel spiro-compound **1**, which was given the trivial name nidemone, and the new dihydrophenanthrene **3**, characterized as 1,5,7-trimethoxy-9,10-dihydrophenanthrene-2,6-diol (Chart 1). The known stilbenoids **2** and **4**−**10** (Chart 1) were also obtained from the active fraction, and their spectral data were in agreement with those reported previously.[2,4−8] In addition, vitexin[9] and nidemin[1] spontaneously crystallized from some inactive fractions.

Compound **1** was isolated as an optically active glassy yellow solid. Its molecular formula was established as $C_{15}H_{14}O_4$ by HREIMS. The nine degrees of unsaturation in this formula could be partially accounted for by two carbonyls, a benzene ring, and one double bond; hence compound **1** was tricyclic. Three partial structures could be constructed that fully accounted for all the atoms in nidemone (**1**). First, a trisubstituted benzene ring moiety was apparent from the ^{1}H NMR spectrum data (Table 1), which showed an ABC system [δ_H 6.72, br dd, J = 7.5, 1 Hz (H-5); 7.39 dd, J = 7.5, 7.5 Hz (H-6); and 6.80, dd, J = 7.5, 1 Hz (H-7)]; these protons correlated with the signals at δ_C 118.9 (C-5), 137.0 (C-6), and 115.8 (C-7), respectively. Additionally, a hydrogen-bonded phenolic hydroxyl group was determined from the signal at δ_H 12.23 in the ^{1}H NMR spectrum. The second partial structure was defined by HMBC (Table 1) as a cyclohexenone, as suggested from absorptions in the IR (1692 cm^{-1}) and ^{13}C NMR spectra (δ_C 202. 7, C-1) for a conjugated carbonyl. The third partial structure consisted of a conjugated cyclopentenone (δ_C 202.3) system with a polarizable double bond (δ_C 189.6 and 101.9) bearing a methoxyl group (δ_H 3.91 s/δ_C 59.1). According to the HMQC experiment, the vinyl carbon resonating at higher field (C-3′) was associated with a proton resonating at δ_H 5.27 (H-3′), while the lower one (C-2′, β-carbon of the enone) was found to bear this methoxyl group. This evidence defined the conjugated system as a vinylogous carboxylic acid methyl ester and explained the unusual polarization of the double bond.[10,11] The connection between the three partial structures in **1** was made on the basis of the analysis of the HMBC data (Table 1). Thus, the HMBC correlations observed between the C-4 methylene hydrogens and the aromatic carbons at δ_C 118.9 (C-5), 145.4 (C-4a), and 116.4 (C-8a), as well as those between the C-3 methylene hydrogens and C-4a and C-1, clearly indicated that the cyclohexenone unit was fused to the benzene ring. Similarly, the long-range correlations of the signals at δ_H 2.47 (H-3A), 2.20 (H-3B), 5.20 (H-3′), 2.48 (H-5′B), and 3.29 (H-5′A) with the signal at δ_C 59.2 (C-2/1′) connected the cyclohexenone and cyclopentenone systems through a spiro-carbon. The NOESY correlations (Table 1) for H-4A, H-4B, H-5, H-5′A, H-5′B, and H-3′ provided further support to this proposal. A systematic conformational search for compound **1** using the Spartan′02 molecular modeling program revealed the two minimum energy conformations **1a** and **1b** depicted in Figure 1 (E_{MMX} = 39.38 and 39.96 kcal/mol, respectively). In **1b**, the six-membered ring adopts an envelope conformation, with the interatomic distances between H4A−H5′A and H4A−H5′B being 2.4084 and 3.1162 Å, respectively. This result is consistent with the strong NOESY correlations observed between H-4A−H-5′A and H-4A−H-5′B (Table 1). Finally, the absolute configuration of compound **1** was proposed as depicted on the basis of the CD data. The CD spectra of α,β-unsaturated ketones, including aromatic ketones, typically exhibit not just two, but three and sometimes four Cotton effects between 185 and 360 nm. Thus, a weak red-shifted ketone carbonyl due to an n → π^* excitation is observed at ∼320−350 nm, and this excitation is followed by one or more $\pi \rightarrow \pi^*$ transitions with one corresponding to the typical UV band between 230 and 260 nm, and a second often overlapping with the first and lying in the 200−220 nm range; in the particular case of aromatic ketones a strong band around ∼290 nm (B band) is also observed. Finally, another, more energetic band is found near 188 nm, and it is thought to involve a n → σ^*

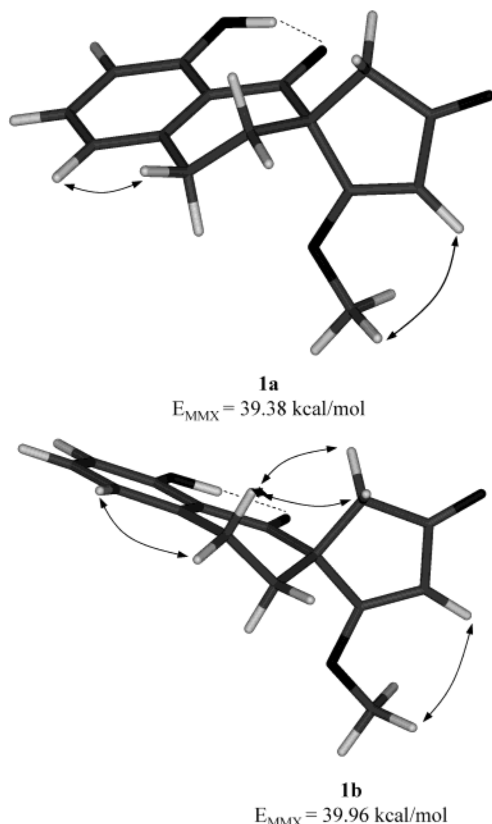


Figure 1. Minimum energy structures of nidemone (**1**) showing relevant NOESY correlations.

transition.[12] The first two sets of bands are the most useful for determining absolute stereochemistry, in particular when inherently dissymmetric chromophores are present in a given molecule. Compound **1** possesses such a chromophore since the cyclopentenone system is nonplanar. According to the Snatzke's helicity rules,[12,13] a cyclopentenone system has a negative helicity if the CD spectrum displays a positive Cotton effect for the $\pi \rightarrow \pi^*$ transition and a negative Cotton effect for the $n \rightarrow \pi^*$. The CD spectrum of **1** exhibited a strong positive Cotton effect around 265 nm and a negative one at 326 nm, correlating with a negative helicity and thus with an *S* absolute stereochemistry at C-2.[13] Furthermore, optical rotations of nidemone (**1**) and (*S*)-4-hydroxycyclopent-2-en-1-one[11] showed a negative sign, suggesting that both compounds have the same absolute configuration.

Compound **3** had the composition $C_{17}H_{18}O_5$ as determined by its MS and ^{13}C NMR data, differing from phenanthrene **4** by two mass units. The NMR spectra of **3** (see Experimental Section) showed the characteristic signals of a 9,10-dihydrophenanthrene[3] and suggested that **3** is the 9,10-dihydro derivative of compound **4**.[2] The most obvious differences between the NMR spectra of the two compounds resulted from the presence of two methylene signals in **3** [δ_H/δ_C 2.8 (2H, m, H-10)/22.5 (C-10) and 2.69 (2H, m, H-9)/29.4 (C-9)] instead of the aromatic resonances attributed to H-9/C-9 and H-10/C-10 in **4**. In addition, the chemical shift values for the aromatic protons and carbons were shifted diamagnetically in comparison to those in **4**. Finally, the HMBC and NOESY correlations supported the position of the substituents along the dihydrophenanthrene core. Thus, in the NOESY spectrum the correlations H-8/OCH_3-7 and H-9, H-4/OCH_3-5 and H-3, and H-10/OCH_3-1 and H-9 were consistent with the placement of the methoxyl groups at C-7, C-5, and C-1 and the hydroxyl at C-2 and C-6. On the other hand, the correlations C-9/H-8, C-4b/H-4, C-4a/H-4, C-1/OMe-1, and C-5/OMe-5 observed in the HMBC spectrum further supported this proposal. On the basis of this evidence, stilbenoid **3** was identified as 1,5,7-trimethoxy-9,10-dihydrophenanthrene-2,6-diol.

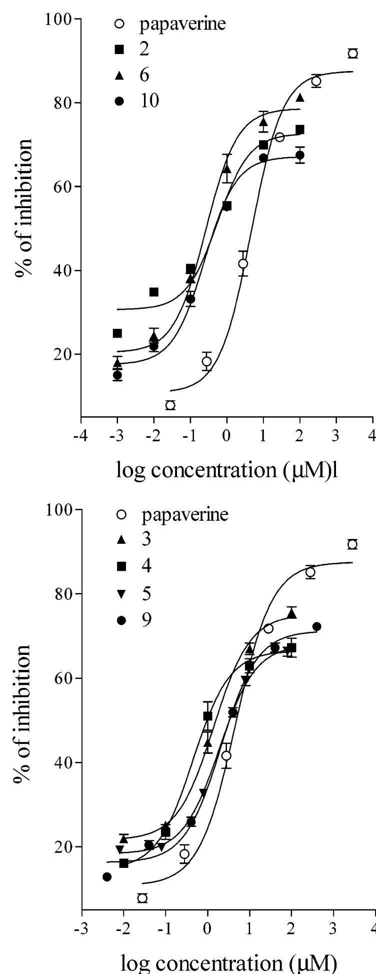


Figure 2. Concentration–response curves showing the relaxant effects of compounds **2**–**6**, **9**, and **10** from *Nidema boothii* on the isolated guinea-pig ileum. Values are expressed as the percentages of inhibition of contractile responses calculated as the mean from six data ± SEM, $p < 0.05$.

Compounds **2**–**6**, **9**, and **10** induced notable concentration-dependent inhibition of the spontaneous contractions of the guinea-pig ileum. Figure 2 shows the concentration–response curves for these compounds. All the isolates were more potent than the crude extract ($IC_{50} = 6.26 \pm 2.5$ μg/mL) and papaverine ($IC_{50} = 4.23 \pm 0.68$ μM). The greatest inhibitory activities were observed for compounds **10** ($IC_{50} = 0.24 \pm 0.11$ μM), **6** ($IC_{50} = 0.26 \pm 0.10$ μM), **9** ($IC_{50} = 0.41 \pm 0.03$ μM), and **4** ($IC_{50} = 0.45 \pm 0.03$ μM). Natural products **1**, **7**, and **8** were not tested due to scarcity of the samples; however, the spasmolytic property of compound **8** was described previously.[2]

To establish the influence of the nature (phenolic vs phenolic methyl ether) and location of the oxygenated substituents along the bibenzyl core on the pharmacological effects of **6** and **10**, compounds **23**–**35** (Chart 2) were synthesized and tested pharmacologically. All compounds but **28** and **33** were obtained using the Wittig reaction, a method widely used for the synthesis of bibenzyls.[14–16] Analogues **28** and **33** were synthesized by catalytic reduction of resveratrol and piceatannol, respectively.[17,19] Bibenzyls **23**, **25**, **28**, and **29** were synthesized as previously described, and their spectral data were in agreement with

Chart 2. Synthetic Analogues of Gigantol (**6**) and Batatasin III (**10**)

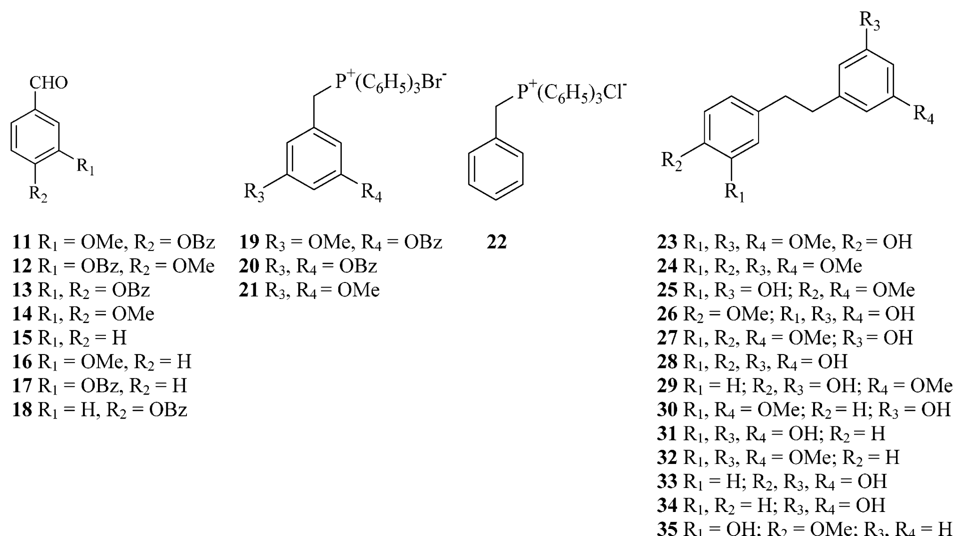


Table 2. Inhibition of the Spontaneous Contraction of Isolated Guinea-Pig Ileum Induced by Gigantol (**6**), Batatasin III (**10**), and Related Compounds[a]

substance	E_{max}	IC_{50} (μM)	potency relative to papaverine
2	73.63 ± 1.18	0.56 ± 0.20	7.65
6	81.30 ± 1.84	0.26 ± 0.10	16.26
10	67.50 ± 4.76	0.24 ± 0.11	17.62
23	76.40 ± 4.76	1.39 ± 0.52	3.05
24	84.94 ± 5.38	0.62 ± 0.03	6.82
25	66.55 ± 1.30	0.14 ± 0.08	31.33
26	51.78 ± 6.06	0.70 ± 0.05	5.99
27	66.00 ± 1.30	0.58 ± 0.08	7.32
28	53.65 ± 6.73	0.85 ± 0.04	4.94
29	85.00 ± 1.79	0.14 ± 0.04	30.85
30	58.59 ± 0.81	1.28 ± 0.40	3.30
31	78.50 ± 1.27	2.36 ± 0.77	1.78
32	75.57 ± 0.21	0.96 ± 0.12	4.41
33	64.10 ± 3.62	1.75 ± 0.17	2.42
34	83.28 ± 4.09	1.49 ± 0.39	2.84
35	82.22 ± 2.50	1.29 ± 0.36	3.28
papaverine	91.76 ± 2.65	4.23 ± 0.68	1
chlorpromazine	81.80 ± 2.30	0.18 ± 0.12	23.50

[a] Values as means ± SEM; $n = 6$; $p < 0.05$. Potency was obtained by the formula IC_{50} (μM) papaverine/IC_{50} (μM) compound assuming a value of 1.00 for papaverine. E_{max} indicates the percentage of maximum inhibition.

those reported.[15−19] Compound **31** was newly synthesized, but it was previously isolated as a natural product from *Dendrobium plicatile*;[20] compounds **25**,[21] **27**,[22] **28**,[23] **29**,[21] **30**,[24] and **33**[25] have also been described as natural products, while bibenzyls **26** and **32** were previously obtained using catalytic reduction and methylation of rhapontigenin and *O*-methylbatatasin III, respectively. The ^{1}H NMR data of **26**,[20] **32**,[24] obtained by the Wittig reaction in the present investigation, and **31**,[26] as well as the complete spectral data for compounds **34**[27,28] and **35**,[29] were in agreement with those previously described. The data presented in Table 2 show that all analogues are potent inhibitors of the spontaneous contractions of the guinea-pig ileum, with IC_{50}'s ranging between 0.14 and 2.36 μM. However, structural differences influenced the potency of this inhibition. Thus, methylation of one or more of the free hydroxyl groups and the presence of additional oxygenated groups in relation to the lead compounds decreased the smooth muscle relaxant activity. Compounds **29**, possessing a free hydroxyl group at C-4, and **25**, in which the location of the hydroxyl and methoxyl groups in ring B is the opposite of gigantol (**6**), were the most potent. Furthermore, the absence of oxygenated substituents in one of the aromatic rings of the stilbenoid moiety, as in the case of compounds **34** and **35**, induced the highest decrease in activity. Hence, it could be inferred that the presence of oxygenated substituents in both aromatic rings is essential for activity within this compound class.

In our initial studies regarding the mode of action of compounds **6** and **10** it was demonstrated that their smooth muscle relaxant effect was blocked by L-NAME when tested at their IC_{50}'s.[2] It was also demonstrated, using a radioimmunoassay procedure, that compound **6** increased *c*GMP content in rat ileal rings. Compound **6**-induced elevation of *c*GMP has been reported to be inhibited by L-NAME and ODQ, suggesting that its spasmolytic effect was mediated by the NO/*c*GMP system.[2] However, on that occasion, the influences of ODQ and different concentrations of L-NAME on the spasmolytic effect of both compounds were not assessed. The results shown in Figure 2 indicated that in the presence of ODQ (100 μM) and L-NAME (500 μM) the concentration−response curves of both compounds were shifted to the right (Figure 3), thus confirming indeed that they modify the nitrergic system.

To provide more information on the mode of action of bibenzyls **6** and **10**, their effect on the regulatory protein calmodulin (CaM) was investigated. CaM is a major Ca^{2+}-binding protein implicated in a variety of cell functions through the regulation of CaM-dependent enzymes, such as *c*AMP phosphodiesterases (PDEs), protein phosphatase, NOS, phosphorylase kinase, kinase I and II, among others.[30,31] Accordingly, CaM influences a number of important biological events, and such agents that inhibit its activity should have profound pharmacological effects. Indeed, certain antipsychotic drugs, smooth muscle relaxants,[30,32] α-adrenergic blocking agents, cytoprotective compounds, and neuropeptides inhibit CaM.[30−32] To study the effect of the bibenzyls on CaM, a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis[33] was first carried out. The results showed that both natural products retarded the mobility of bovine-brain CaM in the presence of Ca^{2+}, suggesting that they are CaM inhibitors. The synthetic analogues **23**−**35** provoked the same electrophoretic effect, and, as an example, Figure 4 illustrates the electrophoretogram showing the activity of compounds **6**, **10**, **25**, **29**, and **34**. Thereafter, to demonstrate if the binding of the bibenzyls with CaM affected its enzyme

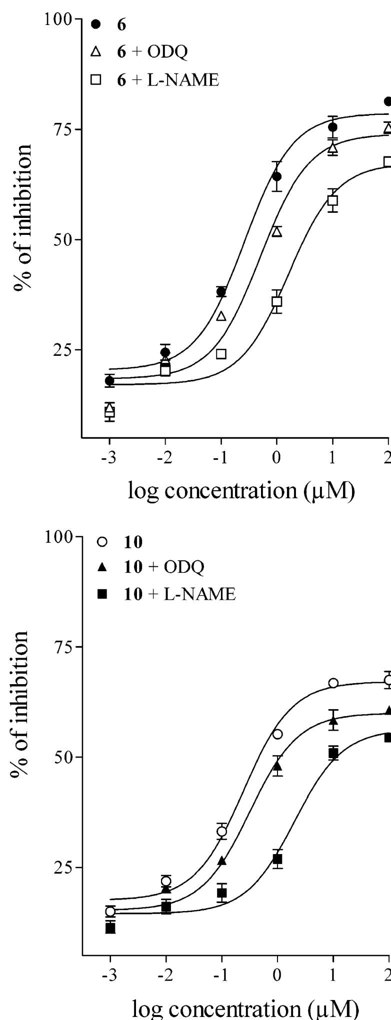


Figure 3. Concentration−response curves showing the relaxant effects of compounds **6** and **10** on the isolated guinea-pig ileum in the presence of ODQ (100 μM) and L-NAME (500 μM). Values are expressed as the percentages of inhibition of contractile responses calculated as the mean from six data ± SEM, $p < 0.05$.

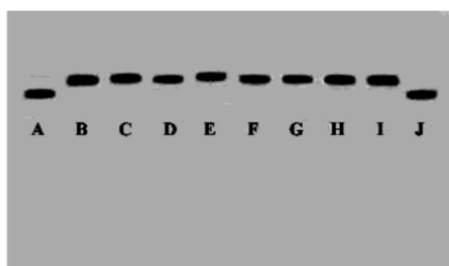


Figure 4. SDS-PAGE of bovine-brain CaM after treatment with compounds **1**, **5**, **6**, **10**, **25**, **29**, **34**, and **35**. Electrophoresis of 2 μg samples of bovine-brain CaM in the presence of 1 mM $CaCl_2$. Pretreatment of the CaM samples: 1.5 h at 30 °C in the presence of $CaCl_2$ (A); chlorpromazine in DMSO (B); **1** (C); **5** (D); **6** (E); **10** (F); **25** (G); **29** (H); **34** (I); and CaM in DMSO (J). In all cases, 0.33 μg of the tested compounds in DMSO was applied.

regulatory properties in vitro, their effect on the activity of CaM-dependent PDE was investigated. CaM-sensitive PDE catalyzes the hydrolysis of cyclic nucleotides to nucleotide monophosphates. Since CaM-sensitive PDE is widely used as a tool to discover CaM inhibitors, and in general to demonstrate the activity of CaM in biochemical studies, we assessed the effect of **6**, **10**, the analogues **25**, **29**, and **34**, and **35** on this enzyme using the method of Sharma and Wang,[34] with the modifications reported by

Table 3. Effect of Selected Stilbenoids on CaM-Dependent PDE in the Presence of 0.2 μg of Bovine-Brain CaM

compound	IC_{50}, μM
chlorpromazine[a]	10.3
1	3.2
5	10.9
6	7.0
10	13.3
25	9.0
29	10.1
34	36.6
35	21.2

[a] Positive control.

Leung and co-workers.[35] The PDE reaction was coupled to the 5′-nucleotidase reaction, and the amount of inorganic phosphate released represented the activity of the PDE; the phosphate produced in the assay was measured by the method of Sumner.[36] Bovine-brain CaM was used as activator of the enzyme. The results summarized in Table 3 indicated that the compounds tested inhibited the activation of PDE in the presence of CaM with IC_{50} values ranging between 3.2 and 36.6 μM. The effect was higher than or comparable to that of chlorpromazine ($IC_{50} = 10.3$ μM), a well-known CaM inhibitor.[30−32] Therefore the bibenzyls tested are CaM inhibitors. Furthermore, when the smooth muscle relaxant effect of compounds **6** and **10** was determined in the presence of chlorpromazine (0.1 μM), their concentration−response curves (Figure 5) were significantly shifted to the left. These results are consistent with chlorpromazine and bibenzyls **6** and **10** being agonists. Since CaM regulates NOS activity in the myenteric plexus, it is highly probable that the nitrergic and the CaM-smooth muscle relaxant effects demonstrated in this investigation are related. Further work is in progress to confirm this hypothesis.

Compounds **1** and **5** were also evaluated as potential CaM inhibitors. The natural products not only modified the electrophoretic mobility of bovine-brain CaM (Figure 4) but also inhibited the activity of CaM-dependent PDE with IC_{50} values of 3.2 and 10.9 μM, respectively. In the case of compound **5** this effect could be also involved in its spasmolytic effect.

To our knowledge the carbocyclic skeleton of nidemone (**1**) is new for a naturally occurring product. However, the 4′-oxo-3′-hydro derivative spiro[cyclopentane-1,2′(1′*H*)-naphthalene], which possesses a similar structural core, has been obtained through the condensation of tetralone with 1,4-dibromobutane.[37] The coexistence of spiro-type natural products with bibenzyls and phenanthrenes has been previously described in *Cannabis sativa* L., although, the spiro-compounds found in *Cannabis* possess an inverse arrangement of the five- and six-membered rings,[38] as the structure of cannabispiradienone (**36**) shows in Chart 3. It has been proposed that the *C. sativa* spirans originate by a direct oxidative phenol coupling (*p-o* fashion) of an appropriate bibenzyl.[38] In the case of compound **1** such a mechanism is not possible. However, it can be envisaged that nidemone (**1**) arises by an oxidative contraction of an aromatic ring of a suitable dihydrophenanthrene. This proposal is supported by the fact that one of the several routes to generate cyclopentane rings from aromatic compounds can be rationalized as an oxidative biological Bayer−Villiger type of reaction. The overall results seem to indicate that the orchid bibenzyls exert their spasmolytic action by inhibiting CaM-mediated processes and/or by a nitrergic mechanism. Whether both effects are related remains to be determined. For maximum spasmolytic

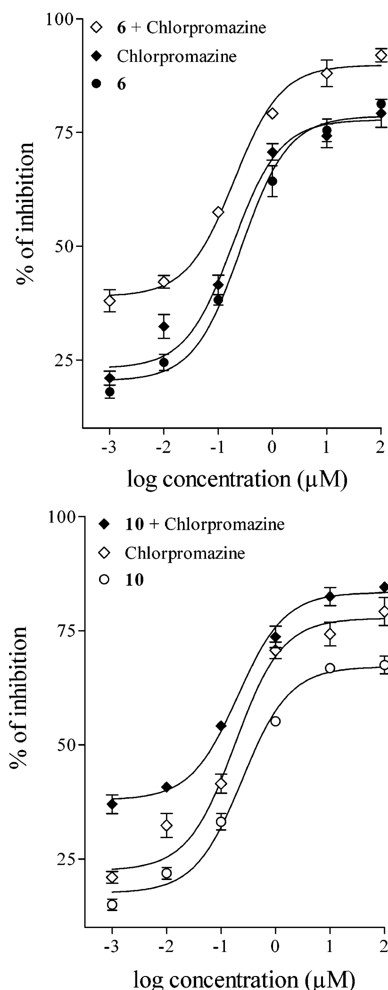
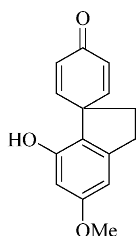


Figure 5. Concentration–response curves showing the relaxant effects of compounds **6** and **10** on the isolated guinea-pig ileum in the presence of chlorpromazine (0.1 μM). Values are expressed as the percentages of inhibition of contractile responses calculated as the mean from six data ± SEM, $p < 0.05$.

Chart 3. Structure of Cannabispiradienone (**36**)



activity, the bibenzyls should have oxygenated substituents on both aromatic rings; methylation of free hydroxyl groups as well as the increment of oxygenated groups in relation to compounds **6** and **10** decreases the resultant smooth muscle relaxant activity.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Optical rotations were taken on a Perkin-Elmer 241 polarimeter. UV spectra were obtained on a Lambda II UV spectrometer in MeOH solution. IR spectra were obtained using KBr disks on a Perkin-Elmer FT 1605 spectrophotometer. The CD spectrum of nidemone (**1**) was recorded on a JASCO 720 spectropolarimeter at 25 °C in MeOH solution. NMR spectra including COSY spectra, NOESY, HMBC, and HMQC experiments were recorded in $CDCl_3$ on a Varian Unity Plus 500 spectrometer either at 500 (^{1}H) or 125 (^{13}C) MHz, using tetramethylsilane (TMS) as an internal standard. Electron-impact mass spectra were registered on a JEOL SX 102 mass spectrometer. Open column chromatography was carried out on silica gel 60 (70–230 mesh, Merck). Analytical and preparative TLC were performed on precoated silica gel 60 F254 plates (Merck). HPLC was carried out with a Waters HPLC instrument equipped with Waters 996 UV photodiode array detector (900) set at 209–214 nm, using a μPorasil column (19 mm i.d. × 300 mm) at a flow rate of 8.3 mL/min. Control of the equipment, data acquisition, processing, and management of chromatographic information were performed by the Millennium 2000 software program (Waters). In all cases purification was achieved using hexane–$CHCl_3$–*i*-PrOH–MeOH (75:23:1:1) as mobile phase.

Plant Material. The whole plant was collected in Catemaco, State of Veracruz, Mexico, in October 1996. An authenticated voucher specimen (G. Carmona-Díaz-96-2) is preserved at the Instituto de Ecología Herbarium (XAL), Xalapa, Veracruz.

Extraction and Isolation. The air-dried plant material (2 kg) was ground into a powder and extracted exhaustively by maceration at room temperature with a mixture of MeOH–$CHCl_3$ (1:1). After filtration, the extract was concentrated in vacuo to yield 174 g of a brown residue. The extract was subjected to column chromatography over silica gel (1 kg) and eluted with a gradient of hexane–EtOAc (10:0 → 0:10) and EtOAc–MeOH (10:0 → 5:5). Three hundred fractions (1 L each) were collected and combined to produce 11 pooled fractions (F-I to F-XI) based on their TLC profiles. According to the pharmacological testing, F-V was the only fraction that inhibited (99%) the spontaneous contraction of the guinea-pig ileum when tested at the IC_{50} of the original extract.

Active fraction F-V (6.0 g, eluted with hexane–EtOAc 8:2) was chromatographed on a silica gel column (80 g) using a gradient of hexane–EtOAc (10:0 → 0:10) to yield eight secondary fractions (FV-1 to FV-8). The most active fraction was FV-8 (600 mg) (100% of inhibition of the spontaneous ileum contraction) eluted with hexane–EtOAc (8:2). HPLC purification (hexane–$CHCl_3$–*i*-PrOH–MeOH, 75:23:1:1) of active fraction FV-8 afforded nidemone (**1**, 4 mg, t_R 14.2 min); aloifol II[7] (**2**, 22 mg, t_R18 min); 1,5,7-trimethoxy-9,10-dihydrophenanthrene-2,6-diol (**3**, 25 mg, t_R 19 min); 1,5,7-trimethoxyphenanthrene-2,6-diol[2] (**4**, 8 mg, t_R 23 min); ephemeranthoquinone[22] (**5**, 5.7 mg, t_R 26 min); gigantol[2] (**6**, 32 mg, t_R 30 min); ephemeranthol B[22] (**7**, 4 mg, t_R 32 min); 2,4-dimethoxyphenanthrene-3,7-diol[6] (**8**, 3 mg, t_R 35 min); lusianthridin[8] (**9**, 55 mg, t_R 50 min); and batatasin III[16] (**10**, 19 mg, t_R 62 min). From inactive fraction III crystallized the known triterpenoid nidemin, identical to a standard sample.[1] Finally, from inactive fraction X spontaneously crystallized a yellow powder characterized (IR, NMR, MS) as vitexin.[9]

Nidemone (1): vitreous solid; $[\alpha]_D$ −114° (*c* 1.6, MeOH); CD (MeOH) $\Delta\epsilon$ (nm) -1.96×10^6 (221), -6×10^5 (246), 9.3×10^5 (265), -1×10^6 (290), -6×10^5 (326); UV (MeOH) λ_{max} (log ϵ) 343 (3.54), 296 (3.10), 226 (4.30); IR (KBr) ν_{max} 1692, 1626, 1596, 1452, 1420, 1358, 1158, 992 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); EIMS *m/z* 258 [M$^+$(78)], 241 (12), 229 (10), 226 (15), 215 (30), 186 (100), 173 (13), 134 (14); HREIMS *m/z* 258.2694 (calcd for $C_{15}H_{14}O_4$, 258.2693).

3,5,7-Trimethoxy-9,10-dihydrophenanthrene-2,6-diol (3): glassy solid; UV (MeOH) λ_{max} (log ϵ) 265 (3.42), 279 (3.30), 300 (2.44); IR (KBr) ν_{max} 3407, 2928, 1614, 1580, 1503, 1480, 1458, 1363, 1213, 1104, 1047, 995 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.00 (1H, s, D_2O exchange, −OH), 8.00 (1H, s, D_2O exchange, −OH), 7.98 (1H, d, J = 8.7 Hz, H-4), 6.88 (1H, d, J = 8.7 Hz, H-3), 6.59 (s, H-8), 3.92 (3H, s, CH_3O-7), 3.81 (3H, s, CH_3O-1), 3.70 (3H, s, CH_3O-5), 2.80 (2H, m, H-9), 2.69 (2H, m, H-10); ^{13}C NMR ($CDCl_3$) δ 147.3 (C-1), 145.8 (C-7), 144.8 (C-5), 143.6 (C-2), 137.6 (C-9a), 130.0 (C-4a), 129.0 (C-10a), 125.8 (C-6), 123.9 (C-4), 117.9 (C-4b) 113.1 (C-3), 106.8 (C-8), 29.4 (C-9), 22.5 (C-10); EIMS *m/z* 302 [M$^+$(100)], 287 (15), 255 (23), 244 (5); HREIMS *m/z* 302.3217 (calcd for $C_{17}H_{18}O_5$, 302.3218).

Synthetic Intermediates. Resveratrol, piceatannol, 3,4-dimethoxylbenzaldehyde (**14**), benzaldehyde (**15**), and *m*-anisaldehyde (**16**) were purchased from Sigma (St. Louis, MO). 4-Benzyloxy-3-methoxybenzaldehyde (**11**), 3-benzyloxy-4-methoxybenzaldehyde (**12**), 3,4-dibenzyloxylbenzaldehyde (**13**), 3-benzyloxybenzaldehyde (**17**), 4-benzyloxybenzaldehyde (**18**), 3-benzyloxy-5-methoxybenzyltriphenylphosphonium bromide (**19**), 3,5-dibenzyloxybenzyltriphenyl-phosphonium bromide (**20**), 3,5-dimethoxybenzyltriphenyl-phosphonium bromide (**21**), and benzyltriphenylphosphonium chloride (**22**) were prepared as previously described.[15-17]

Bibenzyls 23−25, 28, 29, and 33. 4′-Hydroxy-3,3′,5-trimethoxybibenzyl (**23**), 3,3′,4′,5-tetramethoxybibenzyl (**24**), 3,3′-dihydroxy-4′,5-dimethoxybibenzyl (**25**), 3,4′-dihydroxy-5-methoxybibenzyl (**29**), 3,3′,4′,5-tetrahydroxybibenzyl (**28**), and 3,4′,5-trihydroxybibenzyl (**33**) were prepared as previously described.[15-19] In all cases, the spectroscopic properties of the synthetic materials were identical to those described in the literature.[15-19]

4-Methoxy-3,3′,5-trihydroxybibenzyl (26). Compound **20** (1 g, 2 μmol) was dissolved in dry tetrahydrofuran (15 mL) under a N_2 atmosphere; NaH (0.0814 g, 2 μmol) and **11** (0.591 g, 1.86 μmol) were added to the mixture, which was stirred during 3 h. After this period of time, water was added and the product extracted with EtOAc. The extract was washed with brine and water, dried, and evaporated to give an oil (1.4 g), which was purified by open column chromatography [silica gel (30 g), hexane−EtOAc (98:2)] to give a mixture (not purified) of *Z*- and *E*-stilbenes (0.54 g, 55%). The mixture was directly hydrogenated at 45 lb/in.^{2} (30 °C) in EtOAc (25 mL) over 10% palladium on carbon for 3 h. Then the catalyst was filtered off and the filtrate evaporated. The final product was purified by HPLC [$CHCl_3$−MeOH−*i*-PrOH (98:1:1)] to yield **26** as a viscous solid (0.22 g, 27%): IR (KBr) ν_{max} 3350, 1600, 1590, 1450, 1320, 1295, 965 cm^{-1}; ^{1}H NMR (C_3D_6O) δ 8.10 (2H, s, D_2O exchange, −OH), 7.40 (1H, s, D_2O exchange, −OH), 6.81 (1H, d, *J* = 8.1 Hz, H-5′), 6.72 (1H, d, *J* = 2.1 Hz, H-2′), 6.63 (1H, dd, *J* = 8.1, 2.1 Hz, H-6′), 6.22 (2H, d *J* = 2.4, Hz, H-2 and H-6), 6.18 (1H, dd, *J* = 2.4, Hz, H-4), 3.78 (3H, s, OCH_3), 2.71 (4H. m, H-7 and H-7′); ^{13}C NMR (C_3D_6O) δ 159.2 (C-3, C-5), 147.2 (C-4′), 146.5 (C-3′), 145.1 (C-1), 135.8 (C-1′), 120.0 (C-6′), 116.1 (C-2′), 112.4 (C-5′), 107.7 (C-2, C-6), 101.2 (C-4), 56.7 (−CH_3O), 38.7 (C-7), 37.6 (C-7′); EIMS *m*/*z* 260 [M$^+$(100)].

3-Hydroxy-3′,4′,5-trimethoxybibenzyl (27). Condensation of Wittig salt **19** (0.6 g, 1.2 μmol), aldehyde **14** (0.170 g, 1 μmol), and NaH (1.2 μmol) gave, after chromatographic workup [open silica gel column, hexane−EtOAc (98:2)], the corresponding *Z*- and *E*-stilbene mixture (0.239 g, 52%), which upon hydrogenation and purification as described for compound **26** yielded **27** as a viscous solid (0.12 g, 39%). The spectral data were consistent with those found in the literature.[19]

3′-*O*-Methylbatatasin III (30). Phosphonium salt **19** (0.650 g, 1.3 μmol), **16** (0.150 g, 1 μmol), and NaH (0.053 g, 1 μmol) were condensed to yield the mixture of stilbenes (75% yield, 0.285 g), which was similarly hydrogenated and purified to afford **30** (0.117 g, 41%) as a vitreous solid. The spectral properties were consistent with those of the natural product isolated from *Coelogyne ovalis*.[24]

3,3′,5-Trihydroxybibenzyl (31). Wittig reaction using **12** (1.16 g, 2 μmol), **17** (0.336 g, 1.6 μmol), and NaH (0.063 g, 1.6 μmol) as described for **26** gave compound **31** as a white powder (0.124 g, 34%): mp 155 °C; IR (KBr) ν_{max} 3350, 1600, 1590, 1450, 1320, 1295, 965 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.22 (1H, s, D_2O exchange, −OH), 9.04 (2H, s, D_2O exchange, −OH), 7.04 (1H, dd, *J* = 7.8, 7.8 Hz, H-5′), 6.64−6.55 (3H, m, H-2′, H-4′, H-6′), 6.07 (dd, *J* = 1.5, 1.5 Hz, H-2, H-6), 6.0 (1H, dd, *J* = 1.5 Hz, H-4), 2.63 (4H, m, H-7, H-7′); ^{13}C NMR (DMSO-d_6) δ 157.9 (C-5, C-3), 157.0 (C-3′), 143.3 (C-1), 142.9 (C-1′), 128.9 (C-5′), 118.8 (C-6′), 115.0 (C-2′), 112.6 (C-4′), 106.21 (C-2, C-6), 100.0 (C-4), 37.0 (C-7), 36.8 (C-7′); EIMS *m*/*z* 230 [M$^+$(100)], 212 (10), 123 (80), 107 (83), 77 (20).

3,3′,5-Trimethoxybibenzyl (32). Wittig reaction using **21** (1 g, 2.4 μmol), **16** (0.329 g, 2.4 μmol), and NaH (0.057 g, 2.4 μmol) and applying the same general strategy as for **26** gave bibenzyl **32** (0.397 g, 60.9%): IR (KBr) ν_{max} 1600, 1594, 1454, 1204, 1150, 1067, 831 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.2 (1H, dd, *J* = 8.4, 7.8, Hz, H-5′), 6.79 (1H, d, *J* = 8.1 Hz, H-6′), 6.76−6.73 (2H, m, H-2′, H-4′), 6.35 (2H, d, *J* = 2.4 Hz, H-2, H-6), 6.31 (1H, dd, *J* = 2.4, 2.4 Hz, H-4), 3.76 (3H, s, OMe-3′), 3.74 (6H, s, OMe-3, OMe-5), 2.8 (4H, m, H-7, H-7′); ^{13}C NMR ($CDCl_3$) δ 160.6 (C-5, C-3), 159.5 (C-3′), 144.0 (C-1), 143.2 (C-1′), 129.2 (C-4′), 120.7 (C-5′), 114.1 (C-2′), 111.2 (C-3′), 106.4 (C-2, C-6), 97.9 (C-4), 55.2 (OMe-3, OMe-5), 55.1 (OMe-3′), 38.2 (C-7), 37.8 (C-7′); EIMS *m*/*z* 272 [M$^+$(100)].

3,5-Dihydroxybibenzyl (34). Compounds **20** (0.64 g, 0.011 μmol) and **15** (0.05 g, 0.09 μmol) were condensed in the presence of NaH (0.026 g, 0.011 μmol) to yield a mixture of the corresponding Z- and *E*-stilbene (3.29 g, 65%), which upon catalytic hydrogenation as described for **26** gave **34** (0.05 g, 41%). The spectral data of **34** were consistent with those reported in the literature.[27]

3-Hydroxy-4-methoxybibenzyl (35). Wittig reaction using as starting materials **22** (0.230 mg, 0.65 μmol), **12** (0.329 g, 2.4 μmol), and NaH (0.0156 g, 0.65) and applying the same general strategy as for **26** gave compound **35** (0.067 g, 58%).[29]

Pharmacological Testing. The pharmacological tests employing the guinea-pig ileum model were performed using two different types of experiments, as previously described.[2,39] In the first one, the crude extract, primary fractions, and natural and synthetic compounds were evaluated for their ability to relax spontaneous ileal contractions. In the second, the effect of **6** and **10** on the contractions of the ileum in the presence of L-NAME (500 μM), ODQ (100 μM), and chlorpromazine (100 μM) was investigated. Male guinea-pigs (600−800 g) were used. The animals were sacrificed by cervical dislocation. The ileum was dissected and placed in Krebs-Henseleit (KH) solution, pH 7.4, with the following composition (in mM): NaCl 119, KCl 4.6, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.5, $NaHCO_3$ 20, and glucose 11.4. Strips (1 cm long) were dissected and mounted in organ baths containing KH solution gassed with a mixture of 5% CO_2 and 95% O_2 and continuously recorded for isometric tension with a Grass 7D polygraph, as previously described.[39] After a stabilization time of 30 min, a 10 min control period was recorded. The test substances (extract, chromatographic fractions, and compounds), dissolved in dimethyl sulfoxide (DMSO), were added to the bath in a volume of 50 μL at different concentrations (one concentration was used per ileum segment). All the responses were recorded for a 10 min period. The effects of the plant crude extract, fractions, pure compounds, and positive controls were determined by comparing the areas under the curve (AUC) inscribed by the frequency and the amplitude of the test materials. Areas were calculated from the polygraph tracings, using an analog-digital tablet (CPLAB-10) and specially designed software. All the results are expressed as the mean of six experiments ± SEM. Concentration−response curves for the extract and pure compounds were plotted and the experimental data adjusted by the nonlinear curve-fitting program (PRISMA). The statistical significance ($p < 0.05$) of differences between means was assessed by an analysis of variance (ANOVA) followed by a Dunnet's test.[39,40]

Interaction of Compounds with Bovine-Brain CaM. The interaction of the stilbenoids (natural and synthetics) with bovine-brain CaM (Sigma) was performed using a denaturing homogeneous electrophoresis (SDS-PAGE) procedure. The experiment was carried out according to a previously described procedure[30] using a 15% polyacrylamide gel. The interaction of the compounds with CaM was evaluated by observing the difference in electrophoretic mobility in the presence of Ca^{2+}. Each electrophoretic run was done in triplicate, and chlorpromazine was used as positive control. The experimental conditions are described in the legend of Figure 3.

PDE Assay. A PDE assay in the presence of bovine-brain CaM was performed using a modification of the method described by Sharma and Wang.[31] Bovine-brain CaM (0.2 μg) was incubated with 0.015 units of CaM-deficient-CaM-dependent PDE from bovine brain (Sigma) for 3 min in 800 μL of assay solution containing 0.3 units of 5′-nucleotidase (from *Crotalus atrox* venom, Sigma), 45 mM Tris-HCl, 5.6 mM Mg(CH_3COO)$_2$, 45 mM imidazole, and 2.5 mM $CaCl_2$, pH 7.0. The

test compounds were then added to the assay medium at 10, 20, 40, 60, 80, and 100 μM in DMSO, and the samples were incubated for 30 min. Then, 100 μL of 10.8 mM cAMP, pH 7.0, was added to start the assay. After 30 min, the assay was stopped by the addition of 100 μL of 55% trichloroacetic acid solution. All of the above steps were carried out at 30 °C. The PDE reaction was coupled to the 5′-nucleotidase reaction, and the amount of inorganic phosphate released represented the activity of the PDE. The phosphate produced in the assay was measured by the method of Sumner.[30] The wavelength used for the phosphate assay was 660 nm using a CINTRA 5 spectrophotometer. Chlorpromazine was used as a positive control (IC_{50} = 10.2 μM). The results are expressed as IC_{50} values that were determined from the analysis of the concentration-effect (inhibition of the enzyme activity) curves, where each point is the mean (±SEM) of at least three experiments. The concentration−response graphics were analyzed using a curve-fitting program (Microcal Origin 6.0, Professional Software).

Molecular Modeling Calculations. Geometry optimizations and conformational search were carried out using the MMFF94 force-field calculations as implemented in the Spartan'02 molecular modeling software from Wavefunction, Inc. (Irvine, CA).

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References and Notes

(1) Estrada, S.; Acevedo, L.; Rodríguez, M.; Toscano, R. A.; Mata, R. *Nat. Prod. Lett.* **2002**, *16*, 81−86.
(2) Estrada, S.; Rojas, A.; Mathison, Y.; Israel, A.; Mata, R. *Planta Med.* **1999**, *65*, 109−114, and references therein.
(3) Estrada, S.; Toscano, R. A.; Mata, R. *J. Nat. Prod.* **1999**, *62*, 1175−1178.
(4) Tezuka, Y.; Hirano, H.; Kikuchi, T.; Xu, G. J. *Chem. Pharm. Bull.* **1991**, *39*, 593−598.
(5) Jujena, R. K.; Sharma, S. C.; Tandon, J. S. *Phytochemistry* **1987**, *26*, 1123−1126.
(6) Tuchinda, P.; Udchachon, J.; Khumtaveeporn, K.; Taylor, W. C.; Engelhardt, L. M.; White, A. H. *Phytochemistry* **1988**, *27*, 3267−3271.
(7) Min, Z.-D.; Tanaka, T.; Iinuma, M.; Mizuno, M. *J. Nat. Prod.* **1987**, *50*, 1189.
(8) Majumder, P. L.; Lahiri, S. *Phytochemistry* **1990**, *29*, 621−624.
(9) Wollenweber, E.; Dietz, V. H. *Phytochemistry* **1981**, *20*, 869−932.
(10) Furness, M. S.; Robinson, T. P.; Goldsmith, J.; Bowen, J. P. *Tetrahedron Lett.* **1999**, *40*, 459−462.
(11) Demir, A. S.; Sesenoglu, O. *Tetrahedron Asymm.* **2002**, *13*, 667−670.
(12) Lightner D. A.; Gurst J. E. *Organic Conformational Analysis and Stereochemistry from Circular Dichroism Spectroscopy*; Wiley-VCH: New York, 2000; pp 337−393.
(13) Snatzke, G. *Tetrahedron* **1965**, *21*, 421−438.
(14) Gorham, J. *The Biochemistry of the Stilbenoids;* Chapman & Hall: London, 1995; pp 128−133.
(15) Crombie, L.; Jamieson, S. V. J. *Chem. Soc., Perkin Trans. 1* **1982**, 1467−1475.
(16) Hashimoto, T.; Hasegawa, K. *Phytochemistry* **1974**, *13*, 2849−2852.
(17) Reimann, E. *Chem. Ber.* **1969**, *102*, 2881−2888.
(18) Senyavina, L. B.; Sheichenko, V. I.; Sheinker, Y. N.; Dombrovskii, A. V.; Shevchuk, M. I.; Barsukov, L. I.; Bergelson, L. D. *Z. Obshchei Khim.* **1967**, *37*, 499−506.
(19) Mannila, E.; Talvitie, A.; Kolehmainen, E. *Phytochemistry* **1993**, *33*, 813−816.
(20) Honda, Ch.; Yamaki, M. *Nat. Med.* **2001**, *55*, 68−70.
(21) Crombie, L.; Jamieson, S. V. J. *Chem. Soc., Perkin Trans. 1* **1982**, 1455−1466.
(22) Yasuhiro, T.; Hiroyuki, H.; Tohru, K.; Guojun, X. *Chem. Pharm. Bull.* **1991**, *39*, 593−598.
(23) Yishihiko, I.; Masafumi, O.; Hiroshi, T.; Kimiye, B.; Mitsugi, K.; Hideo, N. *Chem. Pharm. Bull.* **1991**, *39*, 3353−3354.
(24) Sachdev, K.; Kulshreshtha, D. K. *Phytochemistry* **1986**, *25*, 499−502.
(25) El-Feraly, F. S. *J. Nat. Prod.* **1984**, *47*, 89−92.
(26) Matsuda, H.; Morikawa, T.; Toguchida, I.; Park, J.-Y.; Harima, S.; Yoshikawa, M. *Bioorg. Med. Chem.* **2001**, *9*, 41−50.
(27) Asakawa, Y.; Kondo, K.; Tori, M.; Hashimoto, T.; Ogawa, S. *Phytochemistry* **1991**, *30*, 1, 219−234.
(28) Takasugi, M.; Kawashima, S.; Monde, K.; Katsui, N.; Masamune, T.; Shirata, A. *Phytochemistry* **1987**, *26*, 37−376.
(29) Scheline, R. R. *Experientia* **1974**, *30*, 880−881.
(30) Ovadi J. In *Progress in Drug Research*; Jucker, E., Ed.; Birkhauser Verlag: Basel, 1989; Vol. 33, pp 353−395.
(31) Hook, S.; Means, A. R. *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41*, 471−505.
(32) Cantabrana, B.; Vallina, J. R. P.; Menendes, L.; Hidalgo, A. *Life Sci.* **1995**, *57*, 1333−1341.
(33) Macías, M.; Ulloa, M.; Gamboa, A.; Mata, R. *J. Nat. Prod.* **2000**, *63*, 757−758.
(34) Sharma, R. K.; Wang, J. H. In *Advances in Cyclic Nucleotide Research*; Greengard, P., Robinson G. A., Eds.; Raven Press: New York, 1979; Vol. 10, pp 187−198.
(35) Leung, P. C.; Taylor, W. A.; Wang, J. H.; Tripton, C. L. *J. Biol. Chem.* **1984**, *259*, 2742−2747.
(36) Sumner, J. B. *Science* **1944**, *100*, 413−415.
(37) Ranu, B. C.; Jana, U. *J. Org. Chem.* **1999**, *64*, 6380−6386.
(38) Crombie, L.; Crombie, M. L. *J. Chem Soc., Perkin Trans.* I **1982**, 1455−1466.
(39) Rojas, A.; Cruz, S.; Rauch, V.; Bye, R.; Linares, E.; Mata, R. *Phytomedicine* **1995**, *2*, 51−55.
(40) Bailey, N. T. J. *Statistical Methods in Biology*; Cambridge University Press: Cambridge, UK, 1995; pp 234−236.

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