Five New Prenylated Stilbenes from the Root Bark of *Lonchocarpus chiricanus*

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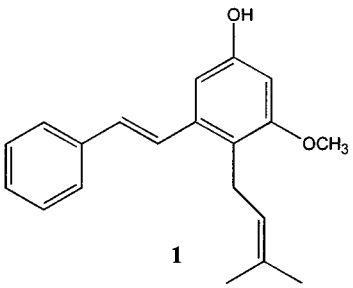
Besides the known compounds longistylines C (**1**), D (**2**), and 3,5-dimethoxystilbene (**5**), five new prenylated stilbenes, named chiricanines A−E (**3**, **4**, **6**−**8**), have been isolated from the root bark of *Lonchocarpus chiricanus*. Their structures were resolved on the basis of spectrometric methods including ^{1}H, ^{13}C, and 2D NMR experiments and mass spectrometry. Compound **3** was the only prenylated stilbene to demonstrate antifungal effects against *Cladosporium cucumerinum*. Four of the isolated compounds showed toxic properties against larvae of the yellow fever-transmitting mosquito *Aedes aegypti*. Compound **5** was found to be as potent as rotenone in larvicidal dilution tests.

Lonchocarpus chiricanus Pittier (Leguminosae) is a tree endemic to Panama that can reach 15 m in height. It is usually found around the coasts on the Pacific Ocean side of Panama.[1] The *Lonchocarpus* genus is well known for its insecticidal properties due to the presence of rotenone derivatives.[2−4] Different types of compounds have also been isolated from *Lonchocarpus* species including flavonoids,[5−15] stilbenes,[14−16] chalcones,[17,21] aurones,[22] triterpenoids,[23] benzoic acid, [24] and dibenzoylmethane derivatives.[25]

In a routine screening for new antifungal and larvicidal lead compounds, the dichloromethane extract of *Lonchocarpus chiricanus* root bark was found to give marked activity against the phytopathogenic fungus *Cladosporium cucumerinum*[26] and the larvae of the yellow fever-transmitting mosquito *Aedes aegypti.*[27] Although several species of the genus have already been investigated for their biological properties,[3,6,12,22−25,28−30] neither activity data nor chemical reports on *L. chiricanus* were found in the literature. Thus, we decided to undertake a phytochemical investigation of the active extract with the aim of isolating both major and bioactive compounds.

Results and Discussion

HPLC/UV/DAD analysis showed the presence of two major compounds (**1** and **2**) in the biologically active dichloromethane extract. Their UV spectra exhibited maxima at around 210 and 280−300 nm, suggesting the presence of stilbene derivatives.[16] After chromatographic fractionation on a silica gel column, the active extract was separated into 28 fractions, which were evaluated for their fungicidal activities against *C. cucumerinum* and their larvicidal effects against *A. aegypti*. Compounds **1** and **2**, respectively obtained in pure form from fractions 14 and 17, were found to be inactive in the *C. cucumerinum* assay. However, these two compounds seemed to be implicated in the larvicidal activity of the extract which was located in fraction 4 and fractions 11−18. Resulting data furnished by ^{1}H and ^{13}C NMR spectroscopy experiments and EI and D/CI mass spectrometry measurements were compared to those of literature data[16] to identify **1** and **2** as longistylines C and D, respectively. These products were previously reported from the root and the bark of *Lonchocarpus violaceus* Jacq.



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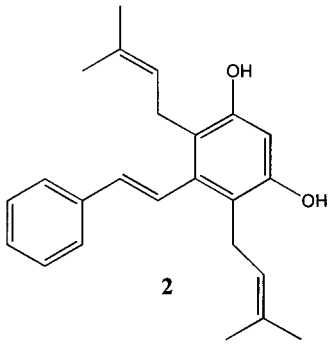

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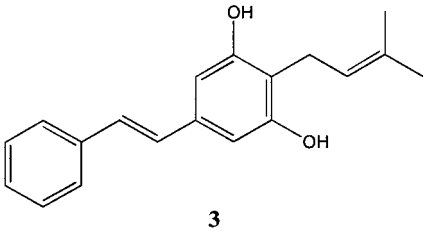

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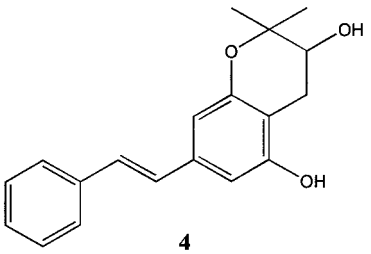

4

Compounds **3** and **4** were successfully isolated from the antifungal fraction 12 after a LPLC separation on a RP-18 column. A repetition of the *C. cucumerinum* test showed

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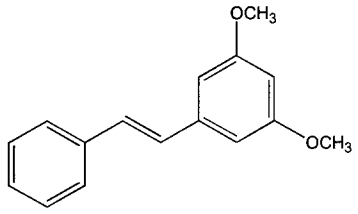
that **3** was the substance responsible for the antifungal properties of the extract, while **4** remained inactive on the targeted mold. Compounds **3** and **4** both showed UV profiles quite similar to those of the previously isolated stilbenes **1** and **2**, with maxima around 200 and 315 nm. Ions observed for **3** and **4** at *m/z* 280 $[M]^{\bullet+}$ and *m/z* 296 $[M]^{\bullet+}$, respectively, in the EI/MS mode and at *m/z* 298 $[M + NH_4]^+$ and *m/z* 298 $[M + NH_4]^+$, respectively, in DCI/MS indicated a molecular formula of $C_{19}H_{20}O_2$ for **3** and $C_{19}H_{20}O_3$ for **4**. Typical aromatic signals of an unsubstituted phenyl moiety appeared between δ 7.2 and 7.5 ppm (H-2,6) in the ^{1}H NMR spectrum of both compounds. Long-distance heteronuclear correlations between ^{13}C NMR resonances at δ 126.5 (C-2 and C-6) and ^{1}H NMR doublets at δ 6.98 ppm (H-7) for **3**, or at δ 7.00 ppm (H-7) for **4**, showed the former phenyl unit to be contiguous to a *trans* double bond (*J* around 16 Hz).[37] Supplementary HMBC cross-peaks between the ^{1}H NMR signal at δ 6.90 ppm (H-8) and ^{13}C NMR resonances located at δ 106.5 ppm (C-2′ and C-6′) for **3**, respectively between the proton observed at δ 6.95 ppm (H-8) and signals at δ 108.0 and 105.0 ppm (C-2′and C-6′) for **4**, gave evidence for a linkage between the second double bond doublet and another aromatic ring. Products **3** and **4** were thus both identified as stilbene derivatives. A substitution symmetry on the latter aromatic ring was noticed in the ^{1}H NMR spectrum of **3** by the presence of singlets at δ 6.57 ppm (H-2′ and H-6′) and δ 5.22 ppm (OH-3′ and OH-5′) correlated to ^{13}C NMR resonances at δ 106.5 ppm (C-2′ and C-6′) and δ 155.0 ppm (C-3′ and C-5′), in the HSQC spectrum. Finally, a (3-methyl)-2-butenyl substituent was identified in the *para* position to the double bond due to typical signals of two methyl groups at δ 1.82 ppm (CH_3-4″) and 1.75 (CH_3-5″), of a methine unit at δ 5.27 ppm (H-2″), and of a methylene group at δ 3.42 ppm (H-1″). These data were in perfect agreement with those of vedelianin, a stilbene derivative isolated from *Macaranga vedeliana* Muell.-Arg. (Euphorbiaceae), which showed an identical aromatic moiety substitution.[31] As no reference to this compound was found in the literature, compound **3** is, to our knowledge, a new natural product. This antifungal prenylated stilbene was named chiricanine A. A growth inhibition of *C. cucumerinum* was obtained with 5 μg of chircanine A in the bioautographic assay and a concentration limit of 30 μg/mL in the dilution test. Chiricanine A was shown to be about 30 times less active than nystatin, a commercially available antifungal substance used as reference compound in this assay.[32]

Compound **4** was found to possess a structure closely related to that of **3** but with the presence of an asymmetric substitution pattern due to cyclization of the prenylated moiety with one of the *ortho* hydroxyl groups. The resonance appearing at δ 69.3 ppm (C-2″) in the ^{13}C spectrum was explained by the hydroxylation of this prenylated unit. These observations were confirmed by comparison of the ^{1}H and ^{13}C NMR data obtained from compound **4** with those of 3′-(1-hydroxyisopropyl)furanopentacoccol, a phloroglucinol derivative isolated from the leaves of *Bosistoa pentacocca* (F. Muell.) Baillon (Rutaceae), which possesses the same substitution pattern on the aromatic ring.[33] To our knowledge, it is the first time that this compound has been reported from nature. This stilbene derivative was thus named chiricanine B.

As fraction 4 exhibited larvicidal effects, it was subjected to a LPLC separation on a RP-18 column, yielding pure compound **5**. According to its UV spectrum and its typical ^{1}H and ^{13}C NMR data, compound **5** was identified as 3,5-dimethoxystilbene, previously extracted from Jack pine (*Pinus banksiana* Lamb., Pinaceae).[34]



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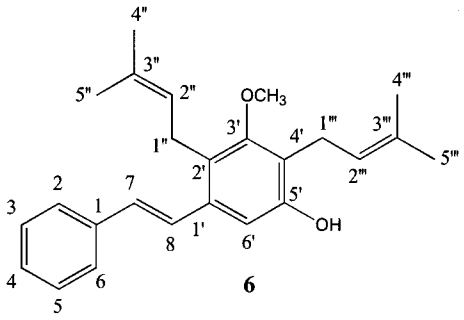
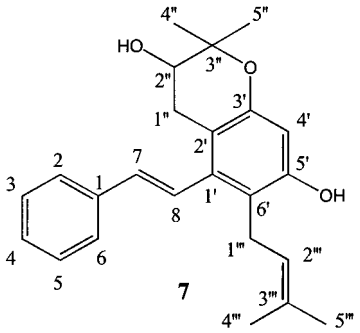


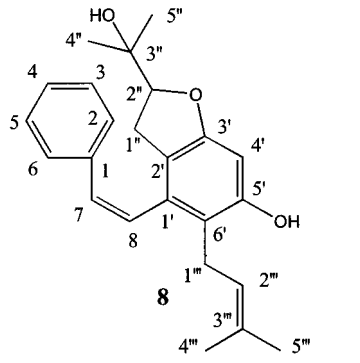
6

Although fraction 5 remained inactive in both antifungal and larvicidal assays, it appeared to contain quantitatively one of the major substances of the studied extract. This compound showed a UV spectrum similar to those of the previously isolated stilbenes and the presence of an ion at *m/z* 363 in a preliminary HPLC/UV/DADMS analysis of the active material. As a literature search for stilbenes with a presumed molecular weight of 362 amu gave no results, the purification of compound **6** was performed using LPLC with a RP-18 column. Confirmation of the suspected molecular weight was achieved after observation of ions at *m/z* 362 $[M]^{\bullet+}$ in EIMS and at *m/z* 380 $[M + NH_4]^+$ in the DCIMS. As in the case of the previous stilbenes, typical ^{1}H and ^{13}C NMR signals of **6** gave evidence for the presence of an unsubstituted phenyl group and a *trans* double bond.[38] Two (3-methyl)-2-butenyl units were also identified. The first of them was shown to be attached to C-2′ by measurement of a long-distance heteronuclear correlation between the C-1′ resonance at δ 136.3 ppm and the ^{1}H NMR signal at δ 3.46 ppm. The aromatic singlet δ 6.96 ppm was assigned to C-6′ after observation of a long-distance heteronuclear correlation between its geminal ^{13}C NMR resonance at δ 108.9 ppm (C-6′) and the double bond doublet at δ 7.28 ppm (H-8). Cross-peaks seen in the NOESY spectrum between the aromatic hydroxyl singlet found at δ 5.28 ppm and signals at δ 6.96 (H-6′) and 3.46 (H-1‴) ppm indicated that the OH unit should be on C-5′ and the second prenylated group attached to C-4′. These assignments were in perfect agreement with the remaining bidimensional NMR data obtained for compound **6**. The determined structure was finally confirmed by ^{1}H and ^{13}C NMR data comparison with gancaonin S,[35] a dihydrostilbene isolated from *Glycyrrhiza uralensis* Fisch. et DC (Leguminosae), with a structure closely related to that of **6**. Compound **6** was indeed found to be a novel natural product and was named chiricanine C.

Finally, two more stilbene derivatives were separated from fractions 18 and 20. Compound **7** was obtained after a LPLC separation of fraction 18 on a RP-18 column. Ions at 365 $[M + H]^+$ in a TSPMS analysis, *m/z* 364 $[M]^{\bullet+}$ in EIMS, and *m/z* 382 $[M + NH_4]^+$ in the DCIMS were consistent with a molecular weight of 364 amu. In agreement with the ^{13}C NMR spectrum, a molecular formula of

$C_{24}H_{28}O_3$ was deduced for compound **7**. As for previously isolated stilbenes, the low-field ^{1}H NMR signals were characteristic of an unsubstituted phenyl group and of a *trans* double bond.[38] According to the ^{1}H and ^{13}C NMR spectra, a (3-methyl)-2-butenyl substituent and a (3-hydroxyl-2,2-dimethyl)pyran moiety due to the cyclization of a hydrated prenylated unit with an aromatic hydroxyl function were identified. HMBC correlations between the ^{13}C NMR resonance at δ 138.7 ppm (C-1′) and ^{1}H NMR signals corresponding to the prenylated methylene groups located at δ 3.44 ppm (C-1‴) and at 2.96 and 2.72 ppm (C-1″) proved these prenylated units to be linked to C-2′ and C-6′. Observation of supplementary HMBC correlations between the same methylene groups at δ 3.44 (C-1‴) and at 2.96 and 2.72 ppm (C-1″) and ^{13}C NMR resonances at δ 154.2 (C-5′) and 151.7 ppm (C-3′), respectively, demonstrated that both prenylated moieties were *ortho* substituted by aromatic hydroxyl groups. The last unattributed ^{1}H NMR signal at δ 6.36 ppm (H-4′) was assigned to a proton *para* to the double bond of the stilbene moiety, in agreement with long-distance correlations between the latter singlet and ^{13}C NMR signals appearing at δ 154.2 (C-5′) and 151.7 ppm (C-3′). The structure hypothesis was finally confirmed by ^{1}H and ^{13}C NMR data comparison with gancaonin T,[35] a dihydrostilbene isolated from *Glycyrrhiza uralensis* Fisch. et DC (Leguminosae), which possesses an identical pattern of substitution. Compound **7** is a new natural product named chiricanine D.





Compound **8** was purified from fraction 20 by semipreparative chromatography using a diol stationary phase. Typical resonances for an unsubstituted phenyl moiety were seen in the ^{1}H and ^{13}C NMR spectra. A coupling constant of 12.1 Hz for doublets observed at δ 6.64 and 6.52 ppm in the ^{1}H NMR experience gave evidence for a *cis* configuration of a double bond.[37] ^{1}H NMR resonances at δ 3.31 (H-1‴), 5.06 (H-2‴), 1.75 (CH_3-4‴), and 1.67 ppm (CH_3-5‴) associated with ^{13}C signals at δ 26.5 (C-1‴), 122.2 (C-2‴), 134.6 (C-3‴), 17.8 (C-4‴), and 25.7 ppm (C-5‴) indicated the presence of a (3-methyl)-2-butenyl unit. The ^{13}C NMR signals at δ 30.2 (C-1″), 89.6 (C-2″), 72.0 (C-3″), 23.4 (C-4″), and 25.4 ppm (C-5″) correlated in the HSQC spectrum to ^{1}H NMR resonances centered at δ 2.67 and 2.48 (H-1″), 4.42 (H-2″), 1.13 (CH_3-4″), and 0.96 ppm (CH_3-5″), respectively, gave evidence for a hydroxyisopropyl−dihydrofuranyl substituent of prenylated origin as in the case of erycristanol A[36] isolated from *Erythrina crista-galli*, a plant belonging to the Leguminosae family. These two prenylated moieties were found to be located on C-2′ and C-6′ through HMBC correlations between their sp^2-hybridized carbons at δ 117.2 and 116.2 ppm and the ^{1}H NMR signal belonging to the double bond at δ 6.52 ppm (H-8). These assignments were in agreement with NOE correlations observed between methylene protons of the prenylated units at δ 2.67, 2.48 (H-1″) and 3.31 ppm (H-1‴) and ^{1}H NMR resonances of the neighboring phenyl moiety at δ 7.09 ppm (H-2 and -6) and of the double bond located at δ 6.52 ppm (H-8). According to the long-distance correlations of ^{13}C NMR resonances at δ 158.6 (C-3′) and 154.6 ppm (C-5′) with the ^{1}H NMR singlet at δ 6.29 ppm, the latter proton was positioned at C-4′. This result was supported by observation of a NOE effect on the ^{1}H NMR signal at δ 6.29 ppm (H-4′) after irradiation of the ^{1}H NMR singlet at 5.21 ppm corresponding to the hydroxyl group. Compound **8** was thus identified as a new natural product that was named chiricanine E.

Chiricanine A (**3**) was responsible for the antifungal properties of the dichloromethane root extract of *L. chiricanus* against *C. cucumerinum*. However, chiricanine A was found to be less active than nystatin on this latter mold in both bioautographic and dilution assays. Longistyline C, longistyline D, chiricanine A, and 3,5-dimethoxystilbene were identified as the *A. aegypti* larvicidal agents of the extract. In dilution tests, 3,5-dimethoxystilbene was even found to be as potent as rotenone, a well-known insecticidal compound originally extracted from species belonging to the *Derris* or *Lonchocarpus* genera. Stilbene derivatives have been identified as the substances responsible for the antifungal and larvicidal activities of the *L. chiricanus* dichloromethane extract. Considering a structure−activity relationship, a high antifungal selectivity was observed against *C. cucumerinum*, as only one of the stilbene analogues, the prenylated product **3**, was responsible for the activity of the extract. On the contrary, the raw extract larvicidal properties were due to prenylated and nonprenylated stilbene derivatives. Compounds exhibiting a cyclization of the prenylated units were found to be inactive against *A. aegypti* larvae. However, these structure−activity considerations should be confirmed by further biological testing of other stilbene derivatives. Results of the bioassays on pure compounds are reported in Table 3.

Experimental Section

General Experimental Procedures. Mp: Mettler-FP-80/82 hot stage apparatus, uncorrected. ^{1}H and ^{13}C NMR: Varian Unity Inova NMR instrument, Palo Alto, CA. ^{1}H and ^{13}C NMR spectra recorded in $CDCl_3$ at 500.00 and 125 MHz, respectively. TMS: internal standard. UV: Varian DMS 100S UV−vis spectrophotometer. UV spectra recorded in MeOH. $[\alpha]_D$: Perkin-Elmer-241 polarimeter. TLC: silica gel 60 F_{254} Al sheets (Merck) using petroleum ether−EtOAc, 1:1, diol HPTLC F_{254} plates (Merck) with hexanes−EtOAc, 8:2, RP-18 HPTLC F_{254} plates (Merck) with MeOH−H_2O in different proportions. CC: silica gel (63−200 μm; 650 × 65 mm i.d., Merck). LPLC: Lobar Lichroprep diol (40−63 μm; 270 × 25 mm i.d.; Merck). Semipreparative HPLC: Shimadzu 9A liquid chromatography pump coupled with a Hewlett-Packard 1040A DAD using a Lichrosorb Diol column (7 μm; 250 × 16 mm i.d.; Merck) and

Table 1. ^{1}H NMR Data for Compounds **3**−**8** ($CDCl_3$)

	3	**4**	**6**	**7**	**8**
2,6	7.46 *d* (7.3)	7.46 *d* (7.4)	7.48 *d* (7.4)	7.50 *d* (7.8)	7.09 *m*
3,5	7.32 *d* (7.3)	7.34 *d* (7.4)	7.35 *d* (7.4)	7.40 *d* (7.8)	7.16 *m*
4	7.24 *t* (7.3)	7.24 *t* (7.3)	7.27 *t* (7.4)	7.32 *t* (7.5)	7.16 *m*
7	6.98 *d* (16.2)[a]	7.00 *d* (16.2)	6.93 *d* (16.4)	6.56 *d* (16.6)	6.64 *d* (12.1)
8	6.90 *d* (16.2)[a]	6.95 *d* (16.5)	7.28 *d* (15.9)	7.04 *d* (15.6)	6.52 *d* (12.1)
2′	6.57 *s*	6.65 *s*			
3′	5.22 *s*				
4′				6.36 *s*	6.29 *s*
5′	5.22 *s*	4.86 *bs*	5.28 *s*	5.26 *s*	5.21 *s*
6′	6.57 *s*	6.52 *s*	6.96 *s*		
1″	3.42 *d* (7.0)	2.93 *dd* (17.0, 5.0)	3.46 *bd* (6.3)[a]	2.96 *dd* (16.7, 5.1)	2.67 *dd* (15.3, 9.5)
		2.72 *dd* (17.0, 5.0)		2.72 *dd* (16.6, 5.6)	2.48 *dd* (15.3, 8.9)
2″	5.27 *t* (7.0)	3.85 *t* (5.0)	5.16 *t* (6.7)[b]	3.78 *t* (5.2)	4.42 *t* (9.0)
CH_3-4″	1.82 *s*	1.39 *s*	1.83 *s*	1.34 *s*	1.13 *s*
CH_3-5″	1.76 *s*	1.33 *s*	1.70 *s*	1.37 *s*	0.96 *s*
1‴			3.46 *bd* (6.3)[a]	3.44 *d* (6.3)	3.31 *d* (6.8)
2‴			5.27 *m*[b]	5.25 *t* (6.0)	5.06 *t* (6.8)
CH_3-4‴			1.85 *s*	1.76 *s*	1.75 *s*
CH_3-5‴			1.77 *s*	1.76 *s*	1.67 *s*
3′-OCH_3			3.72 *s*		
5′-OCH_3			-		

Table 2. ^{13}C NMR Data for Compounds **3**−**8** ($CDCl_3$)

C	**3**	**4**	**6**	**7**	**8**
1	137.1[a]	137.3[a]	137.7	137.1	137.1
2	126.5[b]	126.5[b]	126.5[a]	126.4[a]	128.3[a]
3	128.6[c]	128.7[c]	128.6[b]	128.7[b]	128.4[a]
4	127.6[d]	127.6	127.6	127.8	127.4[b]
5	128.6[c]	128.7[c]	128.6[b]	128.7[b]	128.4[a]
6	126.5[b]	126.5[b]	126.5[a]	126.4[a]	128.3[a]
7	128.0[d]	128.8[d]	129.8	134.8	131.7
8	128.7[d]	128.1[d]	126.3	126.0	127.4[b]
1′	136.8[a]	137.2[a]	136.3	138.7	134.2[c]
2′	106.5	108.0	120.1	109.7	117.2
3′	155.0	154.5[e]	156.9	151.7	158.6
4′	113.3	106.2	125.4	103.5	96.9
5′	155.0	154.0[e]	153.9	154.2	154.6
6′	106.5	105.0	108.9	118.5	116.2
1″	22.5	26.3	25.3[c]	30.4	30.2
2″	121.3	69.3	124.1[d]	70.0	89.6
3″	135.6	76.8	131.3[e]	76.1	72.0
4″	17.9	22.2	18.1[f]	24.7	23.4
5″	25.8	24.5	25.8[g]	21.9	25.4[d]
1‴			23.7[c]	26.6	26.5
2‴			122.0[d]	122.9	122.2
3‴			134.9[e]	133.7	134.6[c]
4‴			17.9[f]	18.0	17.8
5‴			25.7[g]	25.8	25.7[d]
3′-OCH3			61.7		
5′-OCH3					

[a−g] Values in the same column with the same symbol may be interchanged.

Table 3. Antifungal and Larvicidal Activities of the Isolated Compounds

compound	*Cladosporium cucumerinum*[a]	*Cladosporium cucumerinum*[b]	*Aedes aegypti*[c]
1	n.a.[d]	n.t.[e]	50
2	n.a.	n.t.	6
3	5	30	6
4	n.a.	n.t.	>50
5	n.a.	n.t.	3
6	n.a.	n.t.	>50
7	n.a.	n.t.	>50
8	n.t.	n.t.	n.t.
nystatin	0.2	1	
rotenone			3

[a] Minimal amount (μg) of compound to inhibit growth on a silica gel TLC plate. [b] Minimal inhibition concentration MIC (μg/mL) of compound in an agar-dilution assay. [c] Minimal concentration (ppm) of compound required to kill all the larvae after 24 h. [d] n.a. not active at the highest tested amount (100 μg). [e] n.t. not tested.

hexanes−EtOAc (79:21) as the mobile phase. EIMS and D/CIMS: Finnigan MAT TSQ-700 triple stage quadrupole instrument. LC/UV-DAD/TSPMS: LC separation required a Waters (Bedford, MA) 600MS solvent delivery system and a Hewlett-Packard (Waldbronn, Germany) 1050 series on-line photodiode array detector (DAD). MS detection was achieved on a Finnigan MAT TSQ 700 triple quadrupole instrument. Separation of the extract was achieved using acetonitrile−H_2O gradient (35:65→100:0) in 30 min followed by acetonitrile−H_2O, 100:0, for 10 min. LCMS was performed directly after UV-DAD measurements. An aqueous buffer of 0.5 M ammonium acetate was added postcolumn (0.2 mL/min) with a Waters MS 590 pump to help ionization. A thermospray (Finningan MAT, San Jose, CA) interface was used with the following conditions: source temperature 280 °C; vaporizer 95 °C; filament off and positive ion mode. Spectra (150−800 mu) were recorded every 3 s. LC/UV-DAD: the purity control of the isolated compounds was performed by LC/UV-DAD (Hewlett-Packard 1090 Series II) with a Symmetry RP-18 column (4 μm; 250 × 3.9 mm i.d.; Waters) using the same conditions of separation as used for LC/UV-DAD/TSPMS. The detection was set at 210, 254, 280, and 366 nm.

Plant Material. Roots of *Lonchocarpus chiricanus* Pittier were collected in December 1996, in the National Park of Coiba Island, Veraguas, Panama. A voucher is deposited at the National Herbarium of Panama (FLORPAN 2728) and at the Institut de Pharmacognosie et Phytochimie, Lausanne, Switzerland (No. 97019).

Extraction and Isolation. Air-dried powdered root bark of *L. chiricanus* (625 g) was extracted at room temperature with dichloromethane to afford 71.8 g of extract. After homogenization of a part (13.8 g) of this extract with around 60 g of silica gel, a solid introduction was performed on an open silica gel column. This first fractionation was achieved with a petroleum ether−EtOAc gradient step (10:1→0:1) to yield fractions 1−28. Products **1** (2.57 g) and **2** (2.30 g), the major compounds of the active extract, were respectively obtained in a pure form from fractions 14 and 17. Compounds **5** (8.6 mg) and **6** (15.9 mg) were respectively isolated from fractions 4 and 5 by separation on a RP-18 column using a LPLC system with an isocratic mixture of MeOH−H_2O (9:1) as the mobile phase. Compounds **3** (39.8 mg) and **4** (44.7 mg) were successively obtained from fraction 12 after a separation on a LPLC RP-18 column using a MeOH−H_2O gradient step (6:4→1:0) as the mobile phase. An LPLC separation on a RP-18 column with the gradient step previously described was employed to isolate compound **7** (22.0 mg) from fraction 18. The same conditions of separation were applied to fraction 20 to give subfractions 20a−d. Further purification of subfraction 20a on a diol column

using semipreparative chromatography with hexanes−EtOAc (79:21) as the mobile phase gave finally compound **8** (2.0 mg).

Sample Preparation for Bioautographic Assays. Geometric dilutions (concentration range from 1 to 0.015 mg/mL) were obtained from freshly prepared stock solutions of isolated and reference compounds at a concentration of 1 mg/mL in dichloromethane (isolated compounds) or MeOH (nystatine). A 10 μL sample of these solutions was applied on the TLC plates using graduated capillaries.

Bioautographic Assays. Direct bioautography with *C. cucumerinum*: after application of the samples on a silica gel 60 F_{254} Al sheet (Merck), the TLC plates were developed in a petroleum ether−EtOAc, 1:1, solvent system and thoroughly dried for complete removal of solvents. The plate was then sprayed with a suspension of *C. cucumerinum* in a nutritive medium and incubated for 2−3 days in polystyrene boxes with a moist atmosphere. Clear inhibition zones appeared against a dark gray background. Nystatin (Sigma) was used as reference compound.

Dilution Assays. Geometric dilutions of **3** were freshly prepared in DMSO from a stock solution at 3 mg/mL (in DMSO). Aliquots of the dilutions (concentration range from 60 to 3 μg/mL) were added to Sabouraud agar medium (Biokar Diagnostics), which was distributed in 24-well plates. A suspension of *C. cucumerinum* in distilled water was spread over the agar. Well plates were closed hermetically and incubated at 30 °C for 24 h. Control experiments without test compounds were carried out for verification of fungal growth. All samples were measured in duplicate. Nystatin (Sigma) was used as reference compound (concentration range from 10 to 0.1 μg/mL).

Larvicidal Assays. Geometric dilutions of the isolated and reference compounds were freshly prepared from stock solutions at 1 mg/100 μL in DMSO. Aliquots of these dilutions were added to a graduated tube containing approximately 10 larvae of *A. aegypti* in tap water, and the final volume was adjusted to 10 mL to cover a concentration range from 50 to 1 ppm. The tubes were incubated in darkness at 26−28 °C for 24 h. Larvae lethality was observed under lab light. All samples were measured in duplicate. Rotenone (Sigma) was used as reference compound.

Longistyline C (**1**): yellow amorphous powder; mp 88−91 °C (lit.[16] 99−100 °C); UV λ_{max}^{MeOH} (log ϵ) 210 (4.33), 228 (sh, 4.06), 298 (403); LC/TSPMS *m/z* (rel int): 295 $[M + H]^+$; EIMS *m/z* (rel int) 294 $[M]^{\bullet+}$ (21), 279 (18), 252 (14), 251 (88), 236 (13), 203 (43), 189 (10), 188 (21), 165 (11), 163 (13), 162 (100), 91 (17); D/CIMS *m/z* (rel int) 295 $[M + H]^+$ (100), 251 (24), 203 (12), 162 (36).

Longistyline D (**2**): dark yellow amorphous powder; mp 69−73 °C (lit.[16] 89−91 °C); UV λ_{max}^{MeOH} (log ϵ) 203 (4.51), 279 (3.94); LC/TSPMS *m/z* (rel int) 349 $[M + H]^+$; EIMS *m/z* (rel int) 348 $[M]^{\bullet+}$ (11), 249 (156), 237 (23), 216 (100), 202 (20), 201 (31), 199 (24), 178 (26), 173 (22), 165 (31), 115 (30), 91 (72), 69 (22); D/CIMS *m/z* (rel int) 366 $[M + NH_4]^+$ (12), 365 (24), 349 $[M + H]^+$ (100), 295 (15), 216 (37).

Chiricanine A (**3**): yellow amorphous powder; mp 107−111 °C; UV λ_{max}^{MeOH} (log ϵ) 210 (4.22), 238 (sh, 4.02), 315 (4.28); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; LC/TSP-MS *m/z* (rel int) 281 $[M + H]^+$; EIMS *m/z* (rel int) 280 $[M]^{\bullet+}$ (78), 265 (20), 225 (100), 85 (34), 83 (50); D/CIMS *m/z* (rel int) 298 $[M + NH_4]^+$ (20), 281 $[M + H]^+$ (100).

Chiricanine B (**4**): yellow amorphous powder; UV λ_{max}^{MeOH} (log ϵ) 210 (4.31), 238 (sh, 4.13), 312 (4.29); $[\alpha]_D$ −9° (*c* 0.3, $CHCl_3$); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* (rel int) 296 $[M]^{\bullet+}$ (70), 225 (100), 83 (76). D/CIMS *m/z* (rel int) 314 $[M + NH_4]^+$ (40), 297 $[M + H]^+$ (100), 280 (12).

3,5-Dimethoxystilbene (5): yellow amorphous powder; mp 53−55 °C; UV λ_{max}^{MeOH} (log ϵ) 211 (4.14), 228 (sh, 4.01), 299 (4.20); EIMS *m/z* (rel int) 240 $[M]^{\bullet+}$ (100), 239 (51), 209 (20), 165 (42), 152 (16); D/CIMS *m/z* (rel int) 241 $[M + H]^+$ (100).

Chiricanine C (**6**): yellow amorphous powder; mp 82−85 °C; UV λ_{max}^{MeOH} (log ϵ) 208 (4.22), 230 (sh, 3.96), 304 (3.96); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; LC/TSPMS *m/z* (rel int) 363 $[M + H]^+$; EIMS *m/z* (rel int) 362 $[M]^{\bullet+}$ (31), 271 (17), 263 (38), 252 (19), 231 (20), 230 (100), 215 (51), 203 (15), 178 (17), 175 (40), 174 (20), 165 (19), 91 (26); D/CIMS *m/z* (rel int) 380 $[M + NH_4]^+$ (62), 363 $[M + H]^+$(100).

Chiricanine D (**7**): yellow amorphous powder; mp 69−73 °C; UV λ_{max}^{MeOH} (log ϵ) 206 (4.82), 285 (4.37); $[\alpha]_D$ −12.9° (*c* 0.28, $CHCl_3$); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; LC/TSPMS *m/z* (rel int) 365 $[M + H]^+$; EIMS *m/z* (rel int) 364 $[M]^{\bullet+}$ (61), 321 (20), 320 (22), 249 (35), 237 (31), 232 (100), 161 (27); D/CIMS *m/z* (rel int) 382 $[M + NH_4]^+$ (100), 365 $[M + H]^+$ (97), 338 (35).

Chiricanine E (**8**): yellow amorphous powder; UV λ_{max}^{MeOH} (log ϵ) 201 (4.59), 242 (sh, 4.14), 282 (sh, 3.98); $[\alpha]_D$ −21.5° (*c* 0.2, $CHCl_3$); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; LC/TSPMS *m/z* (rel int) 365 $[M + H]^+$; EIMS *m/z* (rel int) 364 $[M]^{\bullet+}$ (100), 347 (20), 331 (25), 321 (22), 249 (26), 232 (26); D/CIMS *m/z* (rel int) 382 $[M + NH_4]^+$ (100), 365 $[M + H]^+$ (97), 338 (35).

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