Insecticidal Sesquiterpene Pyridine Alkaloids from *Maytenus chiapensis*

Marvin J. Núñez,† Ana Guadaño,‡ Ignacio A. Jiménez,† Angel G. Ravelo,† Azucena González-Coloma,‡ and Isabel L. Bazzocchi*,†

Instituto Universitario de Bio-Orgánica Antonio González, Universidad de La Laguna, Avenida Astrofisico Francisco Sánchez 2, 38206 La Laguna, Tenerife, Canary Islands, Spain, and Centro de Ciencias Medio Ambientales, CSIC, Serrano 115-dpdo, 28006 Madrid, Spain

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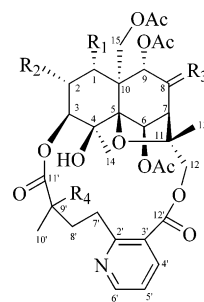
The new sesquiterpene pyridine alkaloids chiapenines ES-I (**1**), ES-II (**2**), ES-III (**3**), and ES-IV (**4**), in addition to the known alkaloids wilfordine (**5**), alatamine (**6**), wilforidine (**7**), alatusinine (**8**), euonine (**9**), euonymine (**10**), ebenifoline E−I (**11**), forrestine (**12**), mayteine (**13**), and 4-hydroxy-7-epi-chuchuhuanine E-V (**14**), were isolated from the leaves of *Maytenus chiapensis*. Their structures were elucidated by 1D and 2D NMR spectroscopy, including homonuclear and heteronuclear correlation (COSY, ROESY, HSQC, and HMBC) experiments. Wilfordine, alatusinine, and euonine exhibited strong antifeedant activity against *Spodoptera littoralis*.

The Celastraceae family is a source of sesquiterpene pyridine alkaloids derived from polyester sesquiterpenes based on the dihydro-β-agarofuran [5,11-epoxy-5β,10α-eudesman-4(14)-ene] skeleton. Sesquiterpenes of this type belong to a family of macrolactones that contain pyridine dicarboxylic acids such as evoninic, isoevoninic, wilfordic, isowilfordic, hydroxywilfordic, cassinic, edulic, or cathaic acids, which bridge the C_3−C_{12} positions of the highly functionalized sesquiterpenoid cores: evoninol, euonyminol, and isoeuonyminol.[1] These alkaloids have also been described in plants of the Hippocrateaceae. This chemical aspect reinforces the recent botanical classification in which the two families Celastraceae and Hippocrateaceae appear to be grouped in the Celastraceae.[2] These alkaloids have also been of interest due to their cytotoxic, insect antifeedant, insecticidal, immunosuppressive, and anti-HIV activities.[3]

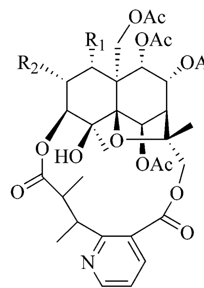
The current investigation is a study into biologically active metabolites from *Maytenus chiapensis* Lundell (Celastraceae). Our earlier work on this species yielded dihydro-β-agarofuran sesquiterpenes[4] with an unusually functionalized C-3,C-12 core, which could be considered to be precursors of the macrocyclic pyridine alkaloids. This paper reports the isolation and structural elucidation of the new sesquiterpene pyridine alkaloids chiapenines ES-I (**1**), ES-II (**2**), ES-III (**3**), and ES-IV (**4**) in addition to 10 known alkaloids (**5**−**14**)[5−14] from the leaves of *M. chiapensis* by application of 1D and 2D NMR spectroscopic techniques, including COSY, HSQC, HMBC, and ROESY experiments. We have investigated the defensive properties (insect antifeedant and toxic effects) of compounds **1**, **2**, **5**, **6**, **8**−**11**, **13**, and **14** against the aphid *Myzus persicae* and the polyphagous lepidopteran *Spodoptera littoralis*, and we have tested the cytotoxicity of these compounds on insect Sf9 and mammalian CHO cells.

Results and Discussion

Repeated chromatography of the CH_2Cl_2 extract of the leaves of *M. chiapensis* on silica gel and Sephadex LH-20 yielded four new sesquiterpene alkaloids named chiapenines ES-I (**1**), ES-II (**2**), ES-III (**3**), and ES-IV (**4**).



1 $R_1 = R_2$ = OBz, R_3 = α-OAc, β-H, R_4 = OH
2 $R_1 = R_2$ = OBz, R_3 = O, R_4 = OH
3 R_1 = OBz, R_2 = OH, R_3 = O, R_4 = OH
4 R_1 = OAc, R_2 = OH, R_3 = O, R_4 = OH
5 R_1 = OAc, R_2 = OBz, R_3 = α-OAc, β-H, R_4 = OH
6 R_1 = OAc, R_2 = OBz, R_3 = O, R_4 = OH
7 R_1 = OAc, R_2 = OH, R_3 = α-OAc, β-H, R_4 = OH
8 $R_1 = R_2$ = OAc, R_3 = α-OAc, β-H, R_4 = OH
9 $R_1 = R_2$ = OAc, R_3 = α-OAc, β-H, R_4 = H



10 $R_1 = R_2$ = OAc
11 R_1 = OBz, R_2 = OH
12 R_1 = OAc, R_2 = OBz
13 R_1 = OBz, R_2 = OAc
14 R_1 = OAc, R_2 = OH

Chiapenine ES-I (**1**) was assigned the molecular formula $C_{48}H_{51}NO_{19}$ by HREIMS. Its IR spectrum showed absorption bands for hydroxyl and ester carbonyl groups, and the

* To whom correspondence should be addressed. Tel: 34 922 318576. Fax: 34 922 318571. E-mail: ilopez@ull.es.
† Instituto Universitario de Bio-Orgánica Antonio González, Universidad de La Laguna.
‡ Centro de Ciencias Medio Ambientales, CSIC.

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Table 1. ^{1}H NMR (δ, $CDCl_3$, J are given in parentheses) Data for Compounds **1**−**4**

proton	**1**	**2**	**3**	**4**
1	6.09 d (3.6)	6.21 d (3.3)	5.94 d (3.0)	5.62 d (2.7)
2	5.53[a]	5.49 dd (3.3, 2.3)	4.09 br s	3.99 br s
3	5.16 d (2.7)	5.25 d (2.3)	5.14 d (3.3)	5.08 d (3.3)
6	6.92 s	6.79 s	6.74 s	6.77 s
7	2.41 d (3.8)	3.07 s	3.04 s	3.04 s
8	5.53[a]			
9	5.46 d (5.8)	5.70 s	5.62 s	5.55 s
12	3.75 d (12.0)	3.78 d (12.0)	3.76 d (11.3)	3.75 d (11.9)
	5.87 d (12.0)	5.95 d (12.0)	5.93 d (11.3)	5.97 d (11.9)
13	1.69 s	1.61 s	1.56 s	1.53 s
14	1.74 s	1.75 s	1.74 s	1.67 s
15	4.61 d (13.2)	4.96 d (13.0)	4.69 d (13.4)	4.99 d (13.3)
	5.72 d (13.2)	5.11 d (13.0)	5.26 d (13.4)	4.62 d (13.3)
4′	8.16 dd (7.8, 1.6)	8.15 dd (8.0, 1.6)	8.15 dd (7.9, 1.9)	8.31 dd (7.9, 1.8)
5′	7.23 dd (7.8, 4.5)	7.23 dd (8.0, 4.8)	7.25 dd (7.9, 4.8)	7.38 dd (7.9, 4.7)
6′	8.71 dd (4.5, 1.6)	8.72 dd (4.8, 1.6)	8.70 dd (4.8, 1.9)	8.74 dd (4.7, 1.8)
7′	4.11 m, 2.90 m	4.08 m, 2.88 m	4.06 m, 2.90 m	4.09 m, 2.94 m
8′	2.55 m, 2.28 m	2.50 m, 2.26 m	2.45 m, 2.25 m	2.54 m, 2.23 m
10′	1.57 s	1.56 s	1.47 s	1.43 s
OAc-1				2.01 s
OAc-6	2.22 s	2.24 s	2.02 s	2.22 s
OAc-8	2.18 s			
OAc-9	1.39 s	1.53 s	1.56 s	2.13 s
OAc-15	2.25 s	2.08 s	2.23 s	2.03 s

[a] Overlapping signals.

UV spectrum revealed the presence of an aromatic ring. The ^{1}H NMR spectrum (Table 1) showed four acetyl groups at δ 1.39, 2.18, 2.22, and 2.25 as singlets; two benzoyl groups between δ 7.33 and 8.07 (10H); and two methyl singlets at δ 1.69 and 1.74. In addition, seven methine protons at δ 2.41, 5.16, 5.46, 5.53 (2H, m overlapping), 6.09 and 6.92 (s) and two sets of methylene protons at δ 4.61, 5.72 (AB_q, J = 13.2 Hz, H-15) and δ 3.75, 5.87 (AB_q, J = 12.0 Hz, H-12), respectively, were observed. On the basis of a ^{1}H−^{1}H COSY experiment, signals at δ 6.09, 5.53, and 5.16 were assigned to H-1, H-2, and H-3, respectively, and signals at δ 2.41, 5.53, and 5.46 were assigned to H-7, H-8, and H-9, respectively, while the remaining singlet at δ 6.92 was assigned to H-6. The presence of a hydroxywilfordic acid moiety was determined by the signals of three aromatic protons corresponding to the 2,3-disubstituted pyridine unit at δ 8.71 (dd, J = 4.5, 1.6 Hz, H-6′), 8.16 (dd, J = 7.8, 1.6 Hz, H-4′), and 7.23 (dd, J = 7.8, 4.5 Hz, H-5′), two methylene protons at δ 4.11, 2.90 (H-7′) and 2.55, 2.28 (H-8′), respectively, all coupled to one another, and a methyl singlet at δ 1.57 (H-10′). These resonances are similar to those for the analogous protons in the wilfordine spectrum (**5**).[5] The ^{13}C NMR spectrum (Table 2) confirmed the presence of eight carbonyl carbons at δ 172.4, 170.3, 170.0, 169.8, 168.9, 168.0, 165.2, and 164.7; moreover, four quaternary carbons were observed at δ 52.2 (C-10), 69.9 (C-4), 85.0 (C-11), and 95.2 (C-5) as well as five aromatic carbons of the 2,3-disubstituted pyridine at δ 164.9 (C-2′), 152.1 (C-6′), 137.9 (C-4′), 125.1 (C-3′), and 120.6 (C-5′). All these data indicate that **1** is a sesquiterpene pyridine alkaloid with a dihydro-β-agarofuran skeleton.

The regiosubstitution of the ester groups around the basic skeleton was solved by an HMBC experiment. The four acetoxy carbonyl resonances at δ_C 169.8, 170.0, 168.9, and 170.3 were correlated with 3H singlets at δ_H 2.22, 2.18, 1.39, and 2.25, respectively, and the attachment of these acetoxy groups at C-6, C-8, C-9, and C-15 was established by defining cross-peaks between the acetoxy carbonyl resonances and proton signals at δ_H 6.92 (H-6), 5.53 (H-8), 5.46 (H-9), and 4.61, 5.72 (H-15). The signals at δ_C 164.7 and 165.2 were assigned to the carbonyl carbons of the benzoate moiety on the basis of their cross-peak with the phenyl *o*-protons at δ_H 8.07 and 7.79. The attachment of the benzoyloxy group at δ_C 165.2 with C-1 was defined by the cross-peak between the carboxyl resonance and the signal at δ_H 6.09 (H-1), and the remaining benzoyloxy group at δ_C 164.7 was located at position C-2. The signal at δ_C 164.9 was assigned to C-2′ on the basis of its cross-peaks with the proton signals at δ_H 4.11, 2.90 (H-7′) and 2.55, 2.28 (H-8′). Further correlations between the carbon signals at δ_C 172.4 (C-11′) and 168.0 (C-12′) with proton signals at δ_H 5.16 (H-3) and 3.75, 5.87 (H-12), respectively, proved the attachment of the hydroxywilfordate moiety at C-3 and C-12. A ROESY experiment showing NOE effects of H-1 to H-2 and H-9, Me-14 to H-3, H-6, and H-15, and H-9 to H-8 and Me-13 enabled the relative position of the substituent groups to be determined. Thus, the relative configurations of the ester groups were determined as 1α, 2α, 6β, 8α, 9α, and 15α. All of these data and comparison with those found in the literature for wilfordine (**5**)[5] established the structure of **1** as 1-benzoyloxy-1-deacetylwilfordine.

In a study of its IR, UV, ^{1}H and ^{13}C NMR data (Tables 1 and 2), and 2D experiments chiapenine ES-II (**2**), with the molecular formula $C_{46}H_{47}NO_{18}$ (HREIMS), was shown to be a sesquiterpene pyridine alkaloid with two benzoate, three acetate, and one carbonyl group. The presence of a hydroxywilfordic acid moiety was determined by comparison of the NMR data with those of **1** (Tables 1 and 2). The regiosubstitution of the ester groups around the basic skeleton was solved by examination of the HMBC experiment. Thus, the acetoxy groups were sited at C-6, C-9, and C-15 and the benzoyloxy groups at C-1 and C-2 by defining cross-peaks between carboxyl resonances and geminal protons. The carbonyl group was sited at C-8, as the signal at δ_C 195.6 was correlated with the signals at δ_H 3.07 (H-7) and 5.70 (H-9). A ROESY experiment enabled the relative configurations of the ester groups to be determined as 1α, 2α, 6β, 9α, and 15α. These data and those given in the literature for alatamine (**6**)[6] and wilfornine E[15] revealed that **2** is 1-benzoyloxy-1-deacetylalatamine.

Chiapenine ES-III (**3**) showed the molecular formula $C_{39}H_{43}NO_{17}$ by HREIMS, and its molecular weight was 104 mass units (C_7H_4O) lower than that of **2**. The ^{1}H and ^{13}C NMR spectra of **2** and **3** were similar (Tables 1 and 2), the main differences being the loss of the signals due to a

Table 2. ^{13}C NMR (δ, $CDCl_3$) Data[a] for Compounds **1**–**4**

carbon	**1**	**2**	**3**	**4**
1	73.1 d	71.4 d	73.7d	73.7 d
2	69.8 d	70.0 d	70.1 d	69.7 d
3	77.2 d	75.9 d	78.2 d	78.2 d
4	69.9 s	69.9 s	70.1 s	70.0 s
5	95.2 s	95.4 s	95.9 s	96.0 s
6	73.7 d	73.5 d	74.2 d	73.5 d
7	51.0 d	62.3 d	62.3 d	62.3 d
8	68.9 d	195.6 s	195.8 s	195.9 s
9	71.4 d	78.8 d	79.2 d	78.6 d
10	52.2 s	52.6 s	53.0 s	52.7 s
11	85.0 s	86.7 s	86.4 s	86.2 s
12	69.9 t	69.9 t	70.1 t	70.0 t
13	17.2 q	18.7 q	18.8 q	18.7 q
14	22.7 q	23.5 q	23.3 q	23.3 q
15	60.8 t	60.8 t	60.7 t	60.7 t
2′	164.9 s	164.9 s	164.8 s	164.3 s
3′	125.1 s	125.1 s	128.7 s	125.2 s
4′	137.9 d	137.9 d	138.5 d	137.8 d
5′	120.6 d	120.7 d	125.0 d	121.1 d
6′	152.1 d	152.3 d	152.2 d	152.4 d
7′	31.4 t	31.5 t	31.5 t	29.7 t
8′	38.4 t	38.8 t	38.8 t	38.7 t
9′	77.7 s	77.8 s	77.6 s	77.2 s
10′	28.2 q	27.8 q	27.5 q	27.7 q
11′	172.4 s	172.2 s	172.5 s	172.6 s
12′	168.0 s	167.9 s	167.2 s	167.3 s
OAc-1				20.5 q, 169.7 s
OAc-6	21.1 q, 169.8 s	21.4 q, 169.2 s	20.5 q, 169.2 s	21.4 q, 169.2 s
OAc-8	20.9 q, 170.0 s			
OAc-9	19.9 q, 168.9 s	19.6 q, 169.4 s	19.6 q, 169.2 s	20.2 q, 169.4 s
OAc-15	21.6 q, 170.3 s	20.5 q, 169.8 s	21.4 q, 169.3 s	20.7 q, 169.6 s

[a] Data are based on DEPT, HSQC, and HMBC experiments.

Table 3. Antifeedant and Nutritional (consumption ΔI and biomass gain ΔB, expressed as percent of the control) Effects of the Test Compounds on *S. littoralis* L6 Larvae: Cytotoxic Effects on Sf9 and CHO Cells

	S. littoralis			LD_{50} (μg/mL)[b]	
compound	EC_{50} (μg/cm^2)[a]	ΔB (%C)	ΔI (%C)	CHO	Sf9
1	3.9×10^{-2} (0.0063, 0.24)[c]	96	87	>100	>100
2	6.6×10^{-2} (0.013, 0.33)	93	92	>100	78.67 (76.30, 81.11)[c]
5	2.3×10^{-4} (1.2×10^{-5}, 0.0044)	93	109	>100	86.22 (73.95, 106.44)
6	5.2×10^{-3} (8.5×10^{-4}, 0.032)	118	117	>100	>100
8	1.6×10^{-4} (1.8×10^{-5}, 0.0014)	111	125	>100	73.22 (70.24, 76.32)
9	1.8×10^{-4} (2.2×10^{-5}, 0.0015)	98	111	>100	48.38 (35.79, 65,39)
10	>50	105	102	>100	49.60 (37.75, 65.17)
11	0.72 (0.26, 2.02)	83	81	>100	78.81 (75.34, 82.44)
13	>50	112	146	>100	>100
14	>50	101	94	>100	57.54 (43.10, 76.82)

[a] EC_{50} = concentration needed to produce 50% feeding inhibition. [b] LD_{50} = concentration needed to produce 50% cell viability. [c] 95% confidence limits.

benzoyloxy group and the upfield shift of H-1 and H-2 (δ_H 6.21 and 5.49 in **2**, versus δ_H 5.94 and 4.09 in **3**). An HMBC experiment established the regiosubstitution partners, and the relative stereochemistry was resolved by analysis of a ROESY experiment showing NOE effects of H-2 to H-1, which indicated the relative position of the hydroxyl group as 2α. All these data and comparison with those found in the literature for alatamine (**6**)[6] and wilfornine E[15] determined the structure of **3** as 1-benzoyloxy-1-deacetyl-2-debenzoylalatamine. Chemical correlation by benzoylation of **3** giving rise to **2** confirmed the proposed structure (see Experimental Section).

The structure of chiapenine ES-IV (**4**) was elucidated by spectroscopic methods, ^1H and ^{13}C NMR studies (Tables 1 and 2), 2D ^1H–^1H and ^1H–^{13}C correlations, a ROESY experiment, chemical correlations, and comparison with data in the literature for wilfornine E.[15] All these data revealed that **4** is 2-deacetylwilfornine E, which was confirmed by acetylation of **4** to give wilfornine E[15] (see Experimental Section).

Ten known compounds were identified from their spectral data upon comparison with values reported in the literature as wilfordine (**5**),[5] alatamine (**6**),[6] wilforidine (**7**),[7] alatusinine (**8**),[8] euonine (**9**),[9] euonymine (**10**),[10] ebenifoline E-I (**11**),[11] forrestine (**12**),[12] mayteine (**13**),[13] and 4-hydroxy-7-epi-chuchuhuanine E-V (**14**).[14]

Compounds **1**, **2**, **5**, **6**, **8**–**11**, **13**, and **14** were evaluated as insect antifeedants against the aphid *Myzus persicae* and the polyphagous lepidopteran *Spodoptera littoralis*, and their cytotoxicity was tested on insect Sf9 and mammalian CHO cells. The antifeedant effects of the test compounds were species- and structure-dependent (Table 3). None of them affected the feeding behavior of the aphid *M. persicae* (data not shown). *S. littoralis* showed the strongest response to **8**, **9**, and **5** followed by **6** > **1**, **2** > **11**. Orally injected *S. littoralis* larvae were not affected by these compounds.

Most of these compounds had selective moderately low cytotoxic effects on insect-derived Sf9 cells (none of them were cytotoxic to mammalian CHO cells). This cytotoxicity

indicates a mode of action other than neurotoxic, and the selectivity between insect and mammalian cells may be related to membrane factors. It has always been supposed that the lipid composition of insect cells significantly differs from that of mammalian cells. It has been shown that plasma membranes of Sf9 cells contain 10 times less cholesterol than membranes isolated from mammalian cells. Furthermore, it has been recently reported that the cholesterol-to-phospholipid ratio in Sf9 cells is lower than in mammalian cells.[16] Compounds **9**, **10**, and **14** were the most active, followed by **2**, **6**, **11**, and **5**. The lack of toxicity of these cytotoxic compounds to *S. littoralis* could be the result of metabolic detoxification or excretion. Cytotoxic effects against tumor cell lines have been described for pyridine alkaloids,[17] and this activity has been linked to the configuration at C-8 with H-8 β-epimers being inactive. However, the insect cytotoxicity of our test compounds did not show such a relationship.

The hydroxywilfordic (**1**−**8**) and wilfordic (**9**) acid esters were the strongest antifeedants, in contrast to the evonic acid esters (**10**−**14**). The number of OAc substituents (**8**, **9**) and the presence of an OAc at C-1 (**5** and **6** versus **1** and **2**) in the dihydro-β-agarofuran core were also important structural requirements for this activity.

Dihydro-β-agarofuran sesquiterpenes and alkaloids have insecticidal effects[18,19] against several insect species. Furthermore, some dihydro-β-agarofuran sesquiterpenes have been described as antifeedants to *S. littoralis*[20] with lower potencies than and a structure−activity relationship similar to those described here. Therefore, the structure of the nicotinic diacid along with the substituents of the dihydro-β-agarofuran determined the antifeedant potency of our test compounds and could be associated with a potential neuronal action of the nicotinic acid in addition to the effect of the sesquiterpene.

Previous studies have shown that nicotinic agonists of insect nicotinic acetylcholine receptors (nAChRs) were mostly insecticidal (toxic), whereas antagonists were antifeedants.[21] A gamma amino butyric acid (GABA)-mediated taste regulation has been proposed for chrysomelids and aphids partially on the basis of the antifeedant/GABA binding action of tricyclic silphinene sesquiterpenes.[22−24] Therefore, pyridine alkaloid-mediated insect taste regulation could involve nAChRs and/or GABA receptors. However, further research is needed to elucidate the neuronal effects of this class of compounds and the direct link between these neuroreceptors and insect taste regulation.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 automatic polarimeter, and the [α_D] are given in 10^{-1} deg cm^2 g^{-1}. IR (film) spectra were recorded on a Bruker IFS 55 spectrophotometer, and UV spectra were collected in absolute EtOH on a JASCO V-560 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-500, a Bruker Avance 400, or a Bruker Avance 300 spectrometer. EIMS and HREIMS were recorded on a Micromass Autospec spectrometer. Purification was performed using silica gel (particle size 40−63 μM, Merck, and HPTLC-Platten-Sil 20 UV$_{254}$, Panreac) and Sephadex LH-20 (Pharmacia).

Plant Material. *Maytenus chiapensis* Lundell (Celastraceae) was collected at the Parque Nacional El Imposible, El Salvador, in August 1999, and was identified by Prof. Edi Montalvo. A voucher specimen (ISB-88) is on file in the Jardin Botánico La Laguna, El Salvador.

Extraction and Isolation. The leaves of *M. chiapensis* (2.1 kg) were extracted with EtOH in a Soxhlet apparatus. Evaporation of the solvent under reduced pressure provided 400.2 g of crude extract, which was partitioned into a CH_2Cl_2−H_2O (1:1, v/v) solution. Removal of the CH_2Cl_2 from the organic-soluble extract under reduced pressure yielded 77.0 g of residue, which was chromatographed on a silica gel column using increasing polarity mixtures of *n*-hexane−EtOAc as an eluant to afford 54 fractions. Fractions 46−54 (8.0 g) were subjected to column chromatography over Sephadex LH-20 (*n*-hexane−$CHCl_3$−MeOH, 2:1:1) and silica gel (CH_2Cl_2−acetone of increasing polarity). Preparative HPTLC developed with benzene−EtO_2 (7:3) was used to purify the new compounds chiapenine ES-I (**1**) (30.0 mg, R_f 0.45), chiapenine ES-II (**2**) (242.0 mg, R_f 0.36), chiapenine ES-III (**3**) (24.0 mg, R_f 0.32), and chiapenine ES-IV (**4**) (6.0 mg, R_f 0.30), in addition to the known compounds wilfordine (**5**) (165.0 mg), alatamine (**6**) (68.0 mg), wilforidine (**7**) (3.2 mg), alatusinine (**8**) (28.0 mg), euonine (**9**) (47.5 mg), euonymine (**10**) (388.0 mg), ebenifoline E-I (**11**) (10.4 mg), forrestine (**12**) (9.6 mg), mayteine (**13**) (38.0 mg), and 4-hydroxy-7-epi-chuchuhuanine E-V (**14**) (45.8 mg).

Chiapenine ES-I (1): colorless lacquer; $[\alpha]^{20}_D$ +15.0° (*c* 1.30, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 268 (3.82), 229 (4.50), 201 (4.57) nm; IR (film) ν_{max} 3461, 2921, 2850, 1740, 1452, 1370, 1247, 1097, 759, 713 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.14 (1H, s, OH-4), OBz [7.33 (2H, m), 7.52 (3H, m), 7.62 (1H, t, J = 7.5 Hz), 7.79 (2H, d, J = 8.1 Hz), 8.07 (2H, d, J = 8.2 Hz)], for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ OBz [128.3 (s), 128.6 (s), 128.8 (2 × d), 129.1 (2 × d), 129.7 (2 × d), 129.9 (2 × d), 133.4 (d), 134.0 (2 × d), 164.7 (s, $-CO_2$-2), 165.2 (s, CO_2-1)], for other signals, see Table 2; EIMS *m*/*z* 945 (M^+, 2), 901 (2), 805 (2), 780 (2), 674 (3), 572 (2), 463 (100), 273 (30), 199 (25), 194 (7), 176 (10), 149 (60), 105 (42); HREIMS *m*/*z* 945.3053 (calcd for $C_{48}H_{51}NO_{19}$, 945.3055).

Chiapenine ES-II (2): colorless lacquer; $[\alpha]^{20}_D$ +60.9° (*c* 4.71, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 269 (3.80), 230 (4.50), 201 (4.50) nm; IR (film) ν_{max} 3459, 2959, 1748, 1451, 1231, 1087, 757, 712 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.28 (1H, s, OH-4), OBz [7.36 (2H, m), 7.53 (3H, m), 7.65 (1H, t, J = 7.8 Hz), 7.85 (2H, d, J = 7.9 Hz), 8.04 (2H, d, J = 7.8 Hz)], for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ OBz [128.3 (s), 128.8 (2 × d), 129.0 (s), 129.0 (2 × d), 129.7 (4 × d), 133.8 (d), 134.1 (2 × d), 164.7 (s, $-CO_2$-2), 164.8 (s, $-CO_2$-1)], for other signals, see Table 2; EIMS *m*/*z* 901 (M^+, 58), 857 (71), 842 (41), 814 (58), 784 (41), 736 (10), 467 (14), 194 (15), 176 (19), 105 (100), 77 (23); HREIMS *m*/*z* 901.2782 (calcd for $C_{46}H_{47}NO_{18}$, 901.2793).

Chiapenine ES-III (3): colorless lacquer; $[\alpha]^{20}_D$ +29.3° (*c* 0.41, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 269 (3.37), 230 (3.98), 201 (4.11) nm; IR (film) ν_{max} 3460, 2925, 2854, 1748, 1452, 1270, 1217, 1060, 758, 713 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.17 (1H, s, OH-4), OBz [7.45 (2H, t, J = 7.6 Hz), 7.58 (2H, t, J = 7.6 Hz), 8.02 (1H, d, J = 7.3 Hz)], for other signals, see Table 1; ^{13}C NMR ($CDCl_3$) δ OBz [128.7 (s), 128.7 (d), 129.9 (d), 133.9 (d), 165.0 (s)], for other signals, see Table 2; EIMS *m*/*z* 797 (M^+, 26), 754 (19), 738 (20), 710 (28), 516 (18), 250 (22), 194 (100), 176 (99), 105 (79), 57 (11); HREIMS *m*/*z* 797.2574 (calcd for $C_{39}H_{43}NO_{17}$, 797.2531).

Benzoylation of 3. Compound **3** (5.0 mg) was dissolved in dry pyridine (0.5 mL) and benzoyl chloride (6 drops), and a catalytic amount of 4-(dimethylamino)pyridine was added under argon atmosphere. The mixture was stirred for 48 h at room temperature, carried to dryness under reduced pressure, and purified by preparative TLC with a mixture of *n*-hexane−EtOAc (6:4) to give a product (3.0 mg) the spectroscopic data of which were identical with those of compound **2**.

Chiapenine ES-IV (4): colorless lacquer; $[\alpha]^{20}_D$ +7.9° (*c* 0.43, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 264 (3.52), 220 (3.89), 201 (4.11) nm; IR (film) ν_{max} 3464, 2926, 1747, 1449, 1373, 1229, 758 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.11 (1H, s, OH-4), for other signals, see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2; EIMS *m*/*z* 735 (M^+, 11), 720 (1), 691 (8), 676 (6), 648 (10), 632 (6), 618 (7), 516 (7), 250 (10), 222 (3), 194 (37), 176 (38), 149 (67), 84 (76); HREIMS *m*/*z* 735.2324 (calcd for $C_{34}H_{41}NO_{17}$, 735.2374).

Acetylation of 4. Ac_2O (4 drops) was added to compound **4** (2.0 mg) dissolved in pyridine (2 drops). The mixture was stirred at room temperature for 16 h, carried to dryness under reduced pressure, and purified by preparative TLC with a

mixture of *n*-hexane−EtOAc (1:1) to yield a product (1.5 mg) whose spectroscopic data were identical with those of wilfornine E.[15]

Insect Bioassays. *Spodoptera littoralis* and *Myzus persicae* colonies were reared on artificial diet and bell pepper (*Capsicum annuum*) plants, respectively, and maintained at 22 ± 1 °C, >70% relative humidity with a photoperiod of 16:8 h (L:D) in a growth chamber.

Choice Feeding Assay. Feeding experiments were conducted with sixth-instar *S. littoralis* larvae and *M. persicae* apterous adults. Percent feeding inhibition (%FI) and percent settling inhibition (%SI) were calculated as previously described.[25] Compounds with an FR/SI > 50% were tested in a dose−response experiment to calculate their relative potency (EC_{50} values, the effective dose for 50% feeding reduction), which was determined from linear regression analysis (%FR or %SI on log dose).

Oral Cannulation. This experiment was performed with preweighed newly molted *S. littoralis* L6-larvae as previously described.[25] An analysis of covariance (ANCOVA) on biomass gains with initial biomass as covariate (covariate $p > 0.05$) showed that initial insect weights were similar among all treatments. A second ANCOVA analysis was performed on biomass gains with food consumption as covariate to test for post-ingestive effects.

Cytotoxicity. Sf9 cells derived from *Spodoptera frugiperda* pupal ovarian tissue (European Collection of Cell Cultures, ECCC) and mammalian chinese hamster ovary cells (CHO, a gift from Dr. Pajares, I. C. Biomédicas, CSIC) were grown as previously described.[26] Cell viability was analyzed by an adaptation of the MTT colorimetric assay method. The relative potency of the active compounds (LD_{50}, effective dose to give 50% cell viability) was determined as previously described.[26]

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