Growth Inhibitory Effects on Fall Armyworm *Spodoptera frugiperda* of Some Limonoids Isolated from *Cedrela* spp. (Meliaceae)

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Dichloromethane extracts of *Cedrela salvadorensis* and *Cedrela dugessi* afforded a photogedunin epimeric mixture, gedunin and cedrelanolide. These compounds and the photogedunin epimeric acetates **3** and **4** at the 23-OH position were evaluated against *Spodoptera frugiperda*. Toosendanin, isolated from *Melia azedarach*, was used as a positive control. When tested for activity on neonate larvae into the no-choice bioassays, gedunin, photogedunin epimeric mixture, and photogedunin acetates mixture caused significant larval mortality with LC_{50} of 39.0, 10.0, and 8.0 ppm at 7 days, respectively, as well as growth reduction. All the compounds tested inhibited larval growth, compared to the control, in a concentration-dependent manner. In addition, it was possible to observe significant reduced pupal weights and adult emergence. All the tested compounds except cedrelanolide showed comparable activity to that of toosendanin.

Keywords: *Cedrela salvadorensis; C. dugessi, Meliaceae; Spodoptera frugiperda; Lepidopterae; limonoids; photogedunin; gedunin; cedrelanolide; toosendanin; insect growth inhibitors*

INTRODUCTION

New plant protection chemicals are needed for modern pest control management due to insect resistance and ecological disorders associated with numerous currently used pesticides (Castillo et al., 1999). Additional experimental work has been carried out with natural products, which are potential models for defensive substances against insect and fungal predators (Crombie, 1999). Insecticides of botanical origin may offer a source of agents for pest control (Berembaum, 1989; Castillo et al., 1998; Crowley et al., 1998; Miyazawa et al., 1998) and may be an efficient alternative to persistent synthetic insecticides (Kubo et al., 1997). The increasing interest in the possible application of secondary metabolites to pest management has directed the investigation toward search for new sources of biologically active natural products with low mammalian toxicity, lack of neurotoxic mode of action, low persistence in the environment, and biodegradability (Jacobson, 1989; Singh et al., 1997), as well as to avoid the development of resistance of the insect pest (González-Coloma et al., 1997). These characteristics may enhance their value as botanical pesticides (Isman et al., 1995; González et al., 1998).

Several efforts to find new pesticides are currently being focused on limonoids from the Meliaceae family due to their potent effects on insect pests and their low toxicity to nontarget organisms (Koul et al., 1992; Kumar et al., 1996; Singh et al., 1997). Although the Meliaceae are widely distributed in the world, only some genera, among them *Melia, Toona, Cedrela, Swietenia, Guarea,* and *Trichilia,* have been investigated for metabolites with insecticide effects (Champagne et al., 1992; Segura et al., 1993; Kraus et al., 1993; Kubo, 1993; Chan et al., 1996; Jimenez et al., 1997). Azadirachtin is the best known example of these limonoids, isolated from *Azadirachta indica* (Ramji et al., 1996) and *Melia azedarach* (Champagne et al., 1989). This compound and its analogues are potent insect antifeedant and ecdysis inhibitors (Kraus, 1995). However, the structural complexity of this compound precludes its synthesis on a commercial scale (Isman, 1996). These facts led us to search for new simpler secondary metabolites with insecticide activity from other Meliaceae plants, such as *Cedrela* spp.

From this genus, a number of limonoids have been isolated belonging to gedunin-type compounds. This group of limonoids consists of natural products which have been formed from azadirone and its derivatives via ring expansion by a Baeyer−Villiger-type reaction to give ring D lactones, having a 4,4,8-trimethyl-17-furanyl steroid skeleton (Kraus, 1995). Examples include gedunin, photogedunin, odoratin, odoratone, odoratol, cedrelanolide, mexicanolide, hydroxytirucalane, and isoodoratol. Several of these compounds have been isolated from the Mexican species *Cedrela oaxacensis* (Lopez, 1996), *Cedrela odorata* (Mezquita et al., 1997) and *Cedrela salvadorensis* Standley (Meliaceae) (Segura et al., 1994; Toscano et al., 1996; Céspedes et al., 1998). These plants are small trees and shrubs which grow on the dry Pacific slope ranging from Jalisco to Chiapas in Mexico, through Central America to northern Panama.

A phytochemical study of *C. odorata* led to the isolation of C-23-epimeric photogedunin **1** and **2** (Figure 1) (Burke et al., 1969) without determination of the stereochemistry at C-23. We have previously reported the isolation of both epimers as a mixture from *C. salvadorensis*, the synthesis of the epimeric acetates **3** and **4** (Figure 1) (Céspedes et al., 1998), and the

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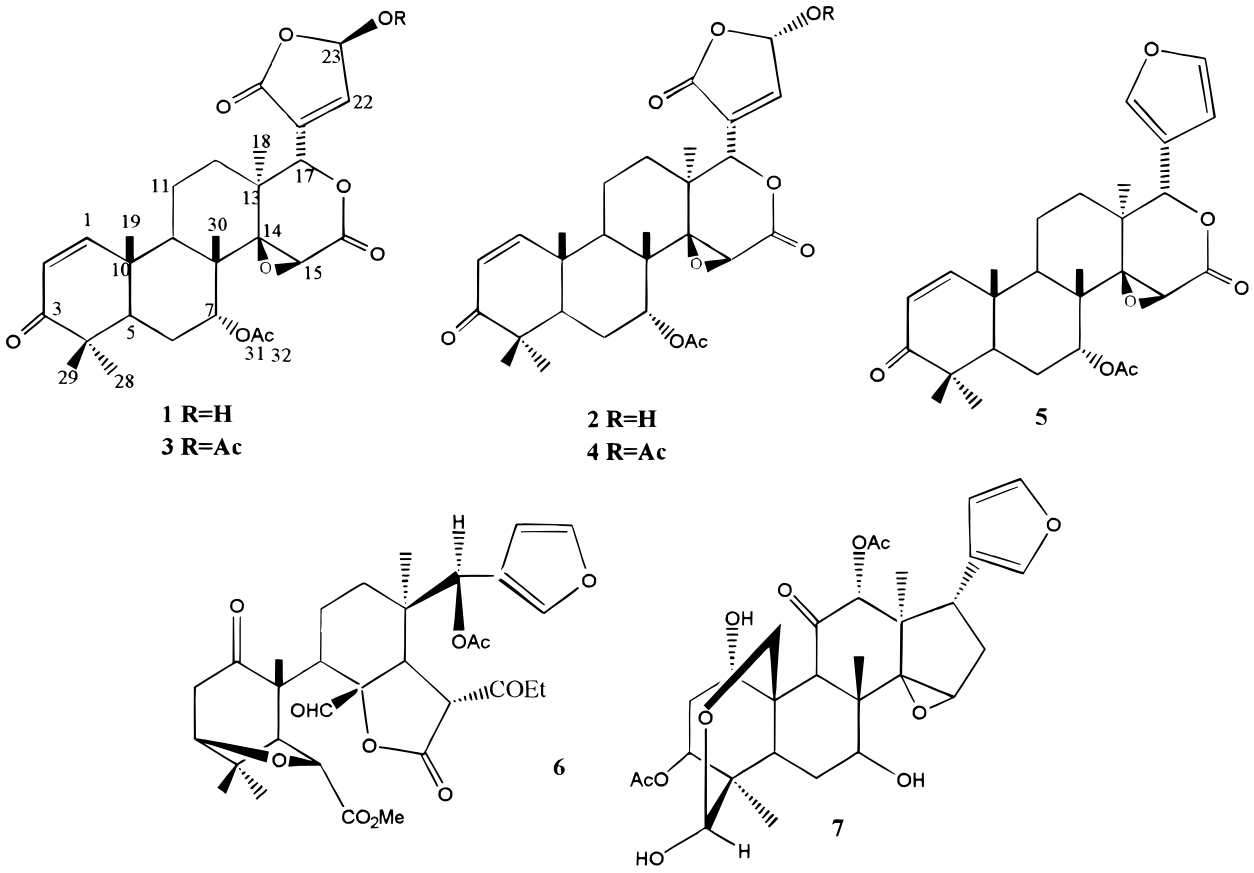

Figure 1. Chemical structures of limonoids.

isolation from *C. ciliolata* of the pure separated epimers (Céspedes et al., 1999).

We have previously described the inhibition of germination and respiration of mono and dicot seeds and electron transport carrier of spinach chloroplasts by photogedunin epimeric mixture **1** and **2** ($\beta + \alpha$) and photogedunin acetate epimeric mixture **3** + **4** ($\beta + \alpha$) (Céspedes et al., 1998, 1999). Gedunin **5** has been found to act as an inhibitor of photophosphorylation in spinach chloroplasts and have antimalaric and larval growth properties against *Ostrinia nubilalis* (Achnine et al., 1999; MacKinnon et al., 1997; Isman et al., 1996), and cedrelanolide **6** was shown as an insect growth regulator against *Ostrinia nubilalis* (Jimenez et al., 1997).

To our knowledge, the effect of **1**, **2**, **3**, **4**, gedunin **5**, and cedrelanolide **6** on *Spodoptera frugiperda* has not been investigated. In this paper, we describe the insect growth regulatory activity of a photogedunin epimeric mixture **1** and **2** ($\beta + \alpha$), a photogedunin acetate epimeric mixture **3** + **4** ($\beta + \alpha$), gedunin **5**, and cedrelanolide **6,** in comparison to toosendanin **7**, a known growth inhibitor against *Spodoptera litura* (Isman et al., 1996), *Peridroma saucia* (Chen et al., 1995), and *O. nubilalis* (Jimenez et al., 1997), against *Spodoptera frugiperda* J. E. Smith (Lepidopterae: Noctuidae), an important pest of several crops in Mexico (Aranda et al., 1996). This pest was selected for our bioassays, since it was the insect available in our lab under artificial conditions.

MATERIALS AND METHODS

Plant Material. Heartwood from *C. dugessi* and *C. salvadorensis* was collected in Morelia, State of Michoacan, in Feb 1997. A voucher sample has been filed at the ethnobotanical collection of the National Herbarium, Instituto de Biología, UNAM. Voucher: MEXU 800.192 and 800.193.

Chemicals and Solvents. All reagents used were commercially available. Agar, acetic acid, ascorbic acid, sorbic acid, *p*-hydroxybenzoic acid methyl ester, calcium pantothenate, choline chloride, formaldehyde, biotin, folic acid, niacinamide, thiamine, riboflavine, vitamin B-12, Vanderzant vitamin mixture for insects, soy meal, corn meal, yeast extract, wheat germ, Wesson salt mixture, streptomycin, and aureomycin were purchased from Sigma Chemical Co. Methanol, $CHCl_3$, CH_2Cl_2, KCl, $CuSO_4$, NH_4Cl, $MgCl_2$, pyridine, acetic anhydride, Silica gel GF_{254} analytical chromatoplates, Silica gel grade 60, 70−230 mesh, 60 Å, for column chromatography were purchased from Merck. Preparative plates were obtained from Macherey-Nagel, precoated TLC plates SIL G-100 UV_{254}, 1.0 mm. Milled ear of corn grain was purchased from "Grupo Gamesa S. A. de C. V.", Mexico.

Apparatus. ^{1}H NMR spectra were recorded at 300 and 500 MHz and ^{13}C NMR at 75 and 125 MHz, respectively, on Varian VXR-300S and VXR-500S spectrometers. Chemical shifts (ppm) are relative to $(CH_3)_4Si$ as an internal reference. $CDCl_3$ and acetone-d_6 from Aldrich Chemical Co. were used as solvents, and coupling constants are quoted in hertz. IR spectra were obtained as KBr pellets on Perkin-Elmer 283-B and FT-IR Nicolet Magna 750 spectrophotometers. Electron impact mass spectra were taken on a JEOL JMS−SX102A instrument (70 eV). UV spectra were determined on a Shimadzu UV-160 spectrophotometer; $CHCl_3$ was used as the solvent. Optical rotations were measured on a JASCO DIP-360 spectropolarimeter; $CHCl_3$ was used as the solvent. Melting points were obtained on a Fisher-Johns hot-plate apparatus and remain uncorrected. Nunc 24-well polystyrene multidishes were purchased from Cole-Parmer. LAB-LINE Chamber model CX14601A, with adjustable Hi-Lo protection thermostats safeguard samples.

Isolation of Limonoids. The compounds were isolated from the CH_2Cl_2 extract of the milled heartwood of young trees

Table 1. Fall Armyworm Bioassay Results of Limonoids of *Cedrela* spp. and Toosendanin (after 7 Days of Incubation)

treatment	concentration μg/mL (ppm)	mean weight gained (mg)[a]	mean length (cm)[b]	larval mortality (%)	LE_{50}
control		75.5 ± 8.24a	0.95 ± 0.2	8.33	
photogedunin epimers **1** + **2**	5.0	15.77 ± 3.67b	0.67 ± 0.15	18.9	10.0
	10.0	13.86 ± 2.1b	1.0 ± 0.1	49.5	
	19.2	7.23 ± 1.17b	0.54 ± 0.19	77.08	
	25.0	3.86 ± 2.3b	0.34 ± 0.16	95.83	
	52.0			100	
photogedunin acetates **3** + **4**	5.0	49.97 ± 5.6a	0.94 ± 0.42	29.2	8.0
	10.0	20.60 ± 2.9b	0.93 ± 0.29	83.3	
	15.0	3.940 ± 3.0b	0.50 ± 0.37	75.0	
	25.0	1.530 ± 0.7c	0.10 ± 0.05	91.0	
	52.0			100	
gedunin **5**	5.0	6.12 ± 6.83a	0.50 ± 0.15	25.0	39.0
	10.0	4.90 ± 1.1b	0.40 ± 0.12	33.3	
	15.0	3.60 ± 1.8b	0.35 ± 0.15	33.3	
	25.0	3.40 ± 1.2b	0.30 ± 0.17	38.3	
	39.0	2.30 ± 1.1b	0.25 ± 0.15	48.3	
	52.0	1.90 ± 1.8c	0.20 ± 0.11	70.8	
cedrelanolide **6**	5.0	27.85 ± 3.2a	1.20 ± 0.25	15	
	25.0	20.80 ± 2.1b	0.95 ± 0.16	21	
	52.0	15.40 ± 3.4b	0.70 ± 0.19	28	
	70.0	9.10 ± 3.6b	0.51 ± 0.17	38	
toosendanin **7**	5.0	5.1 ± 2.5a,b	0.98 ± 0.23	40	7.0
	25.0	2.3 ± 1.7b	0.77 ± 0.18	70	
	52.0	1.0 ± 1.6c	0.57 ± 0.20	89	

[a] Means followed by the same letter within a column are not significantly different in a Student−Newman−Keuls (SNK) test at $P < 0.05$ (treatments are compared to control). [b] Mean length total increase from eclosion.

of *C. salvadorensis* and *C. dugessi*. The heartwood of *C. salvadorensis* (7.5 kg) and *C. dugessi* (1.1 kg) were percolated with CH_2Cl_2 (10 L) and then combined. The resulting crude CH_2Cl_2 extract was evaporated to dryness at room temperature under vacuum. Then the oily residue (432.4 g) was partitioned between *n*-hexane (4 × 50 mL) (F_0-1) and CH_2Cl_2 (F_0-2). Elimination of the solvent yielded residues F_0-1 (369.8 g) and F_0-2 (51.7 g). The original extract, primary fractions, F_0-1, and F_0-2 were evaluated biologically for their potential insect growth inhibitory properties. When fraction F_0-2 was tested by the insect growth inhibitory bioassay and showed significant growth inhibition, it was then chromatographed on a silica gel column (400 × 80 mm, Si Gel grade 60, 70−230 mesh, 1000 g, gravity flow) and eluted with *n*-hexane/ethyl acetate mixtures with increasing gradient polarity starting with hexane only. A total, of 210 frs (400 mL each) were collected. Inhibitory active samples were collected from two groups of fractions (121−129 (F_0-3, 17.9 g) and 198−210 (F_0-4, 10.3)). Further purification by CC (300 × 65 mm, Si Gel grade 60, 70−230 mesh, 500 g, gravity flow, eluted with *n*-hexane/ethyl acetate increasing gradient polarity) of F_0-3 yielded photogedunin epimeric mixture (6.08 g) and gedunin (4.93 g); their spectral and R_f data were identical with an authentic sample obtained earlier (Toscano et al., 1996; Céspedes et al., 1998, 1999).

The β- and α-acetate isomers (**3** and **4)** were obtained by acetylation of epimeric photogedunin and purified by TLC (preparative plates Macherey-Nagel, precoated TLC plates Sil-G-100, UV_{254}, 1.0 mm, eluted with *n*-hexane/ethyl acetate (80:20) solvent system, using UV_{254} detection), and their structures were established by high-resolution spectroscopic methods and mass spectrometry as reported (Céspedes et al., 1998). Cedrelanolide was previously isolated as reported (Segura et al., 1994). Toosendanin was a gift from Prof. M. B. Isman.

^{1}H NMR, ^{13}C NMR, and other physical data of **1**−**4** are given in Céspedes et al. (1998, 1999).

Bioassays with Fall Armyworm. Larvae used for the experiments were obtained from the culture at the Centro de Investigación en Biotecnología at the Universidad Autónoma del Estado de Morelos, Cuernavaca, Morelos, México, maintained under previously described conditions (Aranda et al., 1996). An artificial diet containing 800 mL of sterile water, 10.0 g of Agar, 50.0 g of soy meal, 96.0 g of corn meal, 40.0 g of yeast extract, 4.0 g of wheat germ, 2.0 g of sorbic acid, 2.0 g of choline chloride, 4.0 g of ascorbic acid, 2.5 g of *p*-hydroxybenzoic acid methyl ester, 7.0 mL of Wesson salt mixture, 15.0 mL of Vanderzant vitamin mixture for insects, 2.5 mL of formaldehyde, 0.1 unit of streptomycin, 5.0 g of aureomycin, and 20.0 g of milled ear of corn grain (for 1 kg of diet) were used for the bioassay, which was prepared by the procedure described earlier (Mihm, 1987). 24-Well polystyrene multidishes were filled with the liquid diet, then left for 20 min at room temperature under sterile conditions. The 3.4 mL wells measure 17 mm in depth × 15 mm in diameter with a 1.9 cm^2 culture area. All test compounds were dissolved in 95% ethanol and layered on top of each well with the artificial diet using up to six concentrations (see Table 1) and a control (1 mL 95% ethanol) allowing evaporation of solvent. For each concentration used and control, a single *S. frugiperda* neonate first instar larva was placed on the diet mixture in each well for 7 days. After 7 days, surviving larvae were measured and weighed and then transferred to separate vials containing fresh stock diet. Larval weight gains and mortality were recorded after 21 days of incubation, since pupation average is 23 ± 1 days (Cisneros-Hernández, 1994). Other lifecycle measurements were recorded, such as time to pupation, weight of pupae, mortality of larvae, and adult emergence and deformities. All treatments were carried out in a controlled environment chamber with an 18L:6D photoperiod, at a 25 °C day and 19 °C night temperature regime and a relative humidity of 80% ± 5%. There were three replications for each assay. Control assays (24-wells) contained the same numbers of larvae, volume of diet, and ethanol as the test solutions.

Data Analysis. Data for all the live insect bioassays were analyzed by SAS ANOVA and GLM procedures (SAS Institute, 1982) ($p < 0.05$). LC_{50} values for each activity were calculated by Probit analysis (Finney, 1971) on the basis of the percentage of mortality obtained from the concentration of the compounds. Differences between treatment means were established with a Student-Newman-Keuls (SNK) test. LC_{50} is the concentration producing 50% mortality.

RESULTS AND DISCUSSION

Photogedunin epimeric acetate mixture (**3** + **4**) showed the highest insecticidal activity at 10.0 ppm with 17% survival; the photogedunin epimeric mixture (**1** + **2**) with 50% survival at 10.0 ppm in a form similar to toosendanin **7** (Table 1) was next; gedunin **5** showed this effect at 39.0 ppm. All tested compounds, except cedrelanolide, specifically inhibited each larval stage, i.e., the growth when incorporated into diets at ca. 52 ppm (Table 1).

Table 2. Activity of 1−7 on Larval Growth Parameters of *Spodoptera frugiperda* (after 21 Days of Incubation)

treatment	toncentration (ppm)	tean weight gained (mg)[a]	% of control	length (mm)	% of control
control		469.5 ± 6.59a	100.0	28.0 ± 4.1	100.0
photogedunin epimers (**1** + **2**)	5.0	159.7 ± 5.9b	34.0	18.9 ± 0.71	67.5
	10.0	48.9 ± 0.58b	10.4	10.2 ± 0.5	36.4
	19.2	2.23 ± 0.61b	0.47	3.4 ± 0.6	12.1
	25.0	1.82 ± 0.85b	0.39	3.4 ± 0.4	12.1
	52.0				
photogedunin acetates (**3** + **4**)	5.0	49.97 ± 0.56a	10.6	9.4 ± 0.42	33.5
	10.0	20.6 ± 2.25b	4.40	7.6 ± 0.3	27.1
	13.0	5.95 ± 0.41a, b	1.27	5.5 ± 0.17	19.6
	15.0	3.1 ± 0.13b	0.66	5.0 ± 0.3	17.9
	25.0	1.75 ± 0.15c	0.37	2.5 ± 0.15	8.9
	52.0				
gedunin **5**	5.0	16.12 ± 0.68a	3.4	2.6 ± 0.5	9.28
	10.0	4.95 ± 0.85b	1.05	2.9 ± 0.4	10.36
	15.0	3.61 ± 0.36b	0.77	2.6 ± 0.63	9.28
	25.0	3.45 ± 0.83b	0.73	2.1 ± 0.16	7.5
	39.0	2.80 ± 0.74b	0.60	2.0 ± 0.7	7.1
	52.0	2.20 ± 0.36c	0.47	2.1 ± 0.20	7.5
cedrelanolide **6**	5	55.7 ± 4.9a	11.9	8.7 ± 0.5	31.1
	25	41.6 ± 5.9b	8.9	6.7 ± 0.6	23.9
	52	40.1 ± 3.6b	8.5	5.3 ± 0.5	18.9
toosendanin **7**	5	21.5 ± 2.5a, b	4.58	7.0 ± 0.5	25.0
	25	17.0 ± 3.1b	3.62	6.1 ± 0.6	21.7
	52	11.2 ± 1.6c	2.38	2.8 ± 0.4	10.0

[a] Means followed by the same letter within a column are not significantly different in a Student−Newman−Keuls (SNK) test at $P < 0.05$ (treatments are compared by concentration to control). Means are ± standard error.

Table 3. Activity of 1−7 on Pupation and Emergence Parameters of *Spodoptera frugiperda*[a]

treatment	concentration (ppm)	mean time to pupation (days)	mean weight of pupae (mg)	pupation (%)	mean emergence (days)	emergences (%)
control		22.0	281.5 ± 10.3a	87.5	33	83.3
photogedunin epimers (**1** + **2**)	5	21.5	231.4 ± 3.7a	66.7	31.0	12.5
	25	22.0	138.1 ± 2.1b	20.8	32.0	8.33
	52					
photogedunin acetates (**3** + **4**)	5	21.0	92.9[c]	25.0	36.0[b]	4.17
	25	23.0[b]	63.5 ± 1.9b	12.5		0
	52					0
gedunin **5**	5	22.0	147.9 ± 7.1c	20.8	33.0	17.9
	25	23.0[b]	122.4 ± 3.3c	16.7	35.0[b]	16.7
	52	24.0[b]	52.0[c]	4.17	36.0[b]	4.17
cedrelanolide **6**	5	22.0	221.9 ± 3.9a	79.2	33	79.2
	25	21.5	191.8 ± 4.5a	66.7	33	62.5
	52	22.5	123.9 ± 3.9c	62.5	33	50.0
toosendanin **7**	5	23.5[b]	95.0 ± 2.1b	17.9	36[b]	16.7
	25	24.0[b]	45.1[c]	4.17	36[b]	4.17
	52	24.0[b]	38.0[c]	4.17	-	0

[a] Means followed by the same letter within a column are not significantly different in a Student−Newman−Keuls (SNK) test at $P < 0.05$ (treatments are compared by concentration to control). Means are ± standard error. [b] Means within a column are significantly different from control in a Kruskal-Wallis chi-squared approximation test at $P < 0.005$. [c] These values correspond to one survival larva.

Cedrelanolide **6,** between 5.0 and 70 ppm, respectively, induced only moderate larval mortalities (<40%) (Table 1). Photogedunin epimeric mixture **1** + **2**, gedunin **5**, photogedunin acetates **3** + **4**, and toosendanin **7** generally produced higher mortalities (>45%) between 10.0 and 52.0 ppm, 39 and 52 ppm, 10.0 and 52 ppm, and 25 and 52 ppm, respectively (Table 1). The surviving larvae produce deformed pupae, which did not survive (data not shown).

Furthermore, at 21 days, this growth reduction was clearly significant in the 52 ppm group (Table 2). Moreover, gedunin **5** was also more active than toosendanin **7** at low concentrations (5.0 ppm), but only photogedunin epimeric mixture (**1** + **2**) and photogedunin acetates (**3** + **4**) showed the highest larval growth inhibition at high concentrations (52.0 ppm).

We do not ignore the fact that these compounds could act as antifeedant because we did not do the election test, but as pupation did not take place (100% of inhibition above 52 ppm), we suggest that the mechanism by which these compounds act may be due to physiological effects. We are developing studies to elucidate the target and mechanism of action of these compounds.

The percentage of larvae that reached pupation decreased in all tested compounds in comparison to the control (Table 3). The most important effect was observed with photogedunin epimeric mixture at 52.0 ppm, photogedunin acetate mixture at 52 ppm, gedunin at 52 ppm, and toosendanin at 25 ppm, which reduced successful pupation to 0%, 0%, 4.17%, and 4.17%, respectively.

None of the substances tested showed any significant delay of pupation time. However, the photogedunin acetates drastically reduced the percentage of adult emergence for all concentrations tested.

The effects of photogedunin epimeric mixture (**1** + **2**), photogedunin acetates (**3** + **4**), and gedunin **5** on reducing insect growth, decreasing the percentage of emergence, and increasing mortality of *S. frugiperda* are similar to those of other limonoids (Arnason et al., 1987). The mode of action of these compounds is being inves-

tigated and may be due to a combination of antifeedant action and/or postdigestive toxicity, as found for other limonoids (Champagne et al., 1992; Isman et al., 1995).

Inspection of the structure of the most active compounds isolated from these plants suggests that the presence of an oxygenated function at C-23 was necessary for the activity displayed by the gedunin-type molecule against *S. frugiperda* (compounds **1**−**5**).

All the tested compounds except cedrelanolide showed comparable activity to the commercial insecticide toosendanin, which suggests potential for further development of these materials. However, no limonoid has been found with the outstanding activity of azadirachtin (Jimenez et al., 1997).

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LITERATURE CITED

Achnine, L.; Mata, R.; Lotina-Hennsen, B. Interference of the natural product 7-oxo-7-deacetoxygedunin with CF_0 of H^+-ATPase of spinach chloroplasts. *Pestic. Biochem. Physiol.* **1999**, *63*, 139−149.

Aranda, E.; Sanchez, J.; Peferoen, M.; Güereca, L.; Bravo, A. Interactions of *Bacillus thuringiensis* crystal proteins with the midgut epithelial cells of *Spodoptera frugiperda* (Lepidopterae: Noctuidae). *J. Invertebr. Pathol.* **1996**, *68*, 203−212.

Arnason, J. T.; Philogène, B. J.; Donskov, N.; Kubo, I. Limonoids from the Meliaceae and Rutaceae reduce feeding, growth and development of *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae). *Entomol. Exp. Appl.* **1987**, *43*, 221−227.

Berenbaum, M. R. North American Ethnobotanicals as Sources of Novel Plant-Based Insecticides. In *Insecticides of Plant Origin*; Arnason, J. T., Philogène, B. J. R., Morand, P., Eds.; ACS Symposium Series 387; Washington, DC, 1989; pp 11−24.

Burke, B.; Chan, W.; Magnus, K.; Taylor, D. Extractives of *Cedrela odorata* L.- III The structure of photogedunin. *Tetrahedron* **1969**, *25*, 5007−5011.

Castillo, M.; Martínez-Pardo, R.; Garcerá, M. D.; Covillaud, F. Biological Activities of Natural Sesquiterpene Lactones and the Effect of Synthetic Sesquiterpene Derivatives on Insect Juvenile Hormone Biosynthesis. *J. Agric. Food Chem.* **1998**, *46*, 2030−2035.

Céspedes, C. L.; Calderón, J. S.; King-Diaz, B.; Lotina-Hennsen, B. Phytochemical and biochemical characterization of epimeric photogedunin derivatives. Their different sites of interaction on redox electron transport carrier of *Spinacea oleracea* L. chloroplasts. *J. Agric. Food Chem.* **1998**, *46*, 2810−2816.

Céspedes, C. L.; Calderón, J. S.; Salazar, R.; King-Diaz, B.; Lotina-Hennsen, B. Allelopathic activity of photogedunin from *Cedrela ciliolata* (Meliaceae). *Bol. Soc. Chil. Quim.* **1999**, *44* (2), 173−183.

Chan, W. R.; Taylor, D. R. Hirtin and deacetylhirtin: new limonoids from *Trichillia hirta*. *J. Chem. Soc., Chem. Commun.* **1996**, 206−207.

Champagne, D. E.; Koul, O.; Isman, M. B.; Scudder, G. G. E.; Towers, G. H. N. Biological activity of limonoids from the Rutales. *Phytochemistry* **1992**, *31*, 377−394.

Champagne, D. E.; Isman, M. B.; Towers, G. H. N. Insecticidal Activity of Phytochemicals and Extracts of the Meliaceae. In *Insecticides of Plant Origin*; Arnason, J. T., Philogène, B. J. R., Morand, P., Eds.; ACS Symposium Series 387; Washington, DC, 1989; pp 95−109.

Chen, W.; Isman, M. B.; Chiu, S. F. Antifeedant and growth inhibitory effects of the limonoid toosendanin and *Melia toosendan* extracts on the variegated cutworm, *Peridroma saucia* (Lep., Noctuidae). *J. Appl. Entomol.* **1995**, *119*, 367−370.

Cisneros-Hernández, J. Reproducción de Lepidopteros de cereales (*Diatraea spp*., *Heliotis zea* [Boddie] y *Spodoptera frugiperda* [Smith] en CIMMYT-Mexico. In *Técnicas para la cría de insectos*; Bautista-Martínez, N.; Véjar-Cota, G.; Carrillo-Sánchez, J. L., Eds.; Colegio de Postgraduados: Chapingo, Mexico, 1994; pp 49−62.

Crombie, L. Natural product chemistry and its part in the defence against insects and fungi in agriculture. *Pestic. Sci.* **1999**, *55* (8), 761−774.

Crowley, P.; Fischer, H.; Devonshire, A. Feed the World. *Chem. Br.* **1998**, 25−28.

Finney, D. J. *Probit Analysis*; University Press: Cambridge, 1971.

González, J. A.; Estévez-Braun, A. Effect of (*E*)-Chalcone on Potato-Cyst Nematodes (*Globodera pallida* and *G. rostochiensis*). *J. Agric. Food Chem.* **1998**, *46*, 1163−1165.

González-Coloma, A.; Gutiérrez, C.; Cabrera, R.; Reina, M. Silphinene Derivatives: Their Effects and Modes of Action on Colorado Potato Beetle. *J. Agric. Food Chem.* **1997**, *45*, 946−950.

Isman, M. B.; Arnason, J. T.; Towers, G. H. N. Chemistry and Biological activity of ingredients of other species of Meliaceae. In *The Neem Tree Azadirachta indica A. Juss and Other Meliaceous Plants. Sources of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other purposes*; Schmutterer, H., Ascher, K. R. S., Isman, M. B., Jacobson, M., Ketkar, C. M., Kraus, W., Rembold, H., Saxena, R., Eds.; GTZ: Eschborn, Germany, 1995; pp 652−666

Isman, M. B.; Matsuura, H.; MacKinnon, S.; Durst, T.; Towers, G. H. N.; Arnason, J. T. Phytochemistry of the Meliaceae, So many terpenoids, So few insecticides. In *Recent Advances in Phytochemistry. Phytochemical Diversity and Redundancy in Ecological Interactions*; Romeo, J. T., Saunders, J. A., Barbosa, P., Eds.; Plenum Press: New York, 1996; Vol. 30, pp 155−178.

Jacobson, M. Botanical Pesticides Past, Present and Future. In *Insecticides of Plant Origin*; Arnason, J. T., Philogène, B. J. R., Morand, P., Eds.; ACS Symposium Series 387, Washington, DC, 1989; pp 95−109.

Jiménez, A.; Mata, R.; Pereda-Miranda, R.; Calderón, J.; Isman, M. B.; Nicol, R.; Arnason, J. T. Insecticidal limonoids from *Swietenia humilis* and *Cedrela salvadorensis*. *J. Chem. Ecol.* **1997**, *23*, 1225−1234.

Koul, O.; Isman, M. B. Toxicity of the limonoid allelochemical cedrelone to noctuid larvae. *Entomol. Exp. Appl.* **1992**, *64*, 281−287.

Kraus, W.; Bokel, M.; Schwinger, M.; Vogler, B.; Soellner, R.; Wendisch, D.; Steffens, R.; Wachendorff, U. The chemistry of azaridachtin and other insecticidal constituents of Meliaceae. In *Phytochemistry and Agriculture*; Van Beek, T. A., Breteler, H., Eds.; Kluwer Publishers: Dordrecht, 1993; pp 18−39.

Kraus, W. Biologically Active Ingredients. In *The Neem Tree Azadirachta indica A. Juss and Other Meliaceous Plants. Sources of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other purposes. XXIII*; Schmutterer, H., Ed.; VCH Verlagsgesellschaft mbH: Weinheim, Germany, 1995; pp 35−88.

Kubo, I.; Klocke, J. Insect Ecdysis Inhibitors. In *Natural Resistance of Plants to Pests*; Gree, M. B., Hedin, P. A., Eds.;

ACS Symposium Series 296; American Chemical Society: Washington, DC, 1986; pp 206−219.

Kubo, I. Insect control agents from tropical plants. In *Phytochemical Potential of Tropical plants*; Downum, K. R., Romeo, J. T., Stafford, H. A., Eds.; Plenum Press: New York, 1993; Vol. 27, pp 133−151.

Kubo, I. Tyrosinase inhibitors from plants. In *Phytochemicals for pest control*; Hedin, P. A., Hollingworth, R., Masler, E., Miyamoto, J., Thompson, D., Eds.; ACS Symposium Series 658; American Chemical Society: Washington, DC, 1997; pp 310−326.

Kumar, J.; Parmar, B. S. Physicochemical and Chemical Variation in Neem Oils and Some Bioactivity Leads against *Spodoptera litura* F. *J. Agric. Food Chem*. **1996**, *44*, 2137−2143.

Lopez-Mayorga, R. M. 1996. Estudio Fitoquímico de Corteza de *Cedrela oaxacensis*. B.Sc. Thesis, Universidad Nacional de Colombia.

MacKinnon, S.; Durst, T.; Arnason, J. T.; Angerhofer, C.; Pezzuto, J.; Sánchez-Vindas, P. E.; Poveda, L. J.; Gbeassor, M. Antimalarial activity of tropical Meliaceae extracts and gedunin derivatives. *J. Nat. Prod*. **1997**, *60*, 336−341.

Mezquita, M. V.; Rivero, B. E. Compuestos Bioactivos de *Cedrela odorata* L. (Meliaceae). B.Sc. Thesis, Universidad Nacional Autónoma de México, 1997.

Mihm, J. A. Mass rearing stem borers, fall armyworms and corn earworms at CIMMYT. In Toward insect resistant maize for the third world. *Proceedings of the International Symposium on methodologies for developing host plant resistance to maize insects*; CIMMYT: Mexico, 1987; pp 5−21.

Miyazawa, M.; Yoshio, K.; Ishikawa, Y.; Kameoka, H. Insecticidal Alkaloids against *Drosophila melanogaster* from *Nuphar japonicum* DC. *J. Agric. Food Chem*. **1998**, *46*, 1059−1063.

Ramji, N.; Venkatakrishnan, K.; Madyastha, K. M. 11-epi-azadirachtin H from *Azadirachta indica*. *Phytochemistry* **1996**, *42*, 561−562.

Segura-Correa, R.; Mata, R.; Anaya, A. L.; Hernandez-Bautista, B.; Villena, R.; Soriano-García, M.; Bye, R.; Linares, E. New tetranortriterpenoids from *Switenia humilis*. *J. Nat. Prod*. **1993**, *56*, 1567−1575.

Segura-Correa, R.; Calderón, J.; Toscano, R.; Gutiérrez, A.; Mata, R. Cedrelanolide I, a New limonoid from *Cedrela salvadorensis*. *Tetrahedron Lett*. **1994**, *35*, 3437−3440.

Singh, M.; Khokhar, S.; Malik, S.; Singh, R. Evaluation of Neem (*Azadirachta indica* A. Juss) Extracts against American Bollworm, *Helicoverpa armigera* (Hubner). *J. Agric. Food Chem*. **1997**, *45*, 3262−3268.

Toscano, R.; Mata, R.; Calderón, J.; Segura, R. Gedunin, a D-*seco* limonoid. *J. Chem. Crystallogr*. **1996**, *26*, 707−711.

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