Effects of Limonoids from *Cipadessa fruticosa* on Fall Armyworm

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Six mexicanolide limonoids isolated from the dichloromethane extract of the fruits of *Cipadessa fruticosa* Blume (Meliaceae) were evaluated against *Spodoptera frugiperda* (J. E. Smith). Gedunin was used as a positive control. When incorporated into an artificial diet of neonates at 50.0 mg kg^{-1}, febrifugin A showed 73.3% mortality. All the compounds showed moderate insecticidal activity, except for ruageanin A, when compared with the control. Febrifugin also showed growth inhibition and antifeedant activities (at 100.0 mg kg^{-1}). The correlation between the insecticidal activity of the isolated compounds and their chemical structure was discussed.

Key words: Limonoids, *Cipadessa fruticosa*, *Spodoptera frugiperda*

Introduction

Insecticides of botanical origin may offer a source of agents for pest control and may be an efficient alternative to persistent synthetic insecticides. The increasing interest in the possible application of secondary metabolites for pest management has directed the investigations toward the search for new sources of biologically active natural products with low mammalian toxicity, lack of neurotoxic action, low persistence in the environment, biodegradability, and no development of resistance by insect pest (Céspedes *et al.*, 2000). After the isolation of azadirachtin, an effective insect antifeedant against the desert locust (Butterworth and Morgan, 1971), from neem seeds, several limonoids from the order Rutales have been screened for their insect antifeedant activity.

Limonoids are modified triterpenes. They present a wide range of biological activities, including insect-antifeeding and growth-regulating properties, a variety of medicinal effects on animals and humans, and antifungal, bactericidal and antiviral properties (Champagne *et al.*, 1992).

Cipadessa fruticosa Blume (Meliaceae) is widely cultivated in the southwest of China. This plant has been reported to contain *ent*-clerodane and labdane diterpenoids (Rojatkar and Nagasampagi, 1994; Rojatkar *et al.*, 1994), limonoids, sterols, sesquiterpenoids, heneicosene derivates and one coumarin (Luo *et al.*, 2000, 2001). Flavonoid glycosides (Liang *et al.*, 1991, 1994) were isolated from *C. cinerascens*, and *C. boivinina* yielded sterols (Mulholland *et al.*, 1999).

In the present paper, mexicanolide-type limonoids, cipadesin A (**1**), ruageanin A (**2**), cipadesin (**3**), febrifugin (**4**), febrifugin A (**5**) and khayasin T (**6**), previously isolated from *C. fruticosa* (Leite *et al.*, 2005; Fig. 1), were examined on the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). *S. frugiperda* is a major pest of many crops in the Americas and one of the most important pests of tropical maize, causing up to 34% reduction in the overall productivity of this crop in Brazil (Cruz *et al.*, 1996).

Material and Methods

Plant material

The fruits of *Cipadessa fruticosa* Blume were collected in Viçosa, Minas Gerais, Brazil, and a voucher specimen (110.664) is deposited in the Herbarium of Instituto de Ciências Biológicas, USP, São Paulo, Brazil.

Extraction and isolation of compounds

The powdered air-dried fruits (990 g) of *C. fruticosa* were subsequently extracted with hexane, CH$_2$Cl$_2$ and MeOH. The conc. CH$_2$Cl$_2$ ex-
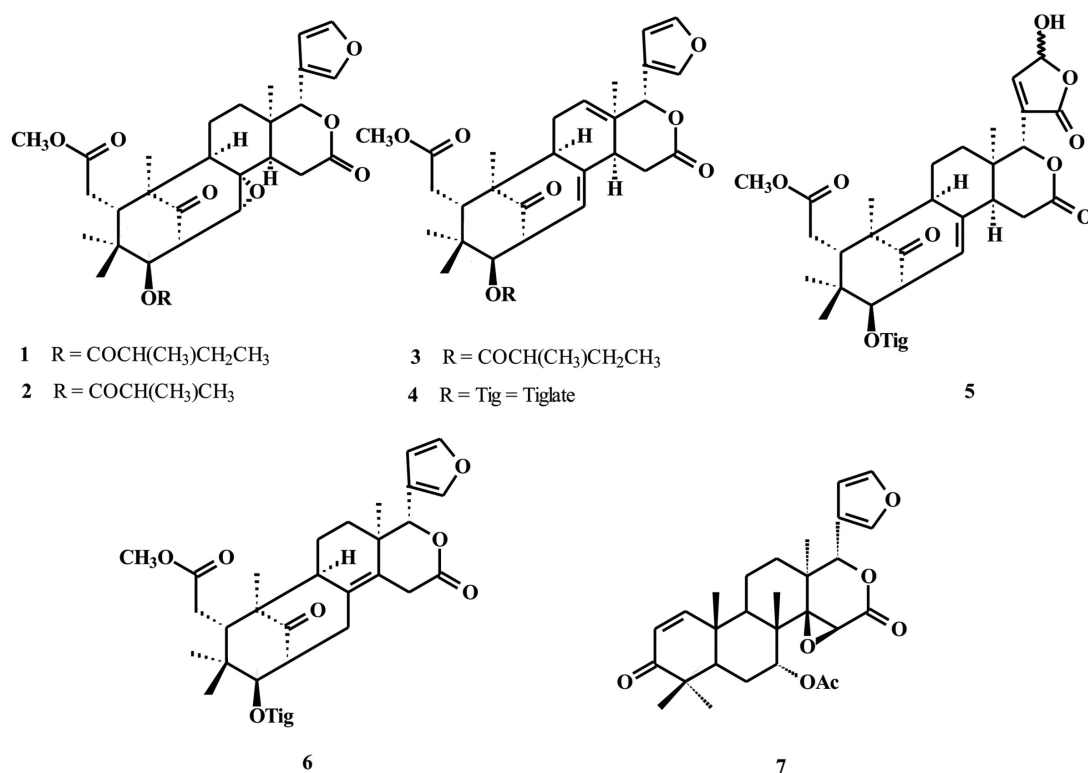
Fig. 1. Chemical structures of limonoids **1–6** from *Cipadessa fruticosa* and gedunin (**7**).

tract (10.9 g) was submitted to vacuum chromatography over silica gel using a hexane/CH$_2$Cl$_2$/EtOAc/MeOH gradient. The ethyl acetate-soluble fraction (2.3 g), rich in limonoids, was chromatographed on silica gel, eluting with a hexane/CH$_2$Cl$_2$/acetone gradient to give 8 fractions (A–H). Fraction C was fractionated as above, using a hexane/EtOAc gradient, affording 11 fractions. Fraction C-4 was eluted with a hexane/CH$_2$Cl$_2$/acetone gradient to give 6 fractions (C-4.1–C-4.6). Fraction C-4.2 was purified by HPLC (detection at λ = 240 nm), using hexane/*iso*-propanol (9:1) at 2.0 ml min^{-1} to yield khayasin T (**6**) (25.2 mg). Fraction C-6 was twice chromatographed on silica gel, eluting with hexane/CH$_2$Cl$_2$/acetone (6:3:1) to give cipadesin A (**1**) (8.5 mg) and cipadesin (**3**) (19.4 mg). Fraction D was chromatographed as above, using a hexane/EtOAc gradient, to afford 4 fractions. Fraction D-3 was twice chromatographed with a hexane/CH$_2$Cl$_2$/acetone gradient yielding a fraction containing compound **2**, which

was purified by HPLC on silica gel (detection at λ = 220 nm), using hexane/*iso*-propanol (85:15) with a flow rate of 1.5 ml min^{-1}, to yield ruageanin A (**2**) (9.3 mg). Fraction E was twice subjected to column chromatography over silica gel, eluting with a hexane/CH$_2$Cl$_2$/acetone gradient, affording 4 fractions. Fraction E-3 was purified by HPLC over a polymeric column (detection at λ = 240 nm), using hexane/*iso*-propanol (8:2) at 2.0 ml min^{-1}, to give febrifugin A (**5**) (4.7 mg) and febrifugin (**4**) (40.3 mg). ^{1}H NMR, ^{13}C NMR and other physical data of cipadesin A (**1**), ruageanin A (**2**), cipadesin (**3**), febrifugin (**4**), febrifugin A (**5**) and khayasin T (**6**) were given by Leite *et al.* (2005). Gedunin (**7**) was a gift from Dr. José Realino de Paula.

Biological activity

Larvae of *Spodoptera frugiperda* (J. E. Smith) were obtained from the Insect Bioassay Laboratory of Universidade Federal de São Carlos, Bra-

zil, and reared on artificial diets (Kasten *et al.*, 1978; Parra, 1986). They were maintained in an incubation chamber at 12 h light and 12 h dark, (70 ± 5)% relative humidity and (25 ± 1) °C. For each treatment and control, 30 neonate larvae of *S. frugiperda* were used. A solution of limonoid was added to ascorbic acid (1.56 g; an ingredient of the diet). After evaporation, the mixture was incorporated to the artificial diet in which bean and wheat germ were the basic ingredients (Kasten *et al.*, 1978) at final contents of 1.0, 10.0, 50.0 and 100.0 mg kg^{-1} for the limonoids cipadesin A (**1**), cipadesin (**3**) and febrifugin (**4**); for the limonoids ruageanina A (**2**), febrifugin A (**5**) and khayasin T (**6**) final contents of 1.0, 10.0 and 50.0 mg kg^{-1} were utilized. The diet for the control was prepared similarly with no limonoid. Gedunin (**7**) was utilized as positive control (Céspedes *et al.*, 2005). The diets were placed in previously sterilized glass tubes (8.5 cm × 2.5 cm), into which larvae of *S. frugiperda* were introduced individually. The obtained pupae were weighed after pupation and were transferred to plastic cups, where they were kept until the emergence of adults. Daily observations were made and the following parameters were evaluated: duration of larval and pupal phases; weight of pupae, and percentage of dead insects (mortality) at the end of each phase and of the life cycle (larva to emergence of adult).

Statistics

Data were submitted to an analysis of variance (ANOVA; Zar, 1984) and the averages were compared applying the Tukey test ($P \leq 0.05$). Each tube containing one insect, independent of the development phase, was considered as one replicate; therefore, the number of replicates was different for each treatment. For evaluation of the mortality of the larval and pupal phases, the experimental unit was constituted by the mean of five tubes with one larva each, with six replications by treatment.

Results and Discussion

Significant differences were observed for the mortality of larvae, pupae, and total life cycle (larva to emergence of adult) of *S. frugiperda* by increasing the content of febrifugin A (**5**) in the diet (Table I and Fig. 2). The mortality varied from 10.0% (control) to 20.0%, 46.7%, and 50.0% (in the larval phase for 1.0, 10.0 and 50.0 mg kg^{-1}, respectively). In the pupal phase, the mortality varied from 0% (control) to 6.7% (1.0 mg kg^{-1}), 6.7% (10.0 mg kg^{-1}) and 23.3% (50.0 mg kg^{-1}). These variations interfered significantly with the total cycle mortality and the observed average mortality varied from 10.0% (control) to 73.3% (50.0 mg kg^{-1}).
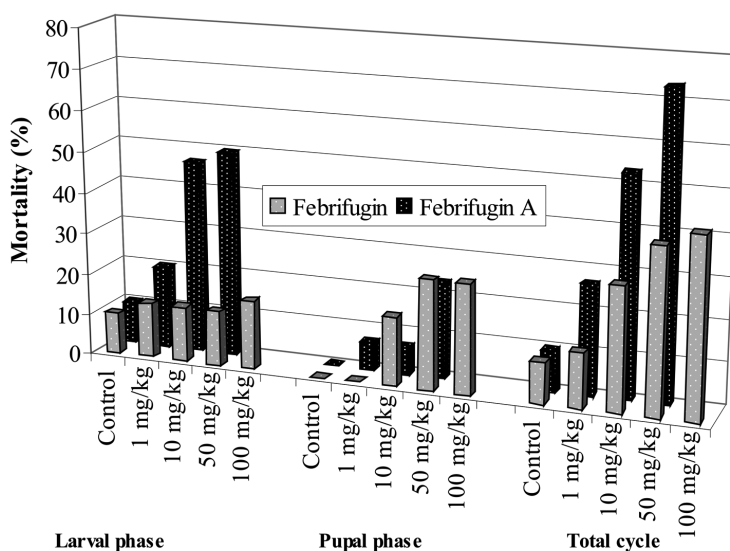

Fig. 2. Mortality of larval and pupal stages and total cycle dependent on febrifugin (**4**) and febrifugin A (**5**) administered with the artificial diet of *Spodoptera frugiperda*.

Table I. Activities of limonoids **1–7** on the mortality of larval and pupal stages and total cycle, mean duration (± SD), and weight of pupae of *Spodoptera frugiperda*.

	Content [mg kg⁻¹]	Mortality (%)[a]			Duration [d] ± SD[a]		Weight [mg][a]
		Larvae	Pupae	Total cycle	Larvae	Pupae	Pupae
Cipadesin A (**1**)	1	16.7 a	3.3 a	20.0 a	20.4 (± 1.1) a	11.1 (± 0.7) ab	279.29 a
	10	30.0 a	6.7 a	36.7 a	20.2 (± 1.4) a	10.3 (± 1.0) b	279.14 a
	50	30.0 a	6.7 a	36.7 a	20.2 (± 1.4) a	10.6 (± 1.0) b	276.19 a
	100	30.0 a	10.0 a	40.0 a	18.6 (± 0.9) b	11.3 (± 1.1) a	276.68 a
Control		10.0 a	0 a	10.0 a	20.7 (± 1.1) a	11.2 (± 1.2) ab	276.96 a
Ruageanin A (**2**)	1	3.3 a	3.3 a	6.7 a	20.3 (± 1.3) a	11.1 (± 0.7) a	273.42 a
	10	6.7 a	6.7 a	13.3 a	20.0 (± 1.2) a	11.0 (± 1.0) a	273.38 a
	50	10.0 a	10.0 a	20.0 a	19.6 (± 1.7) a	11.1 (± 1.0) a	275.44 a
Control		10.0 a	0 a	10.0 a	20.7 (± 1.1) a	11.2 (± 1.2) a	276.96 a
Cipadesin (**3**)	1	10.0 a	6.7 a	16.7 a	20.0 (± 1.5) ab	11.0 (± 0.2) a	279.64 a
	10	30.0 a	6.7 a	36.7 a	19.0 (± 2.1) b	11.7 (± 1.7) a	280.71 a
	50	30.0 a	6.7 a	36.7 a	19.1 (± 1.0) b	11.5 (± 1.0) a	280.00 a
	100	30.0 a	10.0 a	40.0 a	19.9 (± 1.2) ab	10.9 (± 1.3) a	267.52 a
Control		10.0 a	0 a	10.0 a	20.7 (± 1.1) a	11.2 (± 1.2) a	276.96 a
Febrifugin (**4**)	1	13.3 a	0 a	13.3 a	20.7 (± 0.6) b	11.2 (± 0.9) a	277.65 a
	10	13.3 a	16.7 a	30.0 a	21.2 (± 3.3) ab	10.5 (± 1.4) a	262.80 ab
	50	13.3 a	26.7 a	40.0 a	22.2 (± 2.2) ab	11.0 (± 1.1) a	252.73 b
	100	16.7 a	26.7 a	43.3 a	22.5 (± 1.9) a	11.1 (± 1.3) a	244.12 b
Control		10.0 a	0 a	10.0 a	20.7 (± 1.1) bc	11.2 (± 1.2) a	276.96 a
Febrifugin A (**5**)	1	20.0 b	6.7 a	26.7 b	20.7 (± 1.9) a	11.2 (± 1.0) a	273.96 a
	10	46.7 a	6.7 a	53.3 ab	19.6 (± 1.7) a	10.9 (± 1.1) a	269.75 a
	50	50.0 a	23.3 a	73.3 a	19.7 (± 1.1) a	11.1 (± 1.0) a	268.92 a
Control		10.0 b	0 a	10.0 bc	20.7 (± 1.1) a	11.2 (± 1.2) a	276.96 a
Khayasin T (**6**)	1	13.3 a	0 a	13.3 b	20.7 (± 0.7) b	11.1 (± 0.4) a	278.27 a
	10	13.3 a	23.3 a	36.7 ab	20.6 (± 2.1) b	11.0 (± 1.4) ab	268.34 a
	50	26.7 a	23.3 a	50.0 a	21.9 (± 1.8) a	10.0 (± 0.7) b	265.18 a
Control		10.0 a	0 a	10.0 b	20.7 (± 1.1) b	11.2 (± 1.2) a	276.96 a
Gedunin (**7**)	1	16.7 b	3.3 a	20.0 b	16.6 (± 3.1) a	11.8 (± 1.6) a	246.20 a
	10	40.0 b	16.7 a	56.7 a	16.3 (± 3.1) a	12.0 (± 1.1) a	245.63 a
	50	63.3 ab	6.7 a	70.0 a	16.2 (± 2.0) a	12.2 (± 1.0) a	235.27 b
	100	80.0 a	3.3 a	83.3 a	16.0 (± 0.6) a	12.6 (± 0.5) a	238.28 b
Control		10.0 b	6.7 a	16.7 b	14.9 (± 0.8) a	11.4 (± 1.7) a	262.51 a

[a] Means of **1–7** followed by the same letters within the columns indicate no significant difference ($P \leq 0.05$) in the Tukey test.

Nevertheless, febrifugin A (**5**) showed the highest insecticidal activity at 50.0 mg kg^{-1} with 73.3% mortality. Besides, the larvae treated with khayasin T (**6**) showed high mortality of the total cycle with 50.0% at 50.0 mg kg^{-1} when compared with the control (10%).

The compounds cipadesin A (**1**), ruageanin A (**2**), cipadesin (**3**) and febrifugin (**4**) showed no significant total cycle mortalities (≤ 40%) at 50.0 mg kg^{-1} when compared with the control (10%).

The high insecticidal activity of febrifugin A (**5**) reinforces the observation of Suresh *et al.* (2002) that a hydroxy group at C-23 and a carbonyl group at C-21 are important for the activity. These authors demonstrated that the limonoids with intact and *seco* rings and an intact furan ring show a marked increase in the antifeedant activity when compared with the furan ring oxygenated at C-21 and C-23. Céspedes *et al.* (2000) suggested that the presence of an oxygenated function at C-23 is necessary for the activity displayed by photogedunin acetate C-23 epimers and photogedunin C-23 epimers against *S. frugiperda*. These data are confirmed when compared with the moderate mortality showed in the total cycle of larvae treated with febrifugin (**4**) (Fig. 2).

The effect of mexicanolide-type limonoids on the mortality of *S. frugiperda* verified in our study was similar to that of the humilinolides against European corn borer (ECB), *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae), demonstrated by Jimenez *et al.* (1997).

A short prolongation of the larval phase was observed for larvae treated with khayasin T (**6**) and febrifugin (**4**) at 50.0 mg kg^{-1}, for 1.2 and 1.8 days, respectively, when compared with the control. These data suggest that these compounds are acting as larval growth inhibitors. In contrast, the larvae treated with cipadesin A (**1**) at 100.0 mg kg^{-1} and cipadesin (**3**) at 10, 50 and 100.0 mg kg^{-1} showed shortened larval phases of 2.1 and 1.7, 1.6 and 0.8 days, respectively (Table I).

A significant reduction (277–244 mg) in the pupal weight of *S. frugiperda* treated with febrifugin (**4**) was observed compared with the control (Table I). This reduction in the pupal weight suggests that **4** acts as antifeedant. Adults emerging from low weight pupae could be more debilitated and would have a lower capacity of competition for vital activities than individuals from healthy pupae (Batista-Pereira *et al.*, 2002).

Tanzubil and McCaffery (1990) observed that larvae treated with low doses of azadirachtin produced pupae of weights comparable to controls, suggesting that they did not experience any severe feeding inhibition. This was also observed for khayasin T (**6**). Similar cases of alteration in growth without feeding inhibition have been reported by other workers (Seiber and Rembold, 1983; Gaaboub and Hayes, 1984).

A short reduction in the pupal phase was observed for larvae fed with artificial diet treated with cipadesin A (**1**) at 10 and 50.0 mg kg^{-1} and khayasin T (**6**) at 50.0 mg kg^{-1} for 0.9, 0.6 and 1.2 days, respectively, when compared with the control.

Only febrifugin A (**5**) showed activities comparable to gedunin (**7**), which suggests potential for further development of these materials for the control of *S. frugiperda*. Febrifugin A (**5**) showed the highest insecticidal activity at 50.0 mg kg^{-1}. Febrifugin (**4**) besides a moderate insecticidal activity also demonstrated growth inhibition and antifeedant activity at 100.0 mg kg^{-1}.

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