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Full Length Research Paper

Insecticidal activity of kramecyne isolated from *Krameria cytisoides* against *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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ABSTRACT

The discovery and development of insecticides isolated from natural products is a growing area of agricultural research. Here we show that kramecyne, is cyclic peroxide obtained from *Krameria cytisoides,* has insecticidal activity against *Spodoptera frugiperda*. The insecticidal and insectistatic activities of kramecyne against *S. frugiperda* were tested at different concentrations. Larval duration increased by 6.8 d and 18.9 d at doses of 500 and 700 ppm, respectively, whereas the pupal period increased by 3.0 d and 6.5 d under those concentrations. Larval viability was 0%, 16.7%, and 33.3% when the compound was applied at 1000, 700, and 500 ppm, respectively. At 700 and 500 ppm, pupal viability was 25.4% and 50.0%, respectively, and pupal weight decreased by 16.7% and 57.0%. The LV_{50} of kramecyne was 389.3 ppm. Kramecyne isolated from *K. cytisoides* shows potent activity against larvae and pupae of *S. frugiperda* at low concentrations.

Keywords: *Spodoptera frugiperda,* insecticidal activity, *Krameria cytisoides,* kramecyne.

INTRODUCTION

The fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is native to tropical regions of the western hemisphere, with ample distribution from Mexico to South America (Crocomo and Parra, 1985). More than 80 different plants are commonly overrun by this species, though it prefers grasses such as sorghum, wheat, pastures, soybean, and cotton (Pitre and Hogg, 1983). *S. frugiperda* is the most pernicious pest of maize, and has caused incalculable economic loss in many countries (Batista-Pereira, *et al.,* 2006).

Synthetic chemical insecticides are commonly used to control *S. frugiperda*. However, abuse of these products generates chemical resistance (Foster, 1989), kills non-target organisms, and poisons animals and humans. Consequently, much research is now devoted to the discovery and development of new insecticides isolated from natural products. In some cases, these compounds are more selective or specific, less toxic and more biodegradable than synthetic insecticides (Ahmad *et al*., 2010; Nisar *et al.*, 2010a,b,c; 2011; Qayum *et al.*, 2011, 2012; Zia-Ul-Haq *et al.*, 2011; 2012).

Several compounds isolated from plants have been used to combat *S. frugiperda* including the triterpene azadirachtin from *Azadirachta indica* (Capataz *et al.*, 2007). Some eudesmane-type sesquiterpenoids from *Pluchea sagittalis* have been shown to deter larval feeding by *S. frugiperda*. Tavares *et al.* (2011) observed that piperine isolated from the dried fruits of the black pepper plant *Piper nigrum* (Piperaceae) caused 88.8% mortality in recently laid *S. frugiperda* eggs at a concentration of 1%. Ivain IV and 14, 15-dihydroajugapitin were isolated from *Ajuga iva*

(Lamiaceae) and tested at 100 ppm against *S. frugiperda* larvae. Both compounds had antifeedant activity, with feeding indexes of 74% and 61%, respectively (Bondí *et al.*, 2000). The limonoids dumsenin and zumsin were
isolated from *Croton jatrophoides* (Euphobiaceae). These compounds had antifeedant activity against second-instar *S. frugiperda* larvae; the PC_{50} were 1 µg mL^{-1} for dumsenin and 2 µg mL^{-1} for zumsin (Nihei *et al.*, 2002a, b).

Another plant with activity against S. *frugiperda* is *Krameria cytisoides,* a shrub that grows roughly 1.8 m high (Lfts. 3, obovate, to 2 cm long, sericeous. Fls. purplish, showy, sepals 2 cm long). The fruit of *K. cytisoides* are covered with spines, and the roots are used to dye wool. Twenty-one lignans, neolignans, and norneolignans have been isolated from these roots (Achenbach *et al.*, 1987). Recently, the cyclic peroxide krameycne was isolated from *K. cytisoides* leaves (Pérez-Gutiérrez *et al.*, 2011). This compound has demonstrated good anti-inflammatory activity with low toxicity (Pérez-Gutiérrez *et al.*, 2012), and preliminary bioassays have demonstrated that it also has good insecticidal activity. In this study, we present the insecticidal and insectistatic effects of this compound against *S. frugiperda*.

MATERIALS AND METHODS

Plant material

Krameria cytisoides was collected from the rural community Las Comadres Municipality of Guadalcazar, San Luis Potosi, Mexico, in June 2009. The plant was authenticated by taxonomist José García Pérez. A voucher specimen (SPLM44560) was deposited in the Isidro Palacios Herbarium of the Universidad Autónoma de San Luis Potosí. Leaves were separated and dried at room temperature in the dark.

Isolation of krameycne

Dried *K. cystisoides* leaves were ground to a powder. A 200-g sample was defatted with hexane, and the material was extracted into 2 L MeOH. The methanol extract was concentrated to half the original volume under reduced pressure, and a dark brown solid was obtained. The structure was confirmed by FAB-MS, NMR spectroscopy, FT-IR and elemental analysis.

Larvae

Larvae of *S. frugiperda* were reared in plastic cages covered with iron mesh screens at 25±2ºC at 70±5% relative humidity with a light dark cycle of 14:10 h. The artificial diet recommended by the International Maize and Wheat Improvement Center was used as the control diet (Bergvinson and Kumar, 1996).

Bioassays

The insecticidal and insectistatic activities of krameycne against *S. frugiperda* were explored at five concentrations from 300 to 1000 ppm. The tests included a negative control (diet only), and krameycne was admixed with this control diet for experimental trials. Food containing the compound was stored in acrylic glass vials (Bio-Serv catalog no. 9051) at room temperature for 24 h. Each *S. frugiperda* larva was placed in a glass vial at first instar, and the vials were stopper (Bio-Serv catalog no. 9049 stoppers). Pupae were weighed 24 h after pupation and transferred to fresh vials during development to the adult stage. The duration of larval and pupal periods, larval and pupal viability (Rodríguez-Hernández and Vendramim, 1996), pupal weights at 24 h, and larval viability in the presence of krameycne were measured. The larval half-viability levels and VL_{50} values (the concentrations of additive at which 50% of larvae died) were also determined.

Statistical analysis

The completely random experimental design was used. ANOVA analysis and Turkey's test were performed using the SAS statistical analysis program (Delwiche and Slaughter, 2002), and the LV_{50} was calculated using Probit (Raymond, 1985).

RESULTS AND DISCCUSION

Krameycne structure

The compound is a dark brown solid, isolated in a 3% yield that decomposes at 172°C. The compound's purity was determined by thin-layer chromatography and NMR spectroscopy.

The structure of krameycne was reported previously (Pérez-Gutiérrez *et al.*, 2012). The isolated compound was confirmed through experimental and spectroscopy techniques (peroxide test, FAB-MS, FT-IR, one- and two-dimensional NMR spectra and elemental analysis). The compound is a cyclic polymer of the formula $C_{30}H_{48}O_{24}$ (Figure 1a), composed of six monomers of $C_5H_8O_4$. Each monomer is cyclic peroxide with one hydroxymethylene (Figure 1b). The monomers are linked by formation of cyclic ether between atoms 4-9. The three-dimensional structure was modeled and optimized using a DFT approach at the B3LYP/6-31G level of approximation (Figure 2).

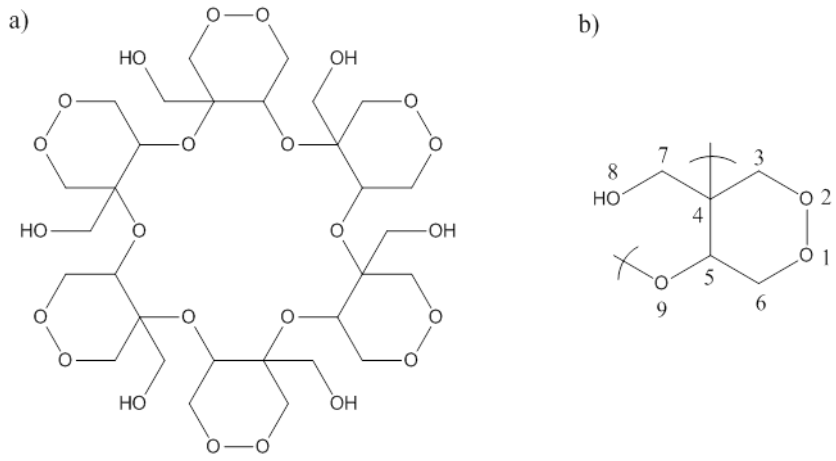

Figure 1. (a) Kramecyne structure; (b) monomer of kramecyne.

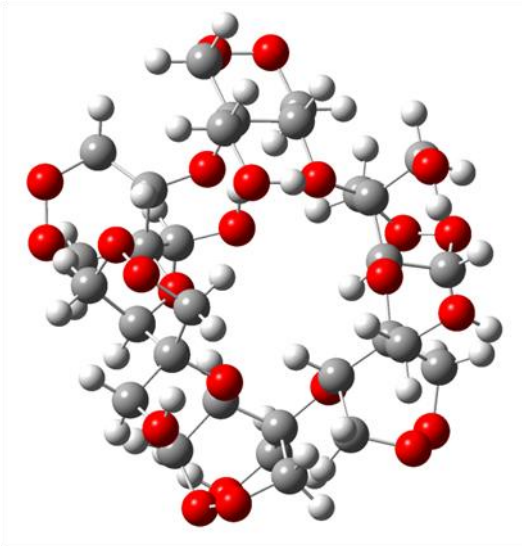

Figure 2. 3D structure of kramecyne

At present, only a small number of naturally abundant peroxides have been investigated for biological activity. For example, peroxides isolated from marine organisms have proved to be ichthyotoxic, antimicrobial, fungicidal, anthelmitic, cytostatic and ant carcinogenic agents (Tolstikov *et al.*, 2006, Rücker, 1997), and some sesquiterpene peroxides have antimalarial activity (Schenkel *et al.*, 2002). However, this is the first report of insecticidal and insectistatic activities for this functional group.

Evaluation of insecticidal and insectistatic activities

The insecticidal and insectistatic activities of kramecyne against *S. frugiperda* are summarized in tables 1 and 2. The maximum insecticidal and insectistatic activities were observed at 1000 ppm (0 % larval viability and 0 % pupae formed). At 500 and 700 ppm, larval viability was 33.3% and 16.7%, respectively, and pupal viability was 50% and 25.4% at these concentrations.

Kramecyne at concentrations of 500 and 700 ppm prolonged the larval phase by 8.8 d and 18.8 d, respectively. The duration of the pupal stage increased to 3.0 d and 6.5 d (table 2) at these two concentrations. Pupal weight was reduced by 16.7% at 500 ppm, and 57% with 700 ppm.

The LV_{50} of this compound was 389.5 ppm. Thus, kramecyne has both insectistatic and insecticide activities against *S. frugiperda*.

In figure 3 is showed 18-day-old *S. frugiperda* larvae fed with a normal diet (3.a) and diet added with 500 ppm

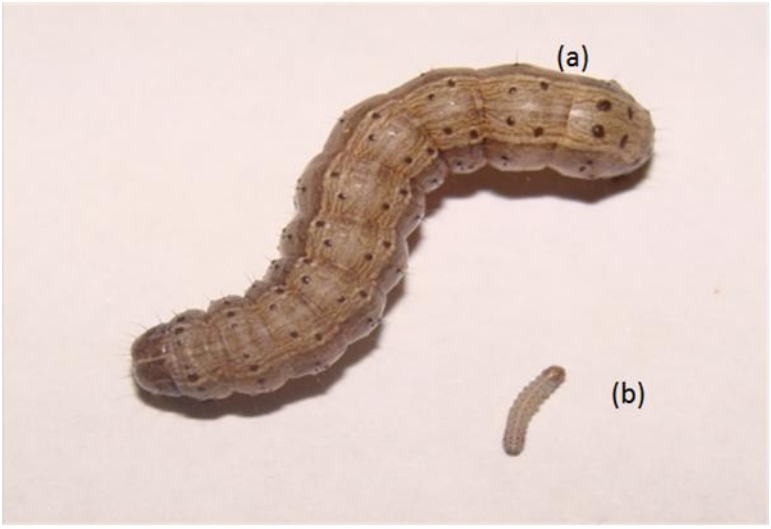

Figure 3. *S. frugiperda* larvae fed with a control diet (a) and a control diet with 500 ppm kramecyne (b).

Figure 4, *S. frugiperda* pupae fed a normal diet (left) and a normal diet with 500 ppm kramecyne (a, b).

of kramecyne (3.b). The larvae fed kramecina not reached its normal growth; these results are supported by data in tables 1 and 2.

Figure 4 showed three *S. frugiperda* pupae 24 h after formation. Pupae were fed a normal diet (4.a) and diet plus 500 ppm of kramecyne (4.b and 4.c). The pupa 4.a has a regular morphology while the pupae 4.b and 4.c showed deformations in morphology, altered color and small size. These changes prevent successful progression to the adult stage and therefore to reproduction.

We have demonstrated, for the first time, that kramecyne, a peroxide isolate of *K. cytisoides*, is active against *S. frugiperda* at low concentrations (though not toxic to mice even at dose of 5,000 mg kg^{-1}) and may thus be used in pest control.

CONCLUSIONS

Kramecyne, a peroxide compound isolated from *K. cytisoides*, has insectistatic and insecticidal activities against *S. frugiperda* at low concentrations, with not toxicity to mice. Therefore, the development of an insecticide based on kramecyne is recommended.

Table 1. Insecticidal activity of kramecyne against *S. frugiperda*.

Concentration (ppm)	Larval Viability (%)	Pupal Viability (%)
1000		
700	16.7±7.8*	25±4.2*
500	33.3±9.8*	50±7.8*
300	58.4±10.3*	64.3±10.1*
0	91.7±5.8	95.5±7.8
VL_{50}	0.3893×10^3 ppm	

Results are the mean of at least 24 determinations ± standard error. * Significantly different from control; P< 0.05.

Table 2. Insectistatic activity of Kramecyne against *S.frugiperda.*

Concentration (ppm)	Larval Phase (d)	Pupal Phase (d)	Pupal weight (mg)
1000			
700	41.3±2.4*	17±0*	97.5±17.4*
500	29.2±1.3*	13.5±0.3*	188.9±8.1*
300	24.4±0.6	11.9±0.3*	211.7±6.2
0	22.4±0.5	10.5±0.2	226.8±3.4

Results are the mean of at least 24 determinations ± standard error. * Significantly different from control; P< 0.05.

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