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ORIGINAL PAPER

Toxic effects of annonaceous acetogenins on *Oncopeltus fasciatus*

Olga Alvarez Colom · Isabel Barrachina · Ildefonso Ayala Mingol · M. Carmen Gonzalez Mas · Pilar Moya Sanz · Adriana Neske · Alicia Bardon

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Abstract From an Argentine collection of *Annona cherimolia* and a Bolivian collection of *Annona montana* (Annonaceae), the acetogenins, squamocin, molvizarin, itrabin, almuñequin, cherimolin-1, annonacin, annonacin-A, densicomacin-1, *cis*-annonacin-10-one and murihexocin-A, were obtained to study their toxicity against the cotton pest *Oncopeltus fasciatus* (Hemiptera: Lygaeidae). Topical ventral application of *O. fasciatus* nymphs with solutions of the mentioned acetogenins produced acute and delayed mortality.

Keywords *Annona cherimolia* · *Annona montana* · Annonaceous acetogenins · Entomotoxicity test · *Oncopeltus fasciatus*

Introduction

Trees of the Annonaceae family, found mainly in tropical areas of America, Africa and Southeast Asia, are important sources of edible fruits and fragrance oils. Extracts from aerial parts are employed in folk medicine for various purposes.

Characteristic constituents from these plants are the annonaceous acetogenins. They are long-chain fatty acid derivatives that possess unique structures and anticancer (Alali et al. 1999; McLaughlin et al. 1993), cytotoxic (Cortes et al. 1993), insecticidal (Alkofahi et al. 1989; Cole 1994; Leatemia and Isman 2004), and antiparasitic actions. These natural products are the most powerful inhibitors of NADH:ubiquinone oxidoreductase (complex I) in the mitochondrial electron transport chain (Gallardo et al. 2000).

Annona cherimolia (common name "chirimoya") is native to Peru and cultivated worldwide. *Annona montana* (common name "sinini") grows in Santa Cruz de la Sierra, Bolivia, where an infusion of its leaves is used for the treatment of lice, influenza and insomnia. Previously, we reported on the isolation of 12 acetogenins from an Argentine collection of *A. cherimolia* (Barrachina et al. 2004), most of which displayed antifeedant and insecticidal effects (Álvarez Colom et al. 2007) on the corn pest *Spodoptera frugiperda* (Lepidoptera). Particularly, the major acetogenin squamocin displays a strong larvicidal effect when incorporated to the larval diet at 50 ppm (Álvarez Colom et al. 2007). We report herein the toxicity of the annonaceous acetogenins squamocin, molvizarin, itrabin, almuñequin and cherimolin-1 from an Argentine collection of *A. cherimolia* and annonacin, annonacin-A, densicomacin-1, *cis*-annonacin-10-one and murihexocin-A from a Bolivian collection of *A. montana*, on *Oncopeltus fasciatus* which is a colored gregarious insect, known as milkweed bug. It is a seedeater, commonly found on seedpods, piercing the wall of the pod to feed on milkweed seeds.

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O. A. Colom · A. Neske · A. Bardon (✉)
Facultad de Bioquímica, Química y Farmacia,
Universidad Nacional de Tucumán,
Ayacucho 471, 4000 Tucuman, Argentina
e-mail: alisan@unt.edu.ar

I. Barrachina
Departamento de Farmacología, Facultad de Farmacia,
Universidad de Valencia, Burjassot, Spain

I. A. Mingol · M. C. G. Mas · P. M. Sanz
Centro de Ecología Química Agrícola (CEQA),
Universidad Politécnica de Valencia,
Campus de Vera s/n. Edificio 9B,
Laboratorio 111, 46022 Valencia, Spain

Materials and methods

Extraction and purification of acetogenins

Dried and powdered seeds of *A. cherimolia* (350 g) were percolated with methanol. Leaves and twigs (combined) of *A. montana* (550 g) were extracted in the same way. After evaporation of the solvent, 15 and 25 g of MeOH extracts were obtained, which were further partitioned between $CHCl_3$ and H_2O. The chloroform extracts were processed by chromatographic techniques including silica gel columns and high performance liquid chromatography (HPLC) with the methodology previously reported (Barrachina et al. 2004). Characterization of acetogenins was assessed by spectroscopic techniques (IR, ^{1}H NMR, ^{13}C NMR, and MS) as well as α_D determination, in comparison with previously reported data (Mc Cloud et al. 1987; Folker et al. 1990; Yu et al. 1992; Zeng et al. 1995; Rieser et al. 1996; Álvarez Colom et al. 2007).

Annonaceous acetogenins

For the present study, the acetogenins **1**, **2**, **3**, **4**, and **5** from an Argentine collection of seeds of *A. cherimolia*; and **6**, **7**, **8**, **9**, and **10** from the leaves and twigs of a Bolivian collection of *A. montana* were obtained and purified by HPLC (Fig. 1). Their structures were determined by comparison of their spectroscopic features with data previously published for the same authentic compounds.

Test insects and diet

Oncopeltus fasciatus Dallas nymphs were maintained at 27 ± 1°C, 50–60% relative humidity, 16/8 h (light/dark) photoperiod and on a diet based on sunflower seeds.

Toxicity assay

The toxicity against *O. fasciatus* was evaluated by topical application, at doses ranging from 10 to 0.1 µg/nymph, in order to obtain the LD_{50} values. One microliter of the appropriate dilution in acetone was applied, using a micropipette, on the ventral surface of the abdomen of ten newly molted fourth-instar nymphs, which had previously been anesthetized with chloroform. Later, treated nymphs were confined in a 9 cm diameter Petri dish with food and water provided ad libitum. Acute toxicity effects were considered according to the number of dead insects after 72 h of exposure to the chemicals. After 72 h, the surviving nymphs were transferred to 500 ml glass flasks and held at standard conditions in order to follow their development. Then, the number of dead adults and nymphs that die in the fifth-instar was determined and reported in Table 1, as "delayed mortality". Controls were carried out in parallel and received the same amount of acetone as treated insects. All the assays were conducted in triplicate. In order to establish a relative level of activity of our more active compounds, the LD_{50} of a very potent insecticide, the piretroid deltamethrin was evaluated against *O. fasciatus* in the same experimental conditions.

Statistical analysis

The results are reported as mean ± SD. Probit analysis (Finney 1971) was used to determine the LD_{50} values (SPSS 10).

Results

Toxicity of annonaceous acetogenins

The delayed mortality of fifth-instar nymphs and adults was evaluated. As shown in Table 1, squamocin and molvizarin were the most toxic, producing 100% mortality on fifth-instar nymphs at 0.1 and 0.25 µg/nymph, respectively. The remaining acetogenins were toxic at higher doses. At 10 µg/nymph, annonacin, annonacin-A, and *cis*-annonacin-10-one killed more than 50% of the adult population and a lower percentage of the population of fifth-instar nymphs. Noteworthy, densicomacin-1 produced 10% acute mortality but did not kill fifth-instar nymphs, however, it killed 28.8% of adults at 10 µg/nymph. Little or no effect was observed for murihexocin-A, and cherimolin-1 in the experimental conditions employed.

As reported in Table 2, for the most active acetogenins, mortality was recorded after 72 h of exposure to different concentrations (ranging from 0.1 to 15 µg/nymph) of the chemicals in order to obtain the lethal dose 50 with its 95% confidence limits, LD_{50} (95% CL). The most potent compounds were squamocin and molvizarin. Although, they are around 25 and 50 times, respectively, less active than deltamethrin, a potent insecticide employed as control to establish a relative level of activity.

Discussion

As shown in Tables 1 and 2, the ventral application of some of the annonaceous acetogenins produced mortality of nymphs and adults at low doses, whereas others had no effects in the experimental conditions employed. Toxic effects of annonaceous acetogenins have been reported on several insect species (Alali et al. 1999; Leatemia and Isman 2004). He et al. (1997) indicated that acetogenins possessing adjacent bis-tetra hydrofurane (THF) rings, with three hydroxyl groups, displayed more potent insec-

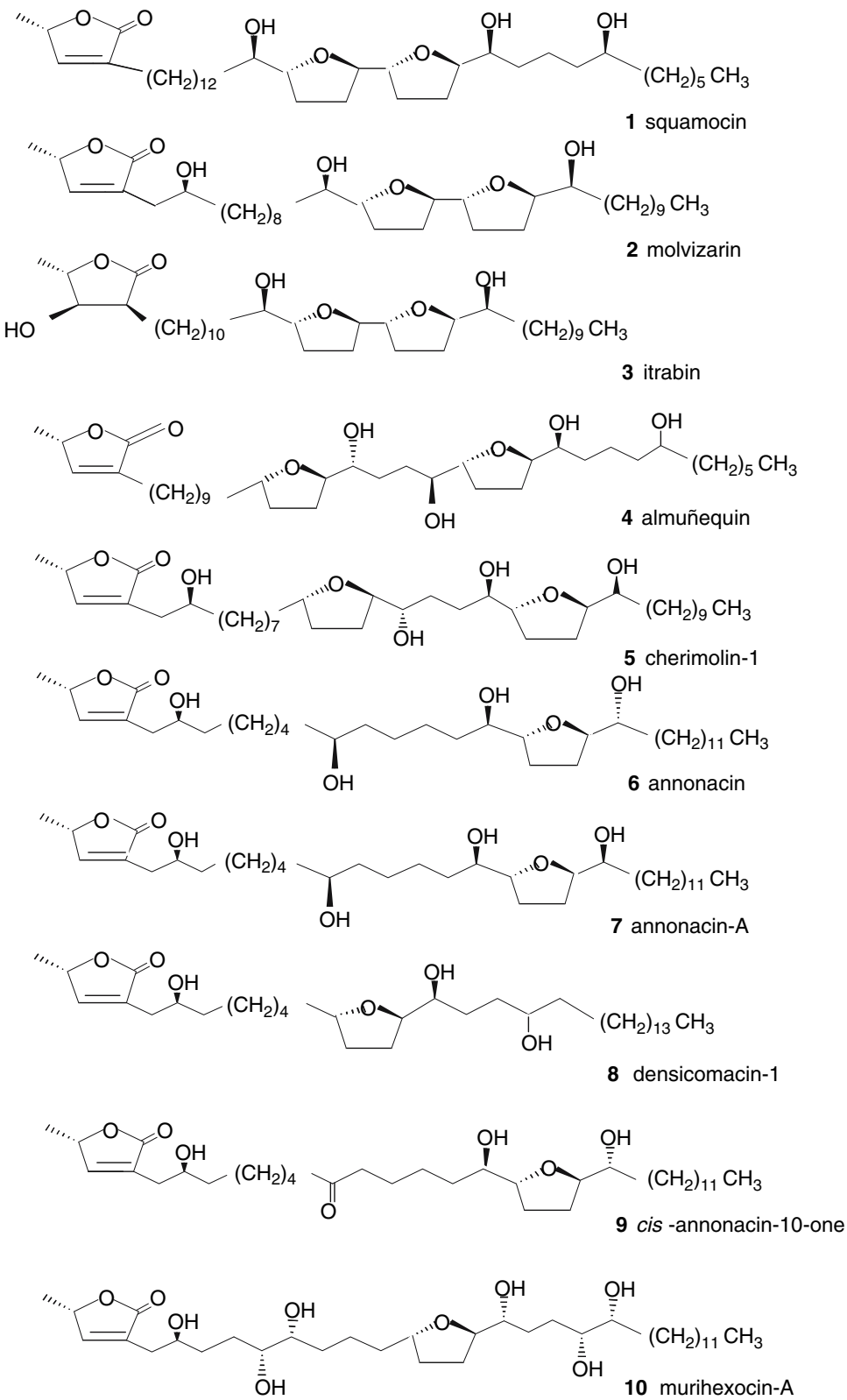

Fig. 1 Acetogenins evaluated for their toxicity against *O. fasciatus*

ticidal effects against the yellow fever mosquito larvae, in a contact assay. Furthermore, squamocin (**1**), a bis-THF-trihydroxy acetogenin, has been described as having ovicidal and larvicidal activity on species of *Drosophila* (Kawasu et al. 1989) and insecticidal effects on *Leptinotarsa decemlineata*, *Myzus persicae* adults (Guadaño et al. 2000) and *Spodoptera frugiperda* (Álvarez Colom et al. 2007).

Table 1 Mortality rates of *O. fasciatus* produced by topical application of acetogenins

	Dose (μg/nymph)	Acute toxicity[a] (%)	Delayed mortality[b]	
			Fifth-instar (%)	Adult (%)
Squamocin	0.1	43.3 ± 3.3	100 ± 0.0	–
Molvizarin	0.25	36.7 ± 3.3	100 ± 0.0	–
	0.1	30.0 ± 0.0	71.4 ± 8.3	100 ± 0.0
Itrabin	10.0	50.0 ± 0.0	73.9 ± 3.9	100 ± 0.0
	5.0	13.3 ± 3.3	61.6 ± 3.2	83.3 ± 4.2
Almuñequin	10.0	40.0 ± 5.0	74.9 ± 8.4	100 ± 0.0
	5.0	5.0 ± 5.0	26.7 ± 6.7	100 ± 0.0
Annonacin	10.0	23.3 ± 3.3	45.9 ± 5.8	72.8 ± 1.1
Annonacin-A	10.0	13.3 ± 3.3	40.3 ± 1.7	56.7 ± 6.7
Densicomacin-1	10.0	10.0 ± 0.0	0.0 ± 0.0	28.8 ± 4.4
cis-annonacin-10-one	10.0	6.7 ± 3.3	6.7 ± 3.3	51.5 ± 4.8
Murihexocin-A	10.0	0.0 ± 0.0	10.0 ± 5.8	10.0 ± 5.8
Cherimolin-1	10.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

[a] Percentage of mortality after 72 h of exposure to the chemical. Values represent means ± standard error of three independent experiments

[b] Percentage of surviving nymphs to the acute toxicity test which could not reach the fifth and adult instars

Table 2 Insecticidal activity of active acetogenins against *O. fasciatus*

	Slope ± ES	LD_{50} (95% CL)[a]	χ^2	*df*	*P*
Squamocin	1.54 ± 0.12	0.16 (0.11, 0.20)	28.88	10	0.001
Molvizarin	1.24 ± 0.11	0.34 (0.26, 0.45)	26.47	10	0.003
Almunequin	6.86 ± 0.82	11.23 (10.02, 14.78)	8.29	4	0.082
Itrabin	2.05 ± 0.19	14.91 (12.08, 20.48)	21.17	7	0.004

Deltamethrin (positive control) data in the same experimental conditions: LD_{50} (95% CL) 7.4 (7.1, 7.7) ng/nymph (Slope ± ES = 9.818 ± 0.605; $\chi^2 = 25.42$; $df = 8$; $P = 0.001$)

[a] Mortality recorded after 72 h of exposure to the chemical. Values, in μg/nymph, are given as the lethal dose 50 with its 95% confidence limits

The present study is the first report on the acute and delayed toxicity of acetogenins of *A. cherimolia* and *A. montana* on *O. fasciatus* nymphs and adults. As shown in Table 1, the acetogenins squamocin, molvizarin and itrabin, carrying in their structures two adjacent THF rings (Fig. 1) are the most toxic among the tested. Noteworthy, squamocin was the most toxic, as for the insects of other families mentioned in the previous paragraph. Apparently the number and location of OH groups, and the presence of adjacent THF moieties are structural features that determine the activity. Almuñequin (**4**), a tetrahydroxy bis-THF-acetogenin (non-adjacent THF) produced 100% of delayed mortality of adults at 10 μg/nymph, displaying a surprisingly higher effect than the structurally related cherimolin-1 (**5**), indicating that the hydroxyl group on C-28, not present in cherimolin-1, is important for the activity. Mono-THF-acetogenins displayed lower but some toxic effects at 10 μg/nymph. The present results are new evidences that acetogenins possess a tremendous potential for the development of new natural pesticides. Extracts of *A. cherimolia* and *A. montana* seeds, which can be easily prepared, could be employed as effective pesticides for several species of insects. On the other hand, squamocin and molvizarin could be considered as lead molecules for further chemical modifications in order to improve their insecticidal activities.

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