Antifeedant *Delphinium* Diterpenoid Alkaloids. Structure−Activity Relationships

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The insect antifeedant and toxic activity of the *Delphinium* diterpene alkaloids 15-acetylcardiopetamine, cardiopetamine along with its amino alcohol, the β,γ unsaturated ketone, and the acetylated ketone derivatives were studied in *Spodoptera littoralis* and *Leptinotarsa decemlineata*. Cardiopetamine and 15-acetylcardiopetamine strongly inhibited the feeding activity of *S. littoralis* and *L. decemlineata*, respectively. Structure−activity studies with *S. littoralis* showed that the C13 and C15 hydroxy substituents are essential features of the active molecule, while a C13 hydroxy and/or a C15 acetate determined their effect on *L. decemlineata*. The C11 benzoate group enhanced the biological effect on both insect species. These alkaloids were not toxic to *S. littoralis*, while their toxicity on *L. decemlineata* was inversely correlated with their antifeedant effects, the β,γ unsaturated ketone derivative being the most toxic. Cardiopetamine showed little antifungal action against several species of plant pathogens and did not have any mutagenic effects on *Salmonella typhimurium* by means of the Ames test.

Keywords: *Diterpene alkaloid; cardiopetamine; 15-acetylcardiopetamine; derivatives; antifeedant; Spodoptera littoralis; Leptinotarsa decemlineata; antifungal; Ames test*

INTRODUCTION

Much attention has been devoted to the diterpenoid alkaloids because of their complex structures and biological activities. Plant species of the genera *Aconitum* and *Delphinium* are known sources of diterpenoid alkaloids of pharmacological importance (Atta-ur-Rahman and Choudary, 1995). *Delphinium* plants, long known to be insecticidal, are a rich source of C-19 norditerpene alkaloids (Jennings et al., 1986; Kukel and Jennings, 1994; Manners et al., 1995). These alkaloids have been investigated in invertebrate (Jennings et al., 1986; Satelle et al., 1989) and vertebrate (Macallan et al., 1988; Namby Aiyar et al., 1979) isolated tissue preparations and found to act as potent nicotinic receptor antagonists. Furthermore, certain C-19 alkaloids could be candidates for insecticide development due to their potency and selectivity as ligands of the insect nicotinic receptor (Kukel and Jennings, 1994). However, little is known about the insecticidal effects of C-20 *Delphinium* diterpene alkaloids.

As part of our ongoing search for natural agrochemicals of plant origin, we have studied the antifeedant and toxic effects of the hetisine subtype diterpene alkaloids cardiopetamine (**4**) and 15-acetylcardiopetamine (**1**), isolated from *Delphinium cardiopetalum* (D.C) (González et al., 1983), against the polyphagous lepidopteran *Spodoptera littoralis* (Boisduval) and the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say). We have conducted structure−activity studies comparing the activity of the natural compounds with that of the amino alcohol (**5**), the β,γ unsaturated ketone (**2**), and the acetylated ketone (**3**) derivatives of **4**. We also describe the toxicity of cardiopetamine against the plant pathogens *Fusarium* spp., *Phytophtora parasitica, Phytium aphanidermatum* and the mutagenic effect of this alkaloid on strains TA98, TA100, and TA102 of *Salmonella typhimurium* by means of the Ames test (Ames et al., 1975).

MATERIALS AND METHODS

General. ^{1}H NMR spectrum was measured on a Bruker AMX 500 MHz spectrometer with pulsed field gradient (chemical shifts reported are relative to residual $CDCl_3$, 7.26 ppm, for ^{1}H). MS were recorded on a Autospec instrument at 70 eV.

Compounds **4** and **1** were isolated from *D. cardiopetalum* (D.C), and the structurally related derivatives of **4**, **2** and **3** (Figure 1), were prepared according to the method of González et al. (1983). Compound **5** was obtained as the hydrolysis product of **4**: 25 mg of **4** was treated overnight at room temperature with 10% KOH in MeOH (10 mL). The reaction mixture was then chromatographed on an alumina column and eluted with EtOAc/MeOH (90:10) to obtain a resin (12.6 mg, 66.0%).

^{13}C NMR data of **5** are given in González et al. (1986); EIMS *m*/*z* (relative intensity), $[M]^+$ 343.1792 (52.3) for $C_{20}H_{25}NO_4$ (calcd 343.1783), 327.1814 (7), 326.1770 (13.0) for $C_{20}H_{24}NO_3$ (calcd 326.1756), 313.1623 (2), 309.1739 (11.6) for $C_{20}H_{23}NO_2$ (calcd 309.1728), 297.1680 (29), 296.1651 (39) for $C_{19}H_{22}NO_2$ (calcd 296.1650), 280.1712 (5.0), 269.1786 (5.1), 227.1815 (26) for $C_{13}H_{25}NO_2$ (calcd 227.1885), 167.0878 (18.0), 91.0575 (62.0) 77.0407 (34), and 57.736 (55.2).

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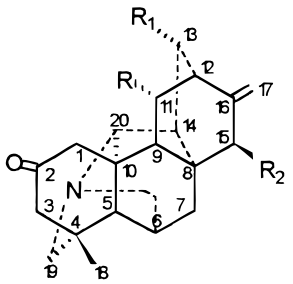

1 R = OBz; R_1 = OH; R_2 = OAc

2 R = OBz; R_1 = O; R_2 = OH

3 R = OBz; R_1 = O; R_2 = OAc

4 R = OBz; R_1 = OH; R_2 = OH

5 R = OH; R_1 = OH; R_2 = OH

Figure 1. Molecular structures of compounds **1**−**5**.

Insect Bioassays. *L. decemlineata* and *S. littoralis* colonies were reared on potato foliage (cv. Desirée) and artificial diet (Poitout and Bues, 1974), respectively, and maintained at 24 ± 1 °C, >70% relative humidity, with a photoperiod of 16:8 h (l:d) in a growth chamber.

Choice Feeding Assays (≤6 h). These experiments were conducted with adult *L. decemlineata* and newly emerged sixth-instar *S. littoralis* larvae. Each treatment consisted of 5−10 plates with three insects each as described in González-Coloma et al. (1995, 1996). The uneaten leaf disk surfaces were measured according to the method of Escoubas et al. (1993) with a computer-interfaced scanner. Percent feeding reduction (%FR) was determined for each arena by the equation %FR = [1 − (treatment consumption/control consumption)] × 100 (Bentley et al., 1984). Compounds with an FR > 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 standard regression analysis (%FR on log dose).

Oral Cannulation. This experiment was performed with preweighed newly emerged *S. littoralis* L6 larvae (average weight 400 mg) under the same environmental conditions as above. Twenty larvae were orally injected with 10 μg of the test compound in 2 μL of DMSO (treatment) or solvent alone (control) using a Hamilton repeating dispensor fitted with a Hamilton 50 μL syringe (50 gauge blunt needle). The needle was gently inserted through the oral cavity into the midgut region where the solution was delivered. At the end of the experiments (72 h), the relative consumption rate (RCR) and the relative growth rate (RGR) were calculated on a dry weight basis [see González-Coloma et al. (1995) for details] according to the method of Farrar et al. (1989). All dry larval weight measures were log-transformed prior to an Anova analysis to test for treatment effects. Differences between treatment means were checked with LSD tests.

Hemolymph Injection. DMSO solutions of the test alkaloids (10 μg each per insect) were injected through the metepimeron suture of the thorax of 20 adult *L. decemlineata* beetles (average weight 130 mg) using a Hamilton repeating dispensor fitted with a Hamilton 50 μL syringe (50 gauge pointed needle). Toxicity symptoms and mortality were recorded up to 3 days after injection by maintenance of beetles on their respective potato leaf foods. Percent mortality was analyzed with contingency table analysis and corrected according to the method of Abbott (1925).

Antifungal Activity Assays. The antifungal activity of the alkaloids was tested at a single dose (0.5 mg/mL) against the plant pathogens *Fusarium oxysporum, Fusarium moniliforme, Fusarium avenaceum, Phytophtora parasitica,* and *Phytium apahanidermatum* and estimated as mycelial growth inhibition (Murabayashi et al., 1991). The experimental conditions were as described in Gonzalez-Coloma et al. (1995).

Mutagenic Evaluation of Cardiopetamine. *S. typhimurium* strains TA98, TA100, and TA102 were kindly supplied by Professor B. N. Ames, University of California, Berkeley, CA. Their genotypes have been described previously (Levin, 1982; Maron and Ames, 1983). Their genetic markers and other characteristics, such as response to positive controls and the number of spontaneous revertants, were routinely verified as described by Maron and Ames (1983).

Metabolic Activation System. The supernatant of the post-mitochondrial liver fraction (S9) from male Wistar rats weighing ∼200 g was used as the metabolic activation system. A combined injection of PB and β-naphthoflavone was used to induce the system (Matshushima et al., 1976). The liver S9 fraction was prepared according to the method described by Maron and Ames (1983). The protein concentration was 47 mg/mL as determined according to the procedure of Lowry et al. (1951). The S9 mix was prepared prior to the mutagenicity tests by the addition of NADP and glucose 6-phosphate to S9.

Salmonella/Mammalian Microsome Test. Overnight cultures of the test strains were prepared by inoculation from the stock cultures into Difco Bacto nutrient broth and shake-incubated for 14 h at 37 °C. The standard plate incorporation test was used (Ames et al., 1975; Maron and Ames, 1983). One hundred microliters of the test chemical solution or DMSO, 100 μL of an overnight culture of the tester strain, and 500 μL of buffer phosphate or S9 mix (containing 4% S9) were added to 2 mL of top agar supplemented with 0.2 mL of a 0.5 mM histidine/biotin solution. The mixture was then spread onto a fresh glucose agar plate. The plates were incubated for 48 h at 37 °C, and the revertants were then counted. All experiments were carried out in triplicate, and results were expressed as the average number of revertants per plate. For each experiment, negative (spontaneous reversion and mutation in response to DMSO) and positive (response to the standard mutagen) controls were included to ensure the validity of the test. The positive controls used per plate were (with corresponding strains in parentheses) 1 μL of methylmethane sulfonate (TA100 and TA102) and 0.5 μg of 4-nitroquinoline *N*-oxide (TA98). When the assays were performed in the presence of S9 mix, 15 μg of 2-aminoanthracene was used for all strains.

RESULTS AND DISCUSSION

Table 1 shows the the ^{1}H NMR (500 MHz, $CDCl_3$) spectral data of alkaloids **1**−**5**.

Table 2 shows the relative potencies (EC_{50}) of compounds **1**−**5**. The diterpenoid alkaloid aconitine, an insect antifeedant and sodium channel antagonist (Wink, 1993), has been included as a positive control for comparison purposes. Compound **4** showed the strongest activity against *S. littoralis* (6 times stronger than aconitine), followed by **5**, while alkaloids **1**−**3** were not effective antifeedants against this insect. Alkaloid **1** had the strongest antifeedant effect on *L. decemlineata*, followed by **4**, **3** (∼4 times stronger than aconitine), and **5**. Compound **5** was as effective as the positive control for both insects.

Table 3 shows the effects of the oral and abdominal injection of compounds **1**−**5** on the target insects. *S. littoralis* consumption and growth rates (RCR and RGR) were not affected by any of the test compounds, while aconitine negatively affected both feeding indices (37% reduction of RCR and 58% reduction of RGR with respect to the control). However, *L. decemlineata*

Table 1. ^{1}H NMR (500 MHz, $CDCl_3$) Spectral Data[a] of Alkaloids 1−5

proton	1	2	3	4	5
H-1α	3.46 dd (13.5,1.8)	2.80 br *d* (14.0)	2.76 d (14.0)	3.48 dd (13.6,1.5)	3.31 br d (14.8)
H-1*β*	2.26 d (13.6)	2.42 d (14.0)	2.39 d (14.0)	2.22 d (13.6)	2.61 d (14.7)
H-3α	2.15 dd (13.9,2.0)	2.11 dd (14.2,2.0)	2.16 br d (14.1)	2.07 dd (13.9,2.2)	2.13 br d (14.2)
H-3*β*	2.28 d (13.6)	2.41 d (13.9)	2.26 d (14.1)	2.26 br d (14.0)	
H-5*β*	2.00 s	2.06 s	2.06 s	2.00 s	2.00 s
H-6	3.31 br s ($W_{1/2}$ = 6.5)	3.41 br s ($W_{1/2}$ = 7.0)	3.38 br s ($W_{1/2}$ = 6.5)	3.32 br s ($W_{1/2}$ = 6.5)	3.41 br s ($W_{1/2}$ = 7.0)
H-7α	1.75 dd (13.6,3.2)	1.86 dd (13.7,3.2)	1.87 dd (13.6,2.9)	1.62 dd (13.8,3.1)	1.57 dd (13.9,3.1)
H-7*β*	1.82 dd (13.6,2.5)	2.25 dd (13.5,2.5)	1.98 dd (13.6,2.5)	2.07 dd (13.8,2.5)	2.02 dd (13.8,2.2)
H-9*β*	2.71 dd (8.9,1.9)	2.92 dd (8.5,1.9)	2.89 dd (10.0,1.8)	2.05 dd (9.2,1.6)	
H-11*β*	5.58 d (8.9)	5.75 d (8.8)	5.66 d (8.4)	5.52 d (8.9)	4.25 d (8.8)
H-12	2.58 d (2.8)	2.76 s	2.78 s	2.51 d (2.8)	2.34 d (2.5)
H-13*β*	4.18 dt (8.8,2.3)			4.0 br d (9.0)	3.93 dt (8.4,2.5)
H-14	2.27 d (8.1)	2.44 br s ($W_{1/2}$ = 6.0)	2.55 br s ($W_{1/2}$ = 5.0)	2.62 dd (9.0,1.8)	
H-15α	5.14 s	4.17 s	5.32 s	3.82 s	3.68 s
H-17z	5.32 s	5.30 s	5.50 s	5.09 s	4.98 s
H-17e	5.26 s	5.28 s	5.48 s	5.06 s	4.97 s
H-18	1.08 s	1.10 s	1.09 s	1.08 s	1.08 s
H-19α	2.67 d (12.8)	2.68 d (12.8)	2.69 d (13.1)	2.59 d (12.7)	2.67 d (12.7)
H-19*β*	2.18 d (12.8)	2.20 d (12.8)	2.20 d (13.1)	2.16 d (12.7)	2.15 d (12.7)
H-20	3.08 s	3.15 s	3.14 s	2.99 s	2.98 s
	7.55 t (7.4)	7.55 t (7.3)	7.56 t (7.0)	7.37 t (8.9)	
OBz	7.43 t (7.6)	7.43 t (7.6)	7.44 t (7.5)	7.48 t (7.5)	
	8.00 d (8.2)	7.95 d (8.0)	7.96 t (8.0)	7.95 d (7.4)	
OAc	2.10 s		2.14 s		

[a] Chemical shift in ppm downfield from TMS and *J* (Hz) in parentheses.

Table 2. Effective Doses (EC_{50}) of the Test Compounds against *S. littoralis* L6 Larvae and *L. decemlineata* Adults in Choice Tests

	EC_{50} values and 95% confidence limits (lower, upper) (nmol/cm^2)	
compd	*S. littoralis*	*L. decemlineata*
1	>100	12.86 (0.16, 25.56)
2	>100	
3	>100	27.25 (22.95, 31.55)
4	5.48 (3.04, 7.92)	22.50 (19.73, 25.27)
5	23.67 (19.37, 27.97)	108.30 (9.97, 116.9)
aconitine	32.35 (19.65, 45.05)	178 (>39)[b]

[a] Concentration required to give an antifeedant index of 50%. [b] From Mullin et al. (1997).

mortality significantly increased when injected with compounds **2**−**5**, the last one showing the highest toxicity. None of the test compounds proved to be as toxic as aconitine on this insect species.

The antifungal and mutagenic activities of alkaloid **4** are shown in Tables 4 and 5, respectively. This compound did not show any significant antifungal (percent inhibition < 50% in all cases) or mutagenic effects in the Ames test (MI < 2.50 in all cases).

Antifeedants are gaining importance as potential components of Integrated Pest Management strategies for insect control. There are numerous reports on the antifeedant, postingestive, and toxic effects of different classes of alkaloids on *Spodoptera* spp. (Aerts et al., 1992; Bentley et al., 1984; Bringmann et al., 1992; González-Coloma et al., 1996; Miller and Feeny, 1983; Reina et al., 1995; Van Dam et al., 1995) and a few on CPB (Mullin et al., 1997; Sinden et al., 1988; Reina et al., 1997). However, this is the first report of the antifeedant effects on insects of hetisine subtype diterpene alkaloids.

The target insects were deterred by at least two of the test alkaloids, cardiopetamine (**4**) being strongly active in both cases, suggesting a shared mode of action for these molecules. Furthermore, *S. littoralis* was more sensitive to alkaloids **4**, **5**, and aconitine than CPB (between 4 and 5 times more sensitive). Previous reports have shown that *L. decemlineata,* a specialist of some alkaloid-containing Solanaceae species (Hsiao, 1986; Mitchell and Harrison, 1985; Sinden et al., 1988), was uniformly less sensitive to alkaloids in terms of feeding deterrency than the western corn rootworm (*Diabrotica virgifera virgifera*), an insect species that does not feed on alkaloid-containing plants (Mullin et al., 1997). The saturated pyrrolizidine alkaloid (PA) 3′-acetyltrachelanthamine from *Heliotropium floridum*, however, had a strong antifeedant effect on CPB without affecting the feeding behavior of *S. littoralis* (Reina et al., 1997), while the opposite pattern was found for the unsaturated PA europine (Reina et al., 1995), indicating that the effect of a given compound on the feeding behavior of an insect cannot be predicted on the basis of its chemical class.

The structure−activity study of the antifeedant action of the test alkaloids showed that the C13 and C15 hydroxy groups are essential features of the active molecule for *S. littoralis* (compounds **1** and **5**), while the presence of a C13 hydroxy and/or a C15 acetate determined their antifeedant effect on *L. decemlineata* (compounds **1**, **3**, and **4**). The C11 benzoate group strongly enhanced this biological action on both insect species.

Similarly, it has been shown that the most potent norditerpene alkaloids acting as inhibitors of mammalian and insect cholinergic receptors have the C18 anthranilic acid esterification, characteristic of the *Delphinium* norditerpene alkaloid methyllycaconitine (MLA), structurally related to aconitine (Jennings et al., 1986; Kukel and Jennings, 1994; Manners et al., 1995). MLA also had antifeedant effects against *Spodoptera eridania* at concentrations as low as 100 ppm (1.34 nmol), with associated postingestive and toxic effects (LC_{50} = 4.13 nmol) at doses within the effective antifeedant dose range of compound **4** (Jennings et al., 1986), suggesting a similar antifeedant mode of action between MLA/aconitine and the C-20 diterpenoid alkaloids studied here.

When the test compounds were orally injected to *S. littoralis* without contacting the mouthpart sensory receptors, the RCR and RGR of larvae treated with the antifeedant C-20 diterpene alkaloids did not decrease,

Table 3. Oral and Hemolymph Injection Effects of Compounds 1−5 on *S. littoralis* L6 Larvae (72 h, RCR and RGR) and *L. decemlineata* Adults (3 Days, % Mortality)

	S. littoralis		*L. decemlineata*		
treatment (10 μg/insect)	RCR[a] (% of control)	RGR[b] (% of control)	N^c	% mortality[d]	N^c
control	100	100	27	0.00	20
1	99.57 ± 4.67	100.10 ± 4.24	19	7.85	10
2	97.17 ± 11.79	105.89 ± 20.32	6	18.08b	18
3	113.90 ± 19.61	87.98 ± 6.65	14	25.16b	18
4	103.32 ± 4.26	110.32 ± 6.22	14	15.75b	20
5	104.54 ± 4.08	106.46 ± 5.68	18	72.20b	15
aconitine	62.80 ± 6.24a	42.30 ± 11.22a	18	100b	14

[a] RCR = $I/(\mathrm{BI})T$, I = mg of food consumed, T = feeding period (days), BI = initial insect weight (mg). Entries followed by "a" or "b" are significantly different from the control. 95% LSD test and contingency table analysis ($p < 0.05$), respectively. [b] RGR = $\Delta B/(\mathrm{BI})T$, ΔB = change in insect body weight (mg). [c] Number of insects. [d] Corrected according to Abbott (1925).

Table 4. Mycelial Growth Inhibition Effect of Compound 4 (0.5 mg/mL) on Several Plant Pathogen Species[a]

	% inhibition[b]				
treatment	*F.o.*	*F.m.*	*F.a.*	*P.h.*	*Pi.a.*
4	35.44 ± 10.05	11.11 ± 9.03	25.00 ± 6.05	14.94 ± 6.08	4.90 ± 10.69
juglone	100	76.80	54.50	53.50	

[a] F.o., *Fusarium oxysporum*; F.m., *F. moniliforme*; F.a., *F. avenaceum*; P.h., *Phytophtora parasitica*, Pi.a., *Phytium aphanidermatum*. [b] $1 - (C/T) \times 100$, where C = diameter of control colony, T = diameter of treated colony.

Table 5. Mutagenic Evaluation of Compound 4 in the Ames Test[a]

	dose of **4** (μg/plate)	TA98[b]	TA100[b]	TA102[b]
−S9[c]	DMSO	38.33 ± 1.70	120.83 ± 6.25	352.33 ± 9.73
	0.1	53.00 ± 1.15	97.00 ± 6.50	366.00 ± 22.60
	1.0	71.66 ± 6.01	90.33 ± 0.88	366.66 ± 9.61
	10	44.66 ± 2.66	109.66 ± 3.18	418.00 ± 39.62
	100	50.00 ± 3.46	113.66 ± 5.81	333.66 ± 8.41
	500	69.00 ± 2.08	112.00 ± 2.88	353.66 ± 18.27
	1000	38.50 ± 6.50	118.00 ± 4.00	
	SR	44.00 ± 2.92	109.00 ± 3.18	357.66 ± 6.93
	SM	508.16 ± 14.35	2185.00 ± 55.38	4230.66 ± 180.58
+S9	DMSO	45.50 ± 1.94	104.50 ± 6.57	433.33 ± 7.05
	0.1	47.66 ± 1.45	98.66 ± 7.51	382.33 ± 7.53
	1.0	73.00 ± 5.85	115.66 ± 7.83	419.33 ± 9.56
	10	76.66 ± 2.40	102.66 ± 1.45	426.00 ± 4.04
	100	85.33 ± 1.76	107.66 ± 6.88	401.66 ± 31.47
	500	82.66 ± 3.71	110.00 ± 3.46	401.66 ± 17.60
	1000	80.00 ± 1.20	97.50 ± 0.50	
	SR	49.50 ± 2.37	115.83 ± 3.47	406.33 ± 17.91
	SM	970.00 ± 28.29	2666.83 ± 125.48	2523.33 ± 182.24

[a] Mean revertants of triplicate plates ± standard error; SR, spontaneous revertants; DMSO, dimethyl sulfoxide; SM, standard mutagen. [b] *S. typhimurium* strain. [c] S9, metabolic activation system.

in contrast to the aconitine-treated larvae. This lack of toxicity and negative postingestive effects on *S. littoralis* suggests that this polyphagous insect can detoxify these chemicals at the dose tested but not aconitine.

However, their toxicity on *L. decemlineata* was inversely correlated to their antifeedant effects and directly related to their polarity, suggesting a different mode of action or receptor affinity at the central nervous system. A similar correlation has been shown between antifeedant effects, toxicity, and polarity of structurally related silphinene sesquiterpenes on CPB and *D. virgifera virgifera* (González-Coloma et al., 1997; Mullin et al., 1997). Such a lack of behavioral and toxicity relationship has been noted for a broad selection of plant allelochemicals (Bernays, 1990, 1991; Bernays and Cornelius, 1992; Wrubel and Bernays, 1990).

Additionally, cardiopetamine (**4**) did not show toxicity against fungal phytopathogens, suggesting a target-specific antifeedant mode of action without associated toxicity to herbivores or plant pathogens.

Delphinium diterpene alkaloids are highly toxic to mammals (Manners et al., 1995); therefore, any attempt to develop a diterpene alkaloid-derived agrochemical must be carefully evaluated for harmful effects. The *Salmonella*/microsome assay with cardiopetamine indicated that this compound does not have any mutagenic effect. However, a structure−activity study conducted with antifeedant unsaturated sesquiterpene dialdehydes showed that derivatization to less polar compounds reduced mutagenicity, while the introduction of hydroxyl groups had the reverse effect (Anke and Sterner, 1991). Therefore, further risk assessment of the cardiopetamine-related alkaloids studied here will be needed.

In summary, the *Delphinium* diterpene alkaloids cardiopetamine (**4**) and 15-acetylcardiopetamine (**1**) have been described as potent insect antifeedants for the first time. These molecules were active on two insect species with different feeding adaptations (a polyphagous Lepidopteran and an oligophagous Chrysomelid beetle), suggesting a potential broad range of antifeedant action for this class of compounds. Their structure−activity study demonstrated the importance of the C13 benzoate group for the antifeedant effect. Neither compound was toxic to the target insects, contrary to aconitine. Furthermore, cardiopetamine did not show antifungal activity and it did not cause any mutagenic effects on *Salmonella* strains, indicating that this antifeedant alkaloid does not have general toxic and genotoxic effects.

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