Antifeedant and Phytotoxic Activity of Cacalolides and Eremophilanolides

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The antifeedant effect of six cacalolides and six eremophilanolides was tested against the herbivorous insects *Spodoptera littoralis*, *Leptinotarsa decemlineata*, and *Myzus persicae*. The test compounds included several natural products isolated from *Senecio madagascariensis* (14-isovaleryloxy-1,2-dehydrocacalol methyl ether, **4**), *S. barba-johannis* (13-hydroxy-14-oxocacalohastine, **5**; 13-acetyloxy-14-oxocacalohastine, **6**) and *S. toluccanus* [6-hydroxyeuryopsin, **7**; 1(10)-epoxy-6-hydroxyeuryopsin, **9**; toluccanolide A, **11**] and the derivatives cacalol methyl ether (**1**); cacalol acetate (**2**); 1-acetyloxy-2-methyloxy-1,2,3,4-tetradehydrocacalol acetate (**3**); 6-acetyloxyeuryopsin (**8**); 6-acetyloxy-1(10)-epoxyeuryopsin (**10**), and toluccanolide A acetate (**12**). Compound **11** and its derivative **12** exhibited moderate antifeedant activity against *S. littoralis*; **2**, **7**–**10**, and **12** showed strong activity against *L. decemlineata*, while the aphid *M. persicae* was moderately deterred in the presence of compounds **1**, **4**, **8**, **10**, and **12**. The phytotoxic activity of **1**–**12** on *Lactuca sativa* was also evaluated. Compounds **2** and **4**–**12** moderately inhibited seed germination at 24 h, while compounds **1**–**4**, **6**, **9**, and **10** had a significant inhibition effect on *L. sativa* radicle length (over 50%).

Key words: Cacalolides, Eremophilanolides, Antifeedant, Phytotoxic

Introduction

Eremophilanolides are sesquiterpenes biogenetically described as rearrangement products derived from farnesylpyrophosphate cyclization (Mann *et al.*, 1994), while cacalolides are Wagner-Meerwein rearrangement products of eremophilanolides. These secondary metabolites, along with pyrrolizidine alkaloids, are the most common natural products isolated from *Senecio* species (Bohlmann *et al.*, 1977; Rizk, 1991) and have been shown to act synergistically against herbivorous insects (*Oreina* spp.) eliciting food avoidance (Hägele and Rowell-Rahier, 2001). Some of these compounds exhibit cytotoxic (Gao *et al.*, 2003; Wu *et al.*, 2005; Zhang *et al.*, 2005), antihyperglycemic (Inman *et al.*, 1999), antimicrobial (Garduño-Ramírez *et al.*, 2001; Wang *et al.*, 2002; Gu *et al.*, 2004; Mohamed and Ahmed, 2005), anti-inflammatory (Jiménez-Estrada *et al.*, 2006) or antioxidant activity (Doe *et al.*, 2004, 2005; Shindo *et al.*, 2004). It should also be noted that cacalolides are not sesquiterpenes, as is erroneously indicated (Doe *et al.*, 2004, 2005; Shindo *et al.*, 2004; Jiménez-Estrada *et al.*, 2006), since they do not hold to the isoprene rule.

As part of our ongoing studies on the structural analysis (Burgueño-Tapia *et al.*, 2001, 2004, 2006; Burgueño-Tapia and Joseph-Nathan, 2003) and plant-defensive properties of cacalolides and eremophilanolides from *Senecio* species (Reina *et al.*, 2001, 2006), we selected *S. madagascariensis*, a plant of South African origin (Humbert, 1923; Scott *et al.*, 1998) which has been the target of many efforts to control infestation levels in pastures (Sindel and Michael, 1992; Anderson and Panetta, 1995). This species is migrated in South America and is currently found in quite distant countries like Argentina and Colombia (Cabrera and Zardini, 1978). In addition we selected *S. barba-johannis* and *S. toluccanus,* two wild species commonly found in central Mexico (Sánchez, 1984).

Here we report on the antifeedant and toxic effects that the natural cacalolides 14-isovaleryloxy-

1,2-dehydrocacalol methyl ether (**4**) (from *S. madagascariensis*), 13-hydroxy-14-oxocacalohastine (**5**), 13-acetyloxy-14-oxocacalohastine (**6**) (from *S. barba-johannis*), as well as the eremophilanolides 6-hydroxyeuryopsin (**7**), 1(10)-epoxy-6-hydroxyeuryopsin (**9**) and toluccanolide A (**11**) (from *S. toluccanus*), and the derivatives cacalol methyl ether (**1**), cacalol acetate (**2**), 1-acetyloxy-2-methyloxy-1,2,3,4-tetradehydrocacalol acetate (**3**), 6-acetyloxyeuryopsin (**8**), 6-acetyloxy-1(10)-epoxyeuryopsin (**10**) and toluccanolide A acetate (**12**) (Fig. 1) have on the herbivorous insects *Spodoptera littoralis*, *Leptinotarsa decemlineata*, and *Myzus persicae.* Their phytotoxic activity on *Lactuca sativa* was also evaluated.

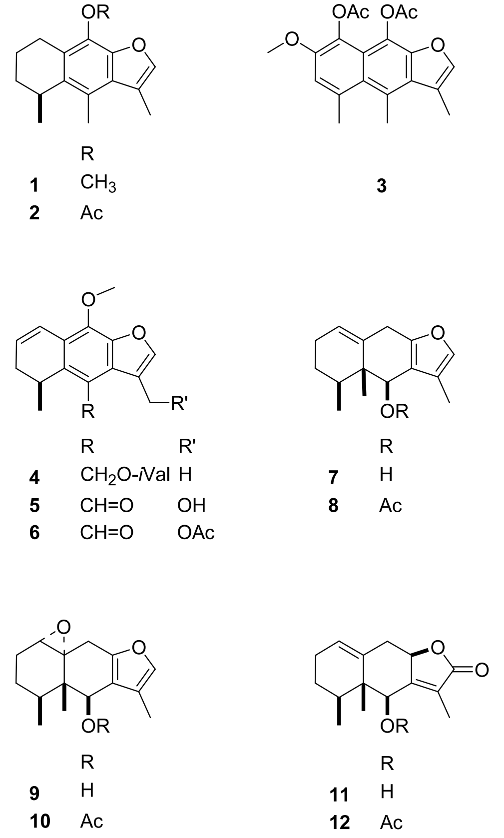

Fig. 1. Studied cacalolides **1**–**6** and eremophilanolides **7**–**12**.

Material and Methods

Compounds

Natural products **4**–**7**, **9**, and **11**, and the derivatives **1**–**3**, **8**, **10**, and **12** were available from previous works (Burgueño-Tapia *et al.*, 2001, 2004, 2006; Burgueño-Tapia and Joseph-Nathan, 2003; Romo and Joseph-Nathan, 1964).

Insect bioassays

S. littoralis, *L. decemlineata*, and *M. persicae* colonies were reared on artificial diet potato foliage (Poitout and Bues, 1974) and bell pepper (*Capsicum annuum*) plants, respectively, and maintained at (24 ± 1) °C, 60–70% relative humidity, with a 16:8 h (l:d) photoperiod in a growth chamber.

Feeding assays

These were conducted with newly emerged *S. littoralis* L6 larvae, and *L. decemlineata* and *M. persicae* adults. Percent feeding inhibition (%FI) was calculated as described in a previous work (Reina *et al.*, 2001).

Oral cannulation

Each experiment consisted of twenty larvae orally dosed with 40 μg of the test compound (Reina *et al.*, 2001). An analysis of covariance (ANCOVA1) on biomass gains with initial biomass as covariate (covariate $p > 0.05$) showed that initial insect weights were similar among all treatments. A second analysis (ANCOVA2) was performed on biomass gains with food consumption as covariate to test for post-ingestive effects (Reina *et al.*, 2001).

Phytotoxic evaluation

These experiments were conducted with *Lactuca sativa* var. Carrascoy seeds as described by Moiteiro *et al.* (2006). The germination was monitored daily and the radicle length measured at the end of the experiment (20 digitalized radicles randomly selected for each experiment) with the application Image J Version 1.37r, 2006 (http://rsb.info.nih.gov./ij/). An analysis of variance (ANOVA) was performed on germination and radicle length data.

Results and Discussion

Table I shows the antifeedant effect of the cacalolides and eremophilanolides tested on *S. littoralis* larvae, *L. decemlineata*, and *M. persicae* adults. Overall, *L. decemlineata* and *M. persicae* responded to a larger number of compounds than *S. littoralis.* Compounds **3**, **6**, **11** and **12** showed

Table I. Antifeedant effects (% FI, dose of 50 μg/cm^2) of cacalolides **1**–**6** and eremophilanolides **7**–**12** against *L. littoralis* L6 larvae and *L. decemlineata* adults and percent settling of *M. persicae* adults on control (% C) and treated (% T) leaf discs (dose of 50 μg/cm^2).

Compound	*S. littoralis*	*L. decemlineata*	*M. persicae*	
	% FI	% FI	% C	% T
1	43.1	63.3*	81	19*
2	16.2	73.4*	–	–
3	53.7*	54.3	56	44
4	44.6	65.8*	60	40*
5	47.2	29.3	46	54
6	53.8*	41.7	51	49
7	38.7	85.5*	44	56
8	48.3	93.3*	60	40*
9	42.8	71.6*	–	–
10	44.5	72.2*	65	35*
11	57.0*	37.7	55	45
12	69.6*	83.9*	65	35*

* $p < 0.05$, Wilcoxon paired rank test.

Table II. Biomass gain (ΔB) and consumption (ΔI) effects (% control) of cacalolides **1**–**6** and eremophilanolides **7**–**12** (40 μg/larvae) on *S. littoralis* larvae.

Compound	ΔB	ΔI	*p*ANCOVA2
1	69*	83	0.145
2	68*	83	0.217
3	85	96	–
4	93	96	–
5	75*	81*	0.745
6	66*	81*	0.229
7	104	103	–
8	59*	73*	0.095
9	53*	59*	0.999
10	95	94	–
11	88	91	–
12	80*	91	0.078

* $p < 0.05$, ANCOVA1 (initial larvae weight as covariate).

moderate activity (% FI > 50) against *S. littoralis*, the toluccanolides **11**, **12** being the most potent compounds. Euryopsin derivatives **7** and **8** were the most active compounds against *L. decemlineata*, followed by eremophilanolides **12**, **9**, **10**, and cacalolides **2**, **1** and **4**. This activity increased when the epoxide in **9** and **10** was reduced to the C1(C10) double bond in **7** and **8**, respectively. Similarly, the activity increased significantly when the lactone in **11** and **12** was reduced to a furane ring in **7** and **8**. Acetylation of the hydroxy group at C-6 increased the antifeedant effect against *L. decemlineata* in all cases, the acetylation of **11** to afford **12** being the most significant example. Compound **1** was a strong aphid antifeedant followed by **4**, **8**, **10**, and **12** which exhibited moderate activity. The acetylation of the hydroxy group at C-6 also increased the antifeedant activity on *M. persicae* (**7** *vs.* **8**, **11** *vs.* **12**), while the reduction of the lactone ring in **11** to afford the furane ring in **7** decreased it. Eremophilanolides with a γ-butyrolactone group, as in **12**, have been reported as strong *M. persicae* antifeedants (Reina *et al.*, 2001). Furthermore, cacalol has been shown to deter generalist insects known to feed on the cacalol-containing *Adenostyles alpina* (Hägele and Rowell-Rahier, 2001).

Table II shows the nutritional effects of **1**–**12** on *S. littoralis* larvae. A covariance analysis (ANCOVA1) of food consumption (ΔI) and biomass gains (ΔB) with initial larval weight as covariate (covariate $p > 0.05$) was performed to test for significant effects of the test compounds on these variables. An additional ANOVA analysis and covariate adjustment on ΔB with ΔI as covariate (ANCOVA2) was performed for those compounds that significantly reduced ΔB in order to gain insight into their post-ingestive mode of action (antifeedant and/or toxic) (Raubenheimer and Simson, 1992; Horton and Redak, 1993; Reina *et al.*, 2001;). Compounds **1**, **2** and **12** had a negative effect on biomass gain (ΔB) but not on consumption (ΔI), while **5**, **6**, **8**, and **9** affected both ΔB and ΔI, acetate **8** and epoxide **9** being the most potent ones. Treatment effects on ΔB disappeared with covariance adjustment, indicating that these compounds are post-ingestive growth inhibitors (**1**, **2**, **12**) or moderate-strong post-ingestive antifeedants (**5**, **6**, **8** and **9**) without any additional toxic effects. Similar to the structure-activity pattern observed for the antifeedant effects, acetylation of C-6 increased the post-ingestive effects except for epoxides **9** and **10**. However, the reduction of the lactone ring in **12** to give the furane ring in **8** increased it.

Cacalol has been shown to reduce the growth of the generalist *Cylindrotoma distinctissima* due to post-ingestive physiological effects and consumption reduction (Hägele and Rowell-Rahier, 2001). Cacalol and its methyl ether and acetate derivatives inhibited ATP synthesis at the electron-transport level (Lotina-Hennsen *et al.*, 1991), and related cacalolides inhibited lipid peroxidation at the mitochondrial and microsomal level (Doe *et al.*,

Table III. Effect in germination and radicular lenght (% control) of cacalolides **1**–**6** and eremophilanolides **7**–**12** (dose of 50 μg/cm^2) on *Lactuca sativa*.

Compound	Germination						Radicular length
	24 h	48 h	72 h	96 h	120 h	144 h	
1	99	99	99	99	99	99	42.8*
2	63*	98	98	98	98	98	44.7*
3	76	99	100	100	100	100	37.2*
4	65*	100	100	100	100	100	43.2*
5	45*	97	99	99	99	99	100.0
6	33*	99	99	99	99	99	47.4*
7	35*	99	10	100	100	100	70.8
8	40*	98	98	98	98	98	74.3
9	23*	99	100	100	100	100	47.4*
10	69*	99	100	100	100	100	40.1*
11	60*	98	98	98	98	98	53.4*
12	47*	100	100	100	100	100	67.9*

* Significantly different from the control, LSD test.

2005). These metabolic effects could explain the insect toxicity observed here.

Table III shows the phytotoxic effects of the test compounds on *L. sativa*. Compounds **5**–**9** and **12** resulted in significant germination inhibition (> 50%) at 24 h, epoxide **9** being the most active molecule, followed by **6** and **7**. The oxidation of the C-1–C-10 double bond in **7** to epoxide **9** increased this activity, while the oxidation of the furane ring in **7** to the lactone ring in **11** reduced it. On the other hand, when comparing the inhibitory capacity of compounds with a free hydroxy group (**5**, **7**, **9**, and **11**) to their respective acetylated derivatives **6**, **8**, **10**, and **12**, the effect depended on the specific structure. Thus, acetylation of **5** and **11** enhanced activity, while acetylation of **7** and **9** decreased it.

Cacalolides **1**–**4** and **6** and eremophilanolides **7**–**12** reduced *L. sativa* radicle length. Compound **3** showed the strongest effect, followed by **10**, **1**, **4**, **2**, **6** and **9** (inhibition > 50%). Oxidation of the C-1–C-10 double bond and the furane ring in **7** to the epoxide ring in **9**, and the lactone ring in **11**, respectively, and acetylation of **5** and **9** increased this activity, while acetylation of **7** and **11** reduced it. It is interesting to note that compound **5** did not show any activity, while its acetylated derivative **6** gave a 47% radicle length reduction.

Cacalol inhibited radicle growth of *Amaranthus hypochondriacus* and *Echinochloa crus galli*, the substitution of the -OH group resulted in a more selective activity (Anaya *et al.*, 1996). This phytotoxic action has been attributed to their inhibition of Hill's reaction in spinach chloroplasts during photosynthesis (Aguilar-Martínez *et al.*, 1996) and the inhibition of ATP synthesis (Lotina-Hennsen *et al.*, 1991). Therefore, we propose a similar mode of action for the phytotoxic effects shown here.

In summary, we have demonstrated that cacalolides **1**–**6** and eremophilanolides **7**–**12** have antifeedant and post-ingestive effects that increase with C-6 acetylation. These compounds are also phytotoxic and this action decreased with acetylation of C-6.

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