Antifeedant and Phytotoxic Activity of Cacalolides and Eremophilanolides

Eleuterio Burgueno-Tapia, Azucena Gonzalez-Coloma, Darío Martín-Benito, and Pedro Joseph-Nathan

Introduction

Eremophilanolides are sesquiterpenes biogenetically described as rearrangement products derived from farnesylylphosphosphate 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 (Bohmang et al., 1977; Rizk, 1991) and have been shown to act synergistically against herbivorous insects (Oreina spp.) eliciting food avoidance (Haguele 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; Jiménez-Estrada et al., 2006), since they do not hold to the isoprene rule.

As part of our ongoing studies on the structural analysis (Burgueno-Tapia et al., 2001, 2004, 2006; Burgueno-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 it 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-1,10-epoxy-6-hydroxyeuryopsin, 1-10; 6-acetyloxy-1(10)-epoxyeuryopsin, 9; toluccanolide A, 11] and the derivatives cacalol methyl ether (1); cacalacetate (2); 1-acetyloxy-2-methyloxy-1,2,3,4-tetrahydrcacaloc acid (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

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1,2-dehydrocacadol 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 cacadol methyl ether (1), cacadol acetate (2), 1-acetyloxy-2-methoxy-1,2,3,4-tetradehydrocacadol acetate (3), 6-acetylxyeuryopsin (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 cacadolides 1–6 and eremophilanolides 7–12.

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### 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, 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*/H11022 ≤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 1.37t, 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 cacadolides 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
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>
<thead>
<tr>
<th>Compound</th>
<th>ΔB</th>
<th>ΔI</th>
<th>pANCOVA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>69*</td>
<td>83</td>
<td>0.145</td>
</tr>
<tr>
<td>2</td>
<td>68*</td>
<td>83</td>
<td>0.217</td>
</tr>
<tr>
<td>3</td>
<td>85</td>
<td>96</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>93</td>
<td>96</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>75*</td>
<td>81*</td>
<td>0.745</td>
</tr>
<tr>
<td>6</td>
<td>66*</td>
<td>81*</td>
<td>0.229</td>
</tr>
<tr>
<td>7</td>
<td>104</td>
<td>103</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>59*</td>
<td>73*</td>
<td>0.095</td>
</tr>
<tr>
<td>9</td>
<td>53*</td>
<td>59*</td>
<td>0.999</td>
</tr>
<tr>
<td>10</td>
<td>95</td>
<td>94</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>88</td>
<td>91</td>
<td>–</td>
</tr>
<tr>
<td>12</td>
<td>80*</td>
<td>91</td>
<td>0.078</td>
</tr>
</tbody>
</table>

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

Acknowledgements

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Table III. Effect in germination and radicular length (% control) of cacalolides 1–6 and eremophilanolides 7–12 (dose of 50 μg/cm²) on Lactuca sativa.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Germination</th>
<th>Radicular length</th>
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<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>1</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>2</td>
<td>63*</td>
<td>98</td>
</tr>
<tr>
<td>3</td>
<td>76</td>
<td>99</td>
</tr>
<tr>
<td>4</td>
<td>65*</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>45*</td>
<td>97</td>
</tr>
<tr>
<td>6</td>
<td>33*</td>
<td>99</td>
</tr>
<tr>
<td>7</td>
<td>35*</td>
<td>99</td>
</tr>
<tr>
<td>8</td>
<td>40*</td>
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<td>69*</td>
<td>99</td>
</tr>
<tr>
<td>11</td>
<td>60*</td>
<td>98</td>
</tr>
<tr>
<td>12</td>
<td>47*</td>
<td>100</td>
</tr>
</tbody>
</table>

* Significantly different from the control, LSD test.


