Antifeedant and Phytotoxic Activity of Hydroxyperezone and Related Molecules

Eleuterio Burgueño-Tapia[a], Azucena González-Coloma[b], Lucía Castillo[b], and Pedro Joseph-Nathan[c,*]

[a] Departamento de Química Orgánica, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Prolongación de Carpio y Plan de Ayala, Col. Santo Tomás, México D. F., 11340 Mexico
[b] Instituto de Ciencias Agrarias – CCMA, CSIC, Serrano 115-bis, 28006 Madrid, España
[c] Departamento de Química, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Apartado 14-740, México D. F., 07000 Mexico.
Fax: +52-55-5747-7137. E-mail: pjoseph@nathan.cinvestav.mx

* Author for correspondence and reprint requests

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The insect antifeedant and toxic activity of hydroxyperezone (**1**), its derivatives **2**–**9**, along with 3-hydroxy- (**10**) and 6-hydroxythymoquinone (**11**) were studied against *Spodoptera littoralis*, *Leptinotarsa decemlineata*, and *Myzus persicae*. The antifeedant tests showed that *L. decemlineata* was the most sensitive insect, followed by *M. persicae*, while *S. littoralis* was not deterred by compounds **1**–**11**. Leucohydroxyperezone tetraacetate (**3**), oxoperezinone (**6**), dihydroleucoperezinone diacetate (**7**), 3-hydroxy- (**10**) and 6-hydroxythymoquinone (**11**) showed strong activity against *L. decemlineata*. **1** and **7** exhibited moderate deterrent activity against *M. persicae*, while **1** and dihydroleucohydroxyperezone tetraacetate (**4**) acted as post-ingestive antifeedants to *S. littoralis*. The phytotoxic activity of compounds **1**–**11** was also evaluated. Hydroxyperezone (**1**) strongly inhibited seed germination at 24 h, while the activity of **3**–**8** and **10** was moderate. The level of radicle growth inhibition obtained with compounds **1**–**5** and **8**–**11** was significant (< 50%).

Key words: Hydroxyperezone Derivatives, Antifeedant, Phytotoxicity

Introduction

Natural products have attracted considerable attention as insect antifeedant (Gonzalez-Coloma *et al*., 2002a, b; Marimoto and Komai, 2006) and phytotoxic (Duke *et al*., 2000; Hiromasa, 2006) compounds. In recent years, natural quinones and derivatives have been isolated as insecticidal and antifeedant agents (Marimoto *et al*., 2002; Xu *et al*., 2003; Wellsow *et al*., 2006). Furthermore, quinones such as juglone and sorgoleone are well known for their phytotoxic activity (Barbosa *et al.*, 2001; Lim *et al.*, 1996; Lima *et al.*, 2003; Hejl and Kosten, 2004a, b; Topal *et al.*, 2007; Willis, 2000).

Hydroxyperezone (**1**) is a sesquiterpene quinone isolated mainly from *Perezia hebeclada* roots (Joseph-Nathan *et al*., 1974) which has been the subject of detailed chemical studies including intramolecular Lewis acid-catalyzed cycloadditions (Joseph-Nathan *et al.*, 1987, 1993) and pioneering ^{13}C NMR studies (Joseph-Nathan *et al*., 1971) that revealed a tautomeric interconversion of two energetically equivalent forms and ^{13}C NMR quinone ring sustituent chemical shifts (Joseph-Nathan *et al.*, 1981; Burgueño-Tapia and Joseph-Nathan, 2000).

As part of our program focusing on the study of the chemical and biological properties of natural products and their derivatives (Gonzalez-Coloma *et al.*, 2005; Reina *et al.*, 2006; Burgueño-Tapia *et al.*, 2007), we have been looking into the antifeedant and toxic effects of hydroxyperezone (**1**) and its derivatives **2**–**9**, along with 3-hydroxy- (**10**) and 6-hydroxythymoquinone (**11**) against *Spodoptera littoralis*, *Leptinotarsa decemlineata*, and *Myzus persicae*. The phytotoxic activity of compounds **1**–**11** against *Lactuca sativa* was likewise evaluated.

Material and Methods

Compounds

Hydroxyperezone (**1**) and its derivatives **2**–**9**, 3-hydroxy- (**10**) and 6-hydroxythymoquinone (**11**) were available from previous studies (Joseph-Nathan *et al*., 1968, 1987; Burgueño-Tapia and Joseph-Nathan, 2000).

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Insect bioassays

Colonies of *S. littoralis*, *L. decemlineata*, and *M. persicae* 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 (1: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* variety 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. The phytotoxin juglone was included as a reference compound (Hejl and Koster, 2004b; Topal *et al.*, 2007; Willis, 2000).

Results and Discussion

The antifeedant effects of hydroxyperezone (**1**) and its derivatives **2**–**9**, 3-hydroxy- (**10**) and 6-hydroxythymoquinone (**11**) (Fig. 1) were tested against *S. littoralis* larvae, *L. decemlineata* and *M. persicae* adults as shown in Table I. In general, *L. decemlineata* was the most sensitive insect and responded to 45% of the tested compounds, followed by *M. persicae* which responded to compounds **7** and **11**, while *S. littoralis* was not affected. Leucohydroxyperezone tetraacetate (**3**), dihydroleucoperezinone diacetate (**7**) and 3-hydroxythymoquinone (**10**) exhibited strong antifeedant activity (% FI > 80) against *L. decemlineata*, **10** being the most potent compound. Oxoperezinone (**6**) and 6-hydroxythymoquinone (**11**) showed moderate activity (% FI > 50).

Antifeedant activity against *L. decemlineata* was significantly reduced when the hydroxy groups of

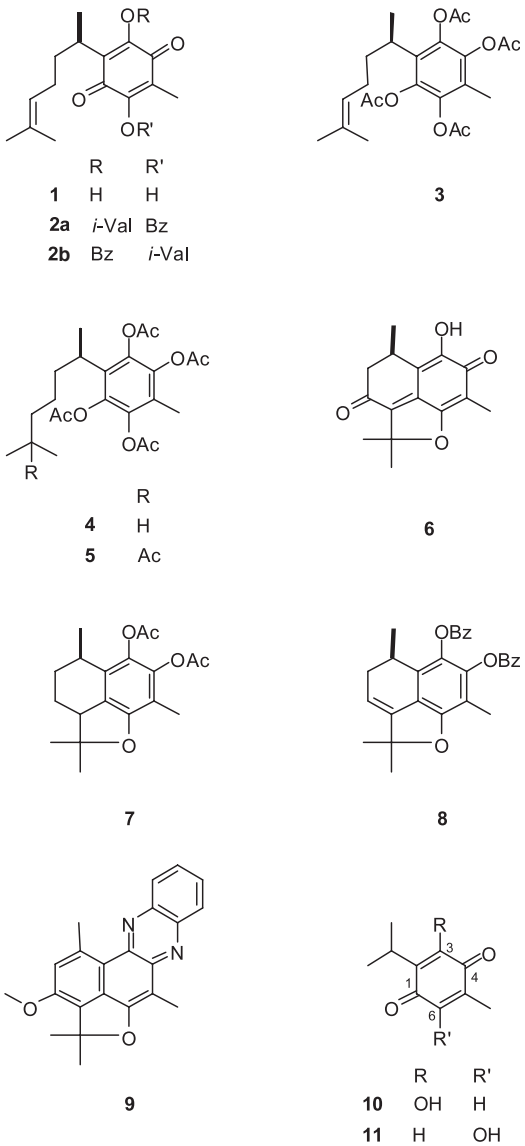

Fig. 1. Studied compounds.

Table I. Antifeedant effects (% FI, dose of 50 μg/cm^2) of **1**–**11** against *S. littoralis* L6 larvae, and *L. decemlineata* and *M. persicae* adults. Percent settling of *M. persicae* adults on control (% C) and treated (% T) leaf disks (dose of 50 μg/cm^2).

Compound	*S. littoralis*	*L. decemlineata*	*M. persicae*	
	% FI	% FI	% C	% T
1	43.8	50.1*	60	40*
2	9.5	17.9	58	42
3	25.7	87.3*	52	48
4	21.3	46.1	48	52
5	46.5	43.2	55	45
6	30.3	71.2*	43	57
7	34.5	82.4*	72	28*
8	53.9*	42.3	58	42
9	24.0	51.8	–	–
10	24.9	89.8*	58	42
11	38.6	68.8*	46	54

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

Table II. Biomass gain (ΔB) and consumption (ΔI) effects (% control) of compounds **1**–**11** (40 μg/larvae) on *S. littoralis* larvae.

Compound	ΔB	ΔI	*p*ANCOVA2
1	62*	65*	0.926
2	82	99	–
3	95	106	–
4	60*	63*	0.433
5	109	98	–
6	97	98	–
7	112	104	–
8	94	93	–
9	90	84	–
10	93	114	–
11	106	110	–

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

hydroxyperezone (**1**) were sterified to afford the inseparable mixture of **2a** and **2b**, while this activity was considerably increased after leucoacetylation to afford **3**. Reduction of the alkyl chain double bond in **3** to give **4** decreased the activity and a similar effect was observed after AcOH Markovnikov addition to the double bond in **3** to afford **5**. Thymoquinone derivatives **10** and **11** showed significant antifeedant activity against *L. decemlineata*, the best activity being observed when the hydroxy group was at C3 as in **10** (% FI = 89.8). We also observed a significant increase in the antifeedant activity following substitution of the benzoyloxy group and reduction of the benzyl double bond in **8** to afford **7**.

Thymol, a positive allosteric modulator of human and *Drosophila* $GABA_A$ receptors (Priestley *et al.*, 2003), acts as a strong insect antifeedant against *L. decemlineata* and moderately against *M. persicae* but has no effect on *S. littoralis* (González-Coloma *et al.*, 2002a, b). The anticonvulsant effects of the thymol derivative thymoquinone, structurally related to **10** and **11**, have been attributed to $GABA_A$ positive modulation (Hosseinzadeh and Parvardeh, 2004; Johnston, 2005). Furthermore, based on the antifeedant action of thymol, silphinenes and picrotoxinin against Chrysomelid beetles and aphids, a GABA-mediated taste regulation has been proposed for these insect species (González-Coloma *et al.*, 2002; Mullin *et al.*, 1997). The ability of silphinenes to reverse the blockage of *Drosophila* neuronal firing induced by GABA (Bloomquist *et al.*, 2007) further supports this hypothesis. A similar mode of antifeedant action could be proposed for antifeedants **3**, **6**, **7**, **10** and **11**.

The nutritional effects of compounds **1**–**11** on *S. littoralis* larvae are shown in Table II. A covariance analysis (ANCOVA1) of food consumption (ΔI) and biomass gain (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 postingestive mode of action (antifeedant and/or toxic) (Raubenheimer and Simpson, 1992; Horton and Redak, 1993; Reina *et al.*, 2001). Hydroxyperezone (**1**) and dihydroleucohydroxyperezone tetraacetate (**4**) had a similar negative effect on biomass gain (ΔB) and on consumption (ΔI). Treatment effects on ΔB disappeared with covariance adjustment, indicating that these compounds are post-ingestive growth inhibitors without any additional toxic effects. The generally low insect toxicity of the test compounds could indicate metabolic detoxification. Sterification of the hydroxy groups in **1** reduced post-ingestive effects, while reduction of the alkyl chain double bond in **3**, to give **4**, considerably increased it.

The effect on *L. sativa* germination and radicle lenght of compounds **1**–**11** is shown in Table III. Compounds **1**, **3**–**8** and **10** resulted in significant germination inhibition at 24 h with hydroxypere-

Table III. Effect on germination and radicle length (% control) of compounds **1**–**11** (50 μg/cm^2) on *Lactuca sativa*.

Compound	Germination			Radicle length
	24 h	48 h	72 h	
1	21*	87*	99	33.9*
2	76*	92*	94	32.8*
3	55*	98	98	43.4*
4	56*	100	100	49.6*
5	56*	97	97	42.7*
6	49*	98	99	68.8*
7	40*	98	98	54.7*
8	57*	99	99	44.2*
9	91*	99	99	43.1*
10	49*	100	100	43.4*
11	99	100	100	37.6*
Juglone	44*	94*	100	29.4*

* Significantly different from the control, $p < 0.05$, LSD test.

zone (**1**) being the most active compound (2-times more active than juglone), followed by **7**, **6** and **10** (germination < 50% of the control). It is interesting to note that **1** and **10**, having a hydroxy group at C3, had a negative effect on germination at 24 h while 6-hydroxythymoquinone (**11**), with the hydroxy group at C6, did not exhibit this effect. Compounds **1**–**5** and **8**–**11** reduced *L. sativa* radicle length, compounds **1**, **2**, and **11** being the most active followed by **5**, **9**, **3**, **10**, **8** and **4** (inhibition > 50%).

Interactions with photosystem II and inhibition of photosynthetic light reactions have been described for various types of quinones (Renger *et al.*, 1988; González *et al.*, 1997; Rimando *et al.*, 1998), including juglone (Hejl *et al.*, 1993) and sorgoleone. These compounds also inhibit H^+-ATPase and water uptake (Hejl and Koster, 2004a, b). 3-Hydroxythymoquinone (**10**) has been shown to be a phytotoxic compound against *Lemna minor* and *Agrostis capillaries* (Van Puyvelde *et al.*, 1999), however, this is the first report on the phytotoxic effects of compounds **1**–**9** and **11**.

In summary, we have demonstrated that hydroxyperezone (**1**) and the quinoxaline **9** have moderate antifeedant activity against *L. decemlineata*, while leucohydroxyperezone tetraacetate (**3**), oxoperezinone (**6**), dihydroleucoperezinone diacetate (**7**), and 3-hydroxythymoquinone (**10**) exhibit strong activity. Compounds **1**–**11** have moderate activity against *M. persicae*, while compounds **1**–**5** and **8**–**11** are phytotoxic with activity levels within the range of juglone for **1** and **7**, regarding germination inhibition, and for **1**, **2**, **10** and **11**, regarding radicle growth**.**

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