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MULTIPLE MODES OF INSECTICIDAL ACTION OF THREE CLASSES OF POLYACETYLENE DERIVATIVES FROM *RUDBECKIA HIRTA*

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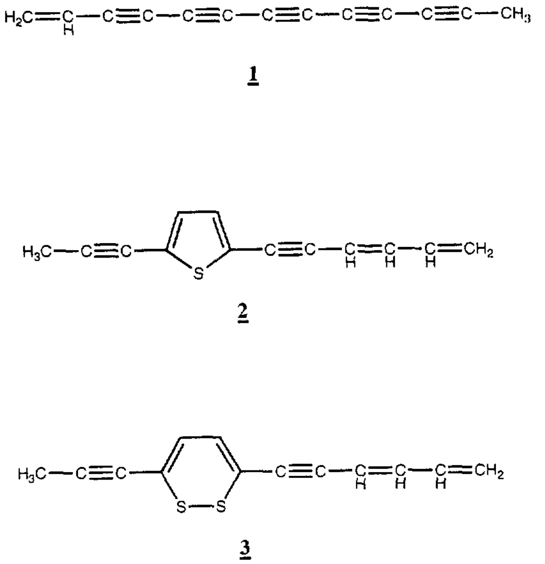
Key Word Index—Asteraceae; phototoxin; polyacetylene; thiophene; thiarubrine; *Rudbeckia hirta*; plant chemical defense; chemical redundancy.

Abstract—Polyacetylene derivatives (PADs) occurring in *Rudbeckia hirta* (Asteraceae) were isolated or chemically synthesized and examined for their insecticidal properties against mosquito larvae under different light regimes: dark (D), visible (VIS) and visible + near-ultra-violet (VIS + UV). A straight chain polyine, 1-tridecene-3,5,7,9,11-pentayne, was highly toxic under all light regimes tested although a thiarubrine, 3-(1-propynyl)-6-(3,5-hexadien-1-ynyl)-1,2-dithiacyclohexa-3,5-diene, and a thiophene, 2-(1-propynyl)-5-(3,5-hexadien-1-ynyl)-thiophene, showed a toxicity that significantly varied between the light regimes. The thiarubrine was more toxic against mosquito larvae under D or VIS + UV conditions while the thiophene had a more pronounced toxicity only in presence of VIS + UV irradiation. The distinctive insecticidal properties in darkness of the thiarubrine compare to the thiophene were also confirmed in trials with larvae of a herbivorous insect, *Manduca sexta*. Such a variability in the light-modulated toxicity to insects for different biosynthetically related PADs emphasizes a diversity in the insecticidal mechanisms of action. © 1997 Elsevier Science Ltd

INTRODUCTION

Over 750 naturally occurring polyacetylene derivatives (PADs) are found in the Asteraceae family [1]. Straight chain PADs are the most common, while thiophenes and thiarubrines (Fig. 1) are sulphur heterocycles that are not as widely distributed. For example, of the approximately 500 species of plants in the subtribe Heliantheae that had been investigated, 204 distinct PADs were reported to occur in 377 species, 55 thiophenes were found in 114 species and 6 different thiarubrines were detected in 37 species [2]. From feeding experiments with radioactive tracers, it has been unequivocally established that straight chain PADs are the precursors of thiophenes and thiarubrines [3–5].

While the light-dependent toxicity (phototoxicity) of thiophenes and some straight chain PADs to insects is well established [6, 7], there is little information on the insecticidal properties of thiarubrines or the effect of light on these activities. There exist only two references in the literature indicating that thiarubrines act as feeding deterrents against the cockroach, *Blatta orientalis*, and European corn borer larvae at concentrations of 10 μg g^{-1} and 100 μg g^{-1}, respectively [8, 9].



$H_2C{=}\underset{H}{C}{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}C{\equiv}C{-}CH_3$

Fig. 1. Polyacetylene derivatives occurring in *Rudbeckia hirta* (Asteraceae).

Rudbeckia hirta L. (Asteraceae), the black-eyed Susan, is a species containing all three classes of PADs (Fig. 1) and which has been reported as exhibiting phototoxic properties in the stem, root and flower [10]. An ethanol extract of *R. hirta* was shown to exert repellent effects against the Japanese beetle [11]. In

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previous studies, we also showed that tortricid and geometrid larvae that attack the phototoxic inflorescences of *R. hirta* in eastern North America are sensitive to the phototoxic properties of this plant [12].

In the present study, we isolated or synthesized and then examined the insecticidal properties of the three related PADs occurring in *R. hirta* (Fig. 1) under different light regimes. The objective was to investigate how different classes of PADs have their toxicity to insects modulated by light. Because of the brief availability of the natural herbivorous larvae, we conducted the present study on the toxic and phototoxic properties of the PADs using laboratory colonies of insects, *Aedes atropalpus* Say (Diptera: Culicidae) and *Manduca sexta* (L.) (Lepidoptera: Sphingidae), that are known to be sensitive to phototoxins [6, 7, 13, 14].

RESULTS AND DISCUSSION

Only the tridecapentaynene (**1**) was highly toxic to *A. atropalpus* larvae under all three light regimes tested (D, VIS and VIS+UV) while the insecticidal effects of compounds **2** and **3** significantly differed between the light treatments (Table 1). The thiophene (**2**) was highly toxic to mosquito larvae only in presence of VIS+UV irradiation and the thiarubrine (**3**) generated the most lethal effects under both D and VIS+UV treatments (Table 1).

The distinct light-dependent insecticidal effects of compound **3** compared to **2** were also confirmed with neonate *M. sexta* larvae. At concentration of 50 μg a.i./g diet, the thiarubrine (**3**) generated a lower weight gain and a higher mortality to *M. sexta* larvae than either the control or the thiophene (**2**) treatments (Table 2).

These variations observed in the insecticidal properties between the three PADs investigated suggest that different mechanisms of toxicity are involved for each compound. Reisch *et al.* [20] have reported that dark toxicity of straight chain polyines against bacteria and fungi is improved by decreasing saturation. As the tridecapentaynene (**1**) is the ultimate form of unsaturation in natural PADs, its dark antibiotic [21] and insecticidal (Tables 1 and 2) activities are not unexpected, although the mechanism of action is unknown and clearly different from light-activated toxicity.

It is also conceivable that a light-induced toxicity of **1** may be involved since straight chain polyacetylenes, which strongly absorb photons in the near-UV wavelengths, generate photoadducts with olefins when exposed to light [22]. Permeabilization of liposomes containing unsaturated lipids when irradiated under near-UV and in presence of straight chain polyacetylenes [23] indeed suggests that the generation of photoadducts may alter the integrity of cellular membranes and eventually lead to mortality as observed with *Escherichia coli* and *Saccharomyces cerevisiae* [24]. In support of such light-induced toxicity with straight chain polyacetylenes, Kagan *et al.* [25] demonstrated that, under a near-UV radiation setup (320–420 nm) similar to the one employed in the present study (300–400 nm), 2-chloro-3,11-tridecadiene-5,7,9-triyn-1-ol induces a photosensitized hemolysis of human erythrocytes under both aerobic and anaerobic conditions. Considering that no phototoxic effect was observed with compound **1** in the present study (Table 1), it is suggested that a competing photodegradation reaction may limit the impact of phototoxicity. This interpretation is supported by the observation of an approximately two orders of magnitude higher rate of photodegradation of the 2-chloro-3,11-tridecadiene-5,7,9-triyn-1-ol than the α-terthiophene when irradiated at 310 nm in 50% ethanol solutions [24].

Enhancement of the toxicity of thiophenes in presence of near-UV light has been reported for different classes of organisms [26] including some insects [7, 13]. This phototoxic process is due to the production of singlet oxygen when thiophenes are irradiated with near-UV light in an oxygenated medium [27] and which ends up in a photooxidative peroxidation of lipids [9].

The dark toxicity of the thiarubrine (**3**) against mosquito larvae was comparable to that observed for the thiophene (**2**) under photosensitizing wavelengths. These light-independent insecticidal properties of a thiarubrine is a new finding that corroborates those obtained with microorganisms (see review in ref. [8]).

Table 1. LC_{50} values (μg a.i. ml^{-1}) for the three polyacetylene derivatives cited in Fig. 1 against second instar *Aedes atropalpus* larvae under different light regimes

Light regime	Tridecapentaynene* (**1**)	Thiophene* (**2**)	Thiarubine* (**3**)
Dark	0.05^{a} (0.03–0.11)	5.51^{a} (2.84–6.92)	0.09^{a} (0.04–0.19)
Visible light	0.19^{a} 90.05–0.29)	2.59^{a} (1.08–4.97)	1.24^{b} (0.77–1.94)
Visible light + near-UV	0.03^{a} (0.01–0.07)	0.14^{b} (0.06–0.21)	0.18^{a} (0.085–0.27)

*Values in parentheses represent the 95% fiducial limits as determined by probit analysis. For each column, values followed by different letters have non-overlapping 95% fiducial limits.

Table 2. Effects on weight gain and mortality of neonate *Manduca sexta* larvae fed for 4 days in darkness with a meridic diet treated with the thiophene (**2**) or the thiarubrine (**3**)

Treatment	Larval weight gain* (mg)	Larval mortality* (%)
Control	18.9^a	5.0^a
	(4.1)	(1.4)
Thiophene (**2**)	15.3^a	10.0^a
(50 μg g^{-1})	(2.1)	(5.1)
Thiarubrine (**3**)	7.2^b	62.0^b
(50 μg g^{-1})	(1.6)	(10.6)

* Values in parentheses represent the standard deviation. For each column, values followed by different letters are significantly different ($P < 0.05$, Tukey's test).

Constabel and Towers [28] have investigated the antibiotic properties of two different thiarubrines, among which one was compound **3** and their related thiophenes. They found that toxicity of thiarubrines to *Saccharomyces cerevisiae*, *Escherichia coli* and *Pseudomonas flavescens*, was slightly lower or comparable in darkness to that under near-UV photosensitizing irradiation, although the two corresponding thiophenes were inactive unless irradiated with near-UV light. The toxicological similarity observed in the present study between the thiophene (**2**) and the thiarubrine (**3**) under both VIS and VIS+UV light regimes is also in agreement with the photo-conversion of thiarubrines to thiophenes through the extrusion of a sulphur atom [15]. However, the mechanism for the dark toxicity of the thiarubrine derivatives has not been elucidated.

The insecticidal results reported here demonstrate different patterns of insecticidal action of the three PADs investigated when exposed under different light regimes. The chemical redundancy of the PADs **1** and **2** in the inflorescences of *R. hirta* could be linked to an evolutionary strategy designed to diversify the mechanisms of their chemical defenses. One potential advantage of such a strategy suggested by Berenbaum *et al.* [29] is synergism, which allows a reduced investment in plant defense compounds. Alternatively, the synthesis of compounds active in the dark such as **1** and **3** may be necessary to counteract an adapted guild of insect herbivores that we have observed to avoid direct light exposure and photoxic effects of PADs [12].

EXPERIMENTAL

Purification and/or synthesis of polyacetylene derivatives. The inflorescences and roots of *R. hirta* collected in the Ottawa/Hull area were separately homogenized in EtOH–H_2O (1:1) filtered and then extracted with hexane. The hexane frn from the inflorescences contained the tridecapentaynene (**1**) which was further purified via repeated TLC (silica gel 60, art. 5721, Merck) using hexane–EtOAc (9:1) as the eluent system. The thiarubrine (**3**) was purified from the root hexane frn as described by Constabel *et al.* (15) while the thiophene (**2**) was synthesized according to a reported procedure [16]. The identity of each compound was confirmed by comparison of UV and MS data with published literature values [1, 15].

Insecticidal activity. Insecticidal activity of the three PADs were evaluated against second instar larvae of *A. atropalpus* as described in Marles *et al.* [7]. Larvae were incubated in 5 ml of distilled H_2O in scintillation vials for 24 h in presence of different concns of the PADs indicated in Fig. 1. Test compounds were added in 25 μl of EtOH and the control received an equal amount of solvent added. To test the light-modulated toxicity of PADs, experiments were performed under three light regimes: (1) darkness, (2) visible light (cool white, 350 μmol photons m^{-2} s^{-1}), (3) visible light (as before) supplemented with near-UV provided by blacklight bulbs (Westinghouse F20T12/BLB, 30 μmol photons m^{-2} s^{-1}) having an emission range between 300–400 nm (17). For each treatment, 3–5 replicates of 20–25 larvae each were performed. The data were submitted to probit analysis to determine the LC_{50} and the 95% fiducial limits [18]. Treatments for which the 95% fiducial limits were not overlapping were considered as significantly different.

Light-independent insecticidal properties of PADs were also determined using *M. sexta* as a phytophagous insect model maintained as described by Stewart and Philogène [19]. The compounds dissolved in EtOH were added (5 μl of EtOH g^{-1} of diet) and mixed into a warm meridic diet (35°) to reach a concn of 50 μg a.i./g of diet. As a comparative basis, the concns of compounds **2** and **3** in root cultures of *R. hirta* are 70 μg g^{-1} dry wt and 440 μg g^{-1} dry wt, respectively [15]. The control received the same EtOH content as did treatments. Neonate larvae (20 per treatment, 3 replicates per treatment) were fed for 4 days in darkness with a diet treated with either compound **2** or **3**. Since HPLC measurements indicated that the half-lives of compounds **2** and **3** in diet were between 2.8 and 4 days, the remaining diets were replaced by freshly prepd ones every 2 days during the experimentation. Using this protocol, it was impossible to test compound **1** because of its instability in the diet (it was completely degraded after 24 hr).

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