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DETERRENT AND INSECTICIDAL CHROMENES AND BENZOFURANS FROM *ENCELIA* (ASTERACEAE)

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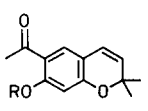
Key Word Index—*Encelia*; Asteraceae; chromenes; benzofurans; encecalin; toxicity; antifeedants; *Peridroma saucia*.

Abstract—Four analogous chromenes and benzofurans that are among the major natural products of the genus *Encelia* were bioassayed for deterrency and toxicity against pest insects. The chromene encecalin has both antifeedant and insecticidal properties towards larvae of the noctuid pests *Peridroma saucia* and *Plusia gamma*. In addition to reducing larval growth and decreasing survivorship of neonate larvae fed artificial diets containing encecalin, this chromene was directly insecticidal to neonate larvae after 4 hr using a residue contact bioassay. Topical application of encecalin to third instar larvae of *Peridroma* led to a reduction in dietary utilization, indicative of a metabolic cost of handling the compound. The demethyl analogue of encecalin is considerably less active, and the benzofurans euparin and methyleuparin, all common constituents of *Encelia*, were essentially inactive or only weakly active in all bioassays against the insects.

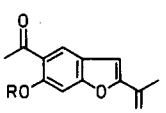
INTRODUCTION

Chromenes (benzopyrans) and benzofurans are common and characteristic natural products of the Asteraceae [1]. To date more than 250 compounds of this type have been isolated from many species in this family. In previous studies we have established that chromenes and benzofurans are also among the principal natural products of the genus *Encelia* Adans. [2–4]. The shrubby species of *Encelia* are common and dominant members of the plant communities of the Sonoran and Mohave deserts. Encecalin (**1**), a major chromene found in several species of *Encelia* has been reported to be insecticidal to nymphs of the milkweed bug, *Oncopeltus fasciatus* [5], and to have antifeedant activity against the corn earworm, *Heliothis zea*, a noctuid caterpillar [6]. Closely related chromenes, precocene I and precocene II, have antihormonal activities in several species of insects [7, 8].

In the present report we examine further deterrent and toxic actions of encecalin against larvae of the variegated cutworm, *Peridroma saucia*, a polyphagous noctuid pest of many agricultural crops. Supplementary bioassays using the looper *Plusia gamma*, another noctuid, are also reported. Although *Peridroma* has not been reported from species of *Encelia* in nature, its range is largely sympatric with many members of *Encelia* and it is known to feed on other asteraceous shrubs, such as *Ambrosia chammisonis* [9]. Also, another noctuid, the cabbage looper *Trichoplusia ni*, is capable of larval development on *Encelia farinosa* [10]. In addition, we have tested another chromene, 7-demethylencecalin (**2**), and two benzofurans, euparin (**4**) and 6-methyleuparin (**3**), all major constituents from *Encelia*.



R = CH_3 Encecalin (1)
R = H 7-Demethylencecalin (2)



R = CH_3 6-Methyleuparin (3)
R = H Euparin (4)

RESULTS AND DISCUSSION

When the four compounds from *Encelia* were added to artificial diets at 3.0 μmol/g fr. wt and fed to neonate *P. saucia* larvae, all but euparin (**4**) significantly reduced larval survival and inhibited larval growth of surviving larvae after 8 days of feeding (Table 1). At this concentration, encecalin (**1**) was the most inhibitory compound; in the case of both the chromenes and the benzofurans, the methoxylated compounds were more inhibitory than their hydroxylated parent compounds. Survival of neonate larvae at 72 hr (time to starvation for non-feeding larvae) on diets containing different concentrations of encecalin was dose-dependent in each of two experiments (Fig. 1). The LD_{50}s obtained from linear regression of the data are 2.3 and 2.4 μmol/g fr. wt respectively. Similar experiments with *P. gamma* indicated that a dietary concentration of 3.0 μmol/g fr. wt was completely lethal to neonate larvae. In contrast, the dietary LD_{50} for neonate *Heliothis zea* was found to be 15 μmol/g fr. wt [6] indicating that this noctuid is considerably less sensitive to encecalin. The natural concentrations of encecalin vary from less than 1 to almost 40 μmol/g fr. wt, with concentrations exceeding 30 μmol/g fr. wt in at least two species, *E. densifolia* and *E. palmeri* [P. Proksch, unpublished data]. These observations suggest that encecalin alone could pose a formidable barrier to the utilization of several species of *Encelia* by *P. saucia* and possibly other noctuids. *E. farinosa*, which can support larval growth and development of the noctuid *Trichoplusia ni*, differs sharply in its encecalin content between various populations analysed and some-

Table 1. Growth and survival of neonate *Peridroma saucia* on artificial diets incorporating chromenes and benzofurans from *Encelia*

Compound*	Larval weight (% of control)	% Survival†
Control	100	80
1, Encecalin	50	23
2, 7-Demethylencecalin	58	57
3, 6-Methyleuparin	77	73
4, Euparin	104	90

*All compounds were tested at a concentration of 3.0 μmol/g fr. wt.

†Based on an eight day feeding period; initial sample size $N = 30$.

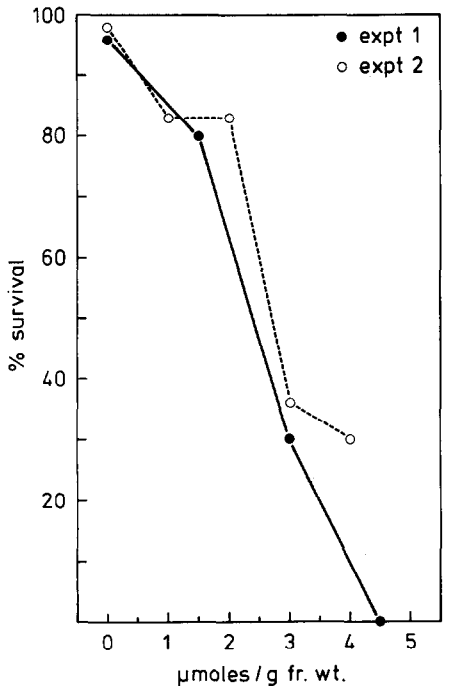


Fig. 1. Dose-response for encecalin in artificial diets offered to neonate *P. saucia*. Survival determined at 72 hr, the approximate time to starvation for non-feeding larvae.

times has very low concentrations, e.g. 0.7–1.1 μmol/g fr. wt. [P. Proksch, unpublished data].

Inhibition of larval growth and survival in *P. saucia* appears to be limited to the earliest larval stages; at 3.0 μmol/g fr. wt neither encecalin nor any of the other three compounds inhibited growth or reduced feeding of third instar larvae (20 mg live wt) relative to controls. In this experiment, larvae consumed an average of 0.95 μmol of encecalin during the 72 hr feeding period. However, at 3.5 μmol/g fr. wt, encecalin reduced feeding of third instar *P. gamma* by 65% with a concomitant 50% reduction in larval growth relative to controls.

Insecticidal activity of the compounds from *Encelia* was investigated using a residue contact bioassay. Only encecalin was active, causing 85% mortality at 1.0 μmol/vial (*ca* 40 cm²) (Table 2). Application of 1.0 μmol of encecalin to the cap alone did not result in significant larval mortality, indicating that toxicity depends on direct contact, rather than volatility as had been previously suggested [5]. Bioassay of several concentrations of encecalin demonstrated a curvalinear dose-response for insecticidal activity towards neonate *P. saucia* (Fig. 2). Doses as low as 0.2 μmol/vial (*ca* 1 μg/cm²) caused significant mortality. In contrast, concentrations exceeding 20 μg/cm² were required to kill neonate nymphs of the milkweed bug *Oncopeltus fasciatus* [5]. This insecticidal activity could be relevant in nature in that several species of *Encelia* exude a copious quantity of resin from their stems and these resins are particularly rich in chromenes and benzofurans (personal observation).

Potential topical toxicity of encecalin to third instar larvae of *P. saucia* was investigated by applying 1.0 μmol

Table 2. Toxicity of chromenes and benzofurans from *Encelia* to neonate *Peridroma saucia* via the residue contact method

Compound*	% Survival†
Control	100
1, Encecalin	15±28
2, 7-Demethylencecalin	80±16
3, 6-Methyleuparin	100
4, Euparin	100
1, Encecalin—applied to cap only	90±15

*Tested at 1.0 μmol/vial (*ca* 5 μg/cm²).

†Mean ± s.d. Determined at 24 hr; initial sample size $N = 50$.

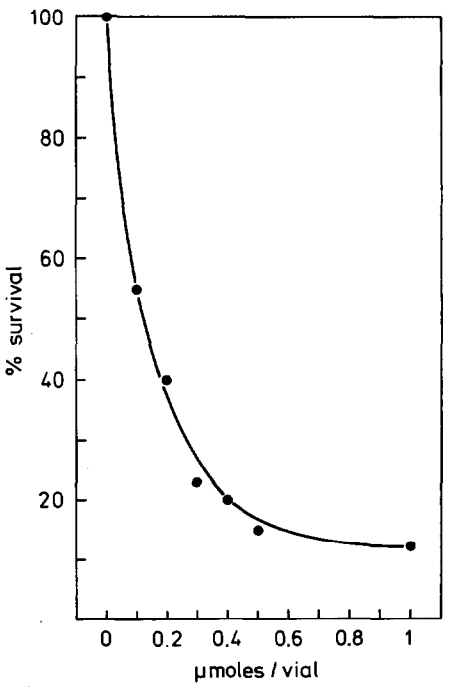


Fig. 2. Survival (24 hr) of neonate *P. saucia* in glass vials coated with encecalin.

in acetone. This quantity was shown earlier to be neither toxic nor deterrent when fed to larvae in artificial diet. However, a single acute topical dose significantly reduced larval growth relative to controls treated with the carrier alone (Table 3). Encecalin applied topically did not have any significant effect on the relative consumption rate, but the reduction in larval growth appears instead to result from a reduction in the net dietary utilization by larvae treated with encecalin. In other words, digested food was not utilized (converted to larval biomass) as efficiently in the presence of encecalin. This metabolic cost cannot be simply attributed to detoxication of encecalin, as *P. saucia* is capable of excreting encecalin intact (unpublished observation). Why encecalin should be benign to third instar larvae when ingested, but deleterious when administered topically is not yet understood, but may reflect a lack of absorption of the compound from the insect's alimentary canal. The fate of encecalin in *P. saucia* and other species following different routes of administration is currently under investigation by one of us (M.B.I.). The potent toxicity and deterrency of encecalin, particularly to neonate larvae of phytophagous noctuids, supports a hypothesis that this compound constitutes an effective adaptation for defense against herbivory on species of *Encelia* producing it in large quantities.

EXPERIMENTAL

The compounds used in this study were isolated from various species of *Encelia* and purified as previously described [2]. For insect feeding experiments, artificial diets were prepared and experiments conducted in the usual manner [11]. Methods for determining growth rates, consumption rates and dietary utilization are well documented [12, 13].

Contact toxicity was assessed by coating the inner walls and bottom of 20 ml glass scintillation vials with the test compound. The compound was dissolved in 0.1 ml of Me_2CO which was poured into the vials; the vials were then rotated by hand until the carrier had completely evaporated. Tests with larger quantities of other chemicals indicate that this method leaves an even coating of residue throughout the inner surfaces of the vial. Five neonate larvae were carefully placed in each vial using a camel hair brush and left undisturbed for 4 hr at which time a 2 g plug of normal diet was placed in each vial. The vials were then held at 27° and surviving larvae counted after 24 hr. Controls consisted of vials treated with the carrier alone. For these bioassays, each treatment consisted of 10 vials with 5 larvae/vial.

Table 3. Feeding, growth and dietary utilization by third instar larvae of *Peridroma saucia* following topical application of encecalin

	Control	Encecalin
Relative growth rate (mg/mg/day)	0.526	0.446*
Relative consumption rate (mg/mg/day)	1.25	1.22
Efficiency of conversion of digested food (net dietary utilization, %)	56.1	46.7*
Net weight gain (mg)	171	102*

*Significant difference, $p < 0.05$ (t-test).

Third instar larvae were treated topically with encecalin by dissolving the compound in Me_2CO at 1 μmol/μl. One μl was then administered to the dorsum using a 50 μl syringe connected to a repeating dispenser. Control larvae were treated with the 1 μl of the carrier alone. Larvae were allowed to feed on normal diet for 72 hr after which feeding and growth were assessed. Sample size, N, = 15 for both treatment and control groups.

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