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ANTIINSECTAN ACTIVITY OF EPILACHNENE, A DEFENSIVE ALKALOID FROM PUPAE OF MEXICAN BEAN BEETLES (*Epilachna varivestis*)

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Abstract—Epilachnene [(5*Z*)-11-propyl-12-azacyclotetradec-5-en-14-olide], the principal component of the secretion of the pupal defensive hairs of the Mexican bean beetle (*Epilachna varivestis*), has antiinsectan activity. Both the *R* enantiomer of epilachnene, and the *S* enantiomer (the natural configuration of the compound), proved deterrent in a feeding bioassay with a predaceous coccinellid beetle (*Harmonia axyridis*). Moreover, both enantiomers proved active in a topical irritancy test with a cockroach (*Periplaneta americana*).

Key Words—Chemical defense, Coccinellidae, alkaloid, azamacrolide, bioassay, feeding deterrent, irritant.

INTRODUCTION

Pupae of the Mexican bean beetle, *Epilachna varivestis* (Coccinellidae), are densely covered with tiny glandular hairs, each bearing a droplet of oily secretion at the tip (Attygalle et al., 1993) (Figure 1). The secretion is presumed to be defensive, and it was indeed noted that when ants (*Leptothorax longispinosus*) come in contact with the hairs, they back away abruptly and engage in intensive cleansing activities (Attygalle et al., 1993). Chemical analyses showed the secretion to consist of a mixture of structurally novel alkaloids, the azamacrolides, of which one, epilachnene [(5*Z*)-11-propyl-12-azacyclotetradec-5-en-14-olide] (**1**), is the major component (Figure 2) of the fluid (Attygalle et al., 1993). Epilachnene has since been synthesized (Rao et al., 1994, 1996; Gribble and Silva, 1996).

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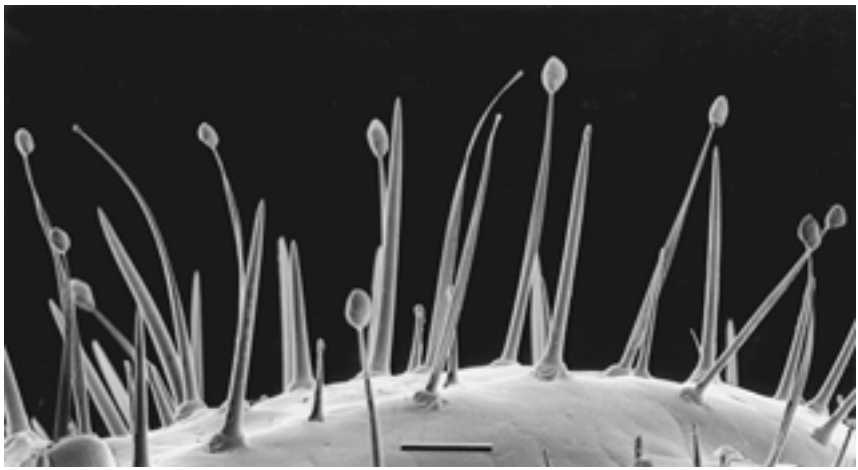

FIG. 1. Glandular hairs (interspersed among nonglandular spines) on surface of pupa of Mexican bean beetle (*Epilachna varivestis*) (bar = 0.1 mm).

Further work showed epilachnene to occur in the secretion as the *S* enantiomer (Farmer et al., 1997) and shed light on the biosynthesis of the compound, demonstrating that it can be derived from oleic acid and serine (Attygalle et al., 1999). We have recently synthesized both (*R*)- and (*S*)-epilachnene (Farmer et al., 1997), and here report that in a feeding bioassay with a coccinellid beetle, *Harmonia axyridis*, both enantiomers proved potently deterrent. Our bioassay was a simple choice test in which individual *H. axyridis* were presented with two offerings of an edible food item (eggs of the arctiid moth *Utetheisa ornatrix*), one of which was treated by topical addition of epilachnene.

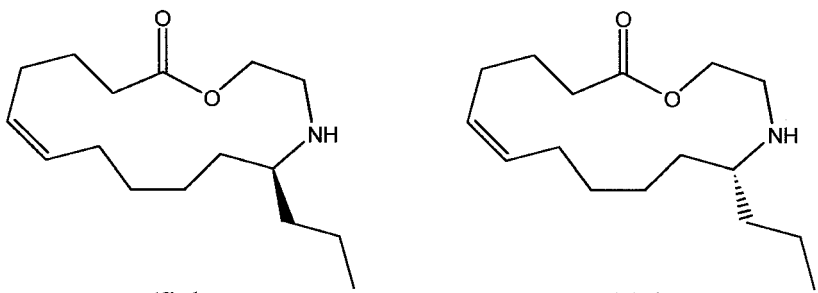

FIG. 2. Chemical structures of (*S*)- and (*R*)-epilachnene.

We also provide evidence that both (*R*)- and (*S*)-epilachnene have the capacity to act as surface irritants to insects, as evidenced by their strong activity in a scratch-reflex test with a cockroach (*Periplaneta americana*).

METHODS AND MATERIALS

H. axyridis. This coccinellid is an exotic species that has become established in much of the eastern United States (Hoebeke and Wheeler, 1996). In the environs of Ithaca, Tompkins County, New York, adults are easily collected in the fall, when they are on the wing in search of overwintering sites. We maintain a colony of the beetle on aphid-infested (*Acyrthosiphon pisum*) alfalfa plants (*Medicago sativa*) in a greenhouse (22 ± 4°C, 16L : 8D photoperiod).

U. ornatrix *Eggs.* The eggs of this moth are ordinarily distasteful to insect predators because of the pyrrolizidine alkaloids they contain (Dussourd et al., 1988). The alkaloids are originally derived by the moth from its larval foodplants (*Crotalaria* spp.; Fabaceae) (Eisner and Meinwald, 1995). If the moth is raised on a diet devoid of pyrrolizidine alklaloids, such as one based on pinto beans that we use in our laboratory (Miller et al., 1976), the eggs are rendered alkaloid-free and palatable to predators (Dussourd et al., 1988; Hare and Eisner, 1993). Such alkaloid-free eggs provided the edible items used in our assay with *H. axyridis*.

To obtain eggs for the assay, we confined mated *U. ornatrix* females in small containers lined with wax paper. They readily oviposited on the paper, laying eggs in dense clusters, as they typically do in nature.

Bioassay (H. axyridis). For testing purposes, adult *H. axyridis* were first starved for 48 hr, during this time they were given water only (soaked cotton wad). They were then confined individually in small Petri dishes (4.8 cm diameter) and presented with two *U. ornatrix* egg offerings. Each offering consisted of 10 eggs still attached to a piece of their wax paper backing. One egg offering was treated by topical addition of epilachnene, administered in ethyl ether solution with a micropipet, at a dosage of 0.8 μg epilachnene (= 1 μl solution) per egg. The other offering (control) was treated by addition of ethyl ether only (1 μl/egg). The fate of the eggs was monitored by observation at 15-min intervals for a period of 2 hr. The data were scored as the fraction of eggs consumed over time per offering. Twenty tests were done with each of the enantiomers.

The fraction of eggs consumed was subjected to arc sine transformation (Snedecor and Cochran, 1989). The transformed data for each enantiomer and its control were subjected to a repeated-measures analysis of variance, with time taken as the repeated measure. Comparison of the effectiveness of the two enantiomers was carried out by the same procedure.

*Scratch Test (*P. americana*)*. When a droplet of an irritant chemical is applied to one side or another of the fifth abdominal tergite of a decapitated nymph of *P. americana*, the animal scratches the site with the hindleg of the

side stimulated. The time interval between application of sample and scratching provides a measure of the irritant potency of the chemical. Details of this assay, which we have used previously for assessment of irritancy of various secretory products of insects and plants, are given elsewhere (Eisner et al., 1976).

We used the assay to test for the potency of both (*R*)- and (*S*)-epilachnene. Sixteen last-instars were used per sample. Droplets were applied at a fixed volume (0.1 μl). Onset of scratching was timed to 1-sec accuracy with a foot-operated stopwatch.

RESULTS

In the *H. axyridis* test, epilachnene was evidently deterrent in both its configurations (Figure 3). The increased consumption rate of the control eggs over the experimentals was significant for both enantiomers ($P \ll 0.01$ for each case), and there was no difference in the consumption rate of the two sets of controls ($P = 0.15$).

While at the dosage tested (0.8 μg/egg) the two enantiomers were equally deterrent ($P = 0.11$), we cannot rule out the possibility that they might have been differentially active at lower concentrations. It would obviously have been desirable to obtain dose–activity data for each enantiomer, but this was precluded by a shortage of synthetic material.

Both enantiomers affected *H. axyridis* visibly. Typically, the beetles backed away promptly when they made oral contact with an epilachnene-treated egg.

Epilachnene also proved active in both configurations in the cockroach scratch test (Figure 4). The mean response times to the two enantiomers [1.9 sec to (*S*)-epilachnene; 2.4 sec to (*R*)-epilachnene] were not significantly different (Mann-Whitney test, $P = 0.80$) and were roughly at a par with response times previously reported for two other oily irritants, hexanal and oleic acid, also known to be produced for defensive purposes by arthropods (Eisner et al., 1996; Bettini, 1978; Blum, 1981).

Epilachnene, in both of its forms, appears to have physical affinity for insect cuticle. In the *P. americana* assay, the test droplets tended to spread instantly and broadly the moment they were applied to the cockroach tergite. Furthermore, because of low volatility, epilachnene has the capacity to inflict enduring irritancy. Indeed, the scratch reflexes elicited by either enantiomer in the cockroach test tended to persist with little interruption for up to 20 min or more following stimulation.

CONCLUSION

Our data are useful in that they provide a first quantitative demonstration of the antiinsectan activity of an azamacrolide. Clearly, epilachnene should be

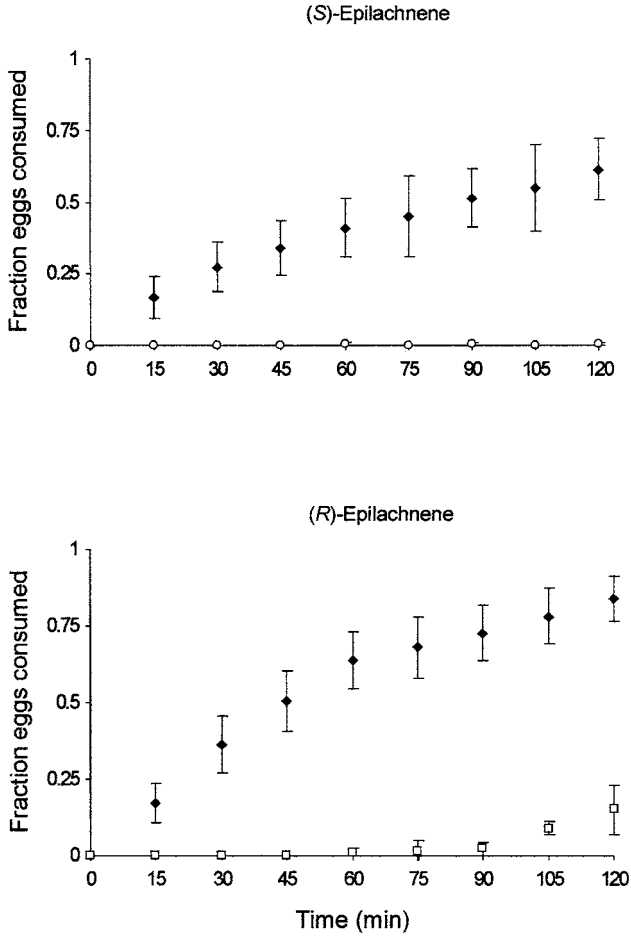

FIG. 3. *H. axyridis* predation rates upon *U. ornatrix* eggs treated with (*S*)-epilachnene (open circles) and (*R*)-epilachnene (open squares), relative to their respective controls (solid diamonds) in choice trials ($N = 20$ beetles tested per enantiomer). Data are given as mean ± SE.

evaluated more broadly. Given the unprecedented chemical structure of this alkaloid, compared to secondary metabolites previously known from natural sources, it is impossible to anticipate what biological properties it might possess. Within the structurally diverse realm of coccinellid alkaloids (Daloze et al., 1995; King and Meinwald, 1996), epilachnene and its congeneric azamacrolides are unique, although biosynthetically all coccinellid alkaloids may share a common origin

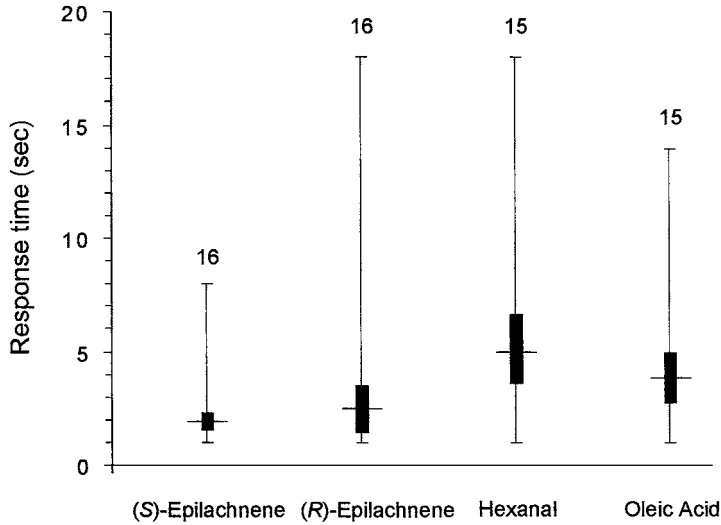

FIG. 4. Sensitivity of *P. americana* to topical application of (*S*)- and (*R*)-epilachnene. Sensitivity is expressed as delay to onset of the scratch reflex induced. Data for hexanal and oleic acid are from Eisner et al. (1996). Data are given as mean ±SE and range. Sample sizes are given above bars.

based on the amination of fatty acids (Daloze et al., 1995; King and Meinwald, 1996).

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