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EFFECTS OF SESQUITERPENE LACTONES ON DEVELOPMENT OF *AEDES ATROPALPUS* AND RELATION TO PARTITION COEFFICIENT[1]

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ABSTRACT.—Six sesquiterpene lactones isolated from plants of the Asteraceae family exhibited chronic toxicity effects to the mosquito *Aedes atropalpus*. Larval development was primarily affected with minimal consequences to pupae, adults, and the F_1 generation. Correlation of activity with the partition coefficient of the compounds as measured by hplc was not strong.

Most of the currently known sesquiterpene lactones have been isolated from plants of the Asteraceae and appear to be characteristic secondary metabolites of this family (1). Recently, the role of these substances as insect allomones has received considerable attention.

Insects as different as armyworms (Lepidoptera), flour beetles (Coleoptera), fruit flies, and mosquitoes (Diptera) have all been shown to be affected by sesquiterpene lactones. Glaucolide-A from *Vernonia* spp. was found to be a feeding deterrent to six lepidopterous larvae (2). Alantolactone (3), helenalin, eupatoriopicrin, and linifolin (4) deterred feeding of *Tribolium confusum*. Four sesquiterpene lactones of *Liriodendron tulipifera* affected the feeding performance of gypsy moth larvae (5). The growth rate and/or survival of lepidopterous larvae was influenced by glaucolide-A (2, 6), that of the fruit fly (*Drosophila melanogaster*) by euponin (7), of the tobacco cutworm (*Spodoptera litura*) and mosquito (*Culex pipiens*) larvae by lupatolide (8). Similarly, inhibition of the confused flour beetle was caused by alantolactone (3), parthenin, coronopilin, and helenalin (9), and inhibition of the fall armyworm (*Spodoptera frugiperda*) was caused by melampodin A (10). Isman and Rodriguez (11) found that the more oxygenated parthenolides were more toxic to *Heliothis zea* than their unsubstituted ambrosanolide analogues.

In the current study, we report effects of six sesquiterpene lactones (Figure 1) on the growth and development of the rock hole breeding mosquito, *Aedes atropalpus* Coquillet.

EXPERIMENTAL

Mosquito larvae were reared as described by Philogène and Labaky (12). Twenty second-instar larvae in 200 ml dechlorinated H_2O in a beaker were treated with the appropriate dose of test substance dissolved in a small volume of EtOH to give the final concentration of 0.1, 1.0, 10, and 100 μg/ml (ppm). Twenty-four hours after treatment, the larvae were washed several times with dechlorinated H_2O, transferred to untreated H_2O, and their subsequent development monitored. The containers were placed in large glass jars to retain the emerging adults. Dead insects were removed daily. Throughout the experiment, the insects were maintained at a photoperiod of L/D: 18/6 (Indorsun fluorescent lighs) and a temperature of 26°. Each test was performed in duplicate.

[1]C.B.R.I. Contribution Number 1450.

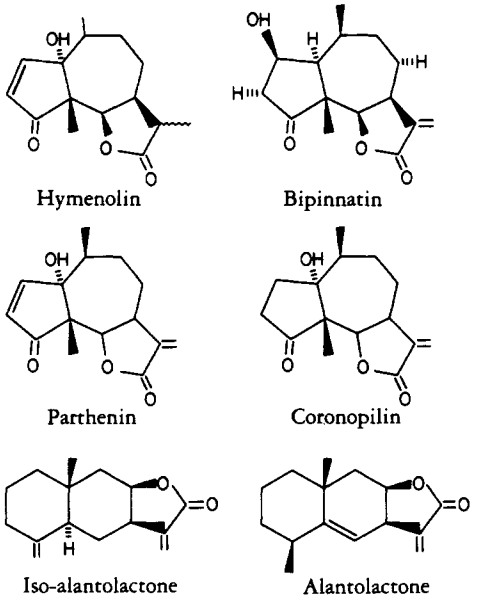

FIGURE 1. Molecular structure of the six sesquiterpene lactones investigated.

Alantolactone and isoalantolactone were separated from a commercial mixture of these two compounds, "Helenin" (Sigma Chemical Co.), by the method of Dupuis *et al.* (13). Coronopilin and parthenin were isolated from dried shoots of *Parthenium hysterophorus* by a method previously described (14). Hymenolin and bipinnatin were isolated from *Hymenoclea salsola* using methods described previously (15, 16). Partition coefficients of sesquiterpene lactones were determined from a standard curve of the logarithm of the octanol-H_2O partition coefficient (log P) plotted against the capacity factor (log K^1) measured by hplc and established for a series of standards with known partition coefficients (17). A Beckman model 332 HPLC was used with a reverse phase ODS column and isocratic elution (60% MeOH, 40% H_2O). Standards with their respective partition values log P, log K^1 were: fenuron (1.52, -0.30), diuron 2.60, 0.493), 2,5 dimethylfuran (1.80, 0.570), toluene (2.59, 0.751), and 1,2'4-trichlorobenzene (4.27, 1.16). The regression line for the standards was log P=1.683 log K^1+1.655, r=0.84.

RESULTS AND DISCUSSION

The six sesquiterpene lactones tested did not exhibit a drastic knockdown effect on second-instar mosquito larvae but affected their overall growth and development to the pupal and adult stages. At 24 h after treatment, only parthenin, alantolactone, and isoalantolactone were significantly toxic, and then only at 10 and 100 ppm. After transfer of the treated larvae to clean H_2O, each substance affected the subsequent development phases (Figure 2). The order of this chronic toxicity was isoalantolactone > alantolactone >> parthenin > coronopilin > bipinnatin > hymenolin. While it took 6.5 days for 50% of control larvae to pupate, 100 μg/ml coronopilin-treated survivors needed 7.5 days. The number of pupae and adults formed in all treated groups (Figure 3) was lower than in the control. There was, however, no evidence of significantly increased mortality or delay in development at these stages. Moreover, there was no significant reduction of fecundity, fertility, or variability of the progeny of the survivors, though they tended to be fewer in number.

The origin of these chronic effects on larval development is probably related to the alkylation of the SH groups in target insect protein as suggested for cardiac-inhibitory activity in grasshoppers, *Melanoplus sanguinipes* (18), and as observed for other types of protein (19, 20). All the tested substances except hymenolin, the least toxic substance, possess the α-methylene-γ-lactone moiety necessary for alkylation and for some ac-

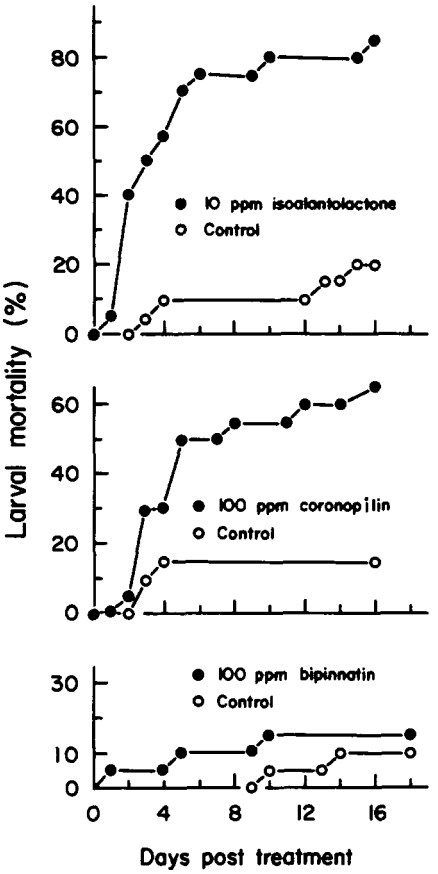

FIGURE 2. Cumulative larval mortality of *Aedes atropalpus* following treatment with isoalantolactone, coronopilin, and bipinnatin.

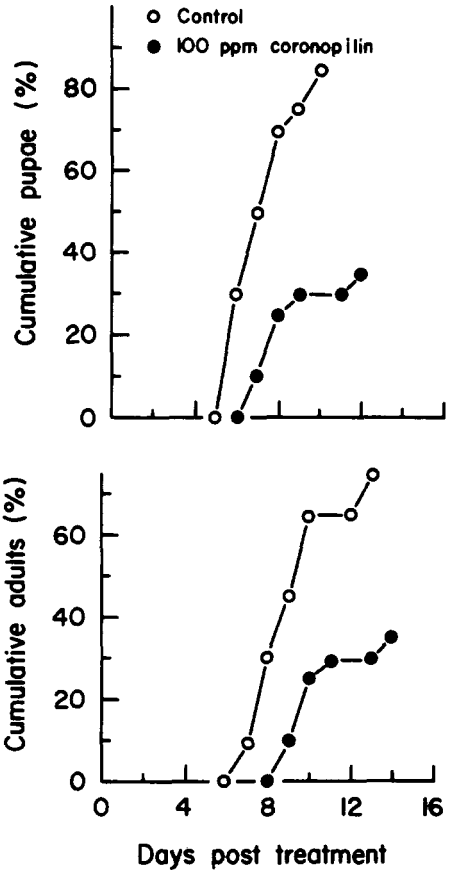

FIGURE 3. Effects of coronopilin on the number of *Aedes atropalpus* pupae and adults following larval treatment with 100 μg/ml of the compound.

tivities of sesquiterpene lactones such as, for example, allergic contact dermatitis (21). It has been suggested that this moiety is responsible for the detrimental properties of parthenin, coronopilin, and helanalin on survival of the confused flour beetle since tenulin which lacks this moiety was not active (9). Mutagenic activity has been recorded for some sesquiterpene lactones (22, 23), but no mutants were observed in these studies.

The order of toxicity of the compounds is similar to that found for some of the compounds used in molluscicidal tests (24) and insecticidal tests (3, 9, 11). However, the order of toxicity remains largely unexplained. Using the hplc method of Konemann *et al.* (25), the logarithm of the partition coefficient (log P) was estimated from the regression of log P versus the logarithm of the capacity factor (Log K^1) derived for a set of standards with known partition coefficients. The data in Table 1 indicate that there is a substantial decrease in the value of log P between the two most toxic (alantolactone and isoalantolactone) and the remaining compounds (parthenin, coronopilin, bipinnatin, and hymenolin). Within the group hymenolin, bipinnatin, parthenin, and coronopilin, there is little difference in the values for the partition coefficient, yet differences in toxicity are evident. While bioaccumulation of the more lipophilic substances may contribute to their toxicity, other structural considerations appear to be more important

TABLE 1. Capacity Factors and Partition Coefficients for Test Compounds

Compound	log K^1	log P
Isoalantolactone	1.05	3.42
Alantolactone	1.03	3.38
Hymenolin	0.025	1.70
Bipinnatin	−0.020	1.62
Coronopilin	−0.14	1.42
Parthenin	−0.17	1.37

determinants of toxicity. For example, parthenin, which is more active than coronopilin, hymenolin, and bipinnatin, has two, rather than one, centers for Michael addition.

The acute toxicity of sesquiterpenes tested here is considerably lower than that recorded earlier for polyacetylenes and their thiophene derivatives which are also secondary metabolites of the Asteraceae (26). This is, in part, due to the higher partition coefficients of these substances and their unique phototoxic mode of action. Our results also indicate that the sesquiterpene lactones tested here are not likely to be useful larvicides.

ACKNOWLEDGMENTS

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