Biological Evaluation of Juvenile Hormone Compounds Against Pupae of the Stable Fly

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Four compounds assayed for juvenile hormone activity against pupae of the stable fly, *Stomoxys calcitrans* (L.), had morphogenetic effects (prevented pupal-adult apolyses) when they were applied topically at doses ranging from 1 to 10 ng as follows: ethyl 10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate; methyl 10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate; ethyl 10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate; and (*E*)-4-[(6,7-epoxy-3,7-dimethyl-2-octenyl)oxy]-1,2-(methylenedioxy)-benzene.

Since Williams (1956) suggested using insect hormones as insecticides, the juvenile hormone has been identified and synthesized from the abdomen of adult male *Hyalophora cecropia* (L.) (Dahm *et al.*, 1967; Röller *et al.*, 1967) and many synthetic or naturally occurring chemicals have been shown to have juvenile hormone activity in a variety of insects (*e.g.*, Bowers, 1968; Bowers *et al.*, 1965; Riddiford, 1970; Schneiderman *et al.*, 1965; Sláma *et al.*, 1968; Wigglesworth, 1969). A previous report from this laboratory established assay procedures that allowed definition of the biological activity associated with juvenile hormones in the stable fly, *Stomoxys calcitrans* (L.) (Wright, 1970). The present paper represents a continuation of this research to define chemicals with potent juvenile hormone activity in the metamorphosis of *S. calcitrans*.

METHODS AND MATERIALS

All compounds were dissolved in acetone (Fisher A. C. S. Spectranalyzed), and 1 μl of the dilution was applied externally to the test insects from the laboratory colony with a microapplicator; control insects received 1 μl of acetone diluent. The insects were treated within 1 hr after the onset of larval-pupal apolysis when the color of the puparium is still white. Large numbers of this stage, referred to hereafter as pupa, were easily hand-picked from rearing pans containing larvae that were 8 to 9 days old. After treatment, the pupae were allowed to air dry, placed in 1-oz paper souffle cups, and held in an incubator at 27° C and about 95% relative humidity. (The humidity must be high or the developing pupa will die and desiccate within the puparium.) At 8 days posttreatment, when the control insects had undergone pupal-adult apolysis, the treated pupae were dissected. Any adults emerging from the experimental pupae were retained and checked for fecundity and fertility.

The following substances (all supplied by personnel of the Pesticide Chemicals Research Branch of the Entomology Research Division unless otherwise noted) were evaluated against stable fly pupae.

I. 11,12-epoxy-4,8,12-trimethyl-3,7-tridecadien-2-one; courtesy of Meyer Schwarz.

II. 11,12-epoxy-8-ethyl-4,12-dimethyl-3,7-tridecadien-2-one; courtesy of Meyer Schwarz.

III. ethyl 10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate; courtesy of John Siddall, Zoecon Corp. This material is a mixture of known isomers (22% trans, cis, trans; 24% trans, trans, cis; 54% trans, trans, trans).

IV. methyl 10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate; courtesy of Martin Jacobson. This material is a mixture of isomers.

V. ethyl 10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate; courtesy of William Bowers.

VI. (*E*)-4-[(6,7-epoxy-3,7-dimethyl-2-octenyl)oxy]-1,2-(methylenedioxy)benzene; courtesy of William Bowers.

VII. 4-[2-(2-butoxyethoxy)ethoxy]-1,2-(methylenedioxy)-benzene; courtesy of Terrence P. McGovern.

VIII. Acetaldehyde 2-(2-butoxyethoxy)ethyl 3,4-(methylenedioxy)phenyl acetal; courtesy of Terrence P. McGovern.

IX. α-[2-(2-butoxyethoxy)ethoxy]-4,5-(methylenedioxy)-2-propyl toluene; (piperonyl butoxide); courtesy of Murray Beadles.

X. 1,2-(methylenedioxy)-4-[2-(octylsulfinyl)propyl]benzene; (sulfoxide); courtesy of Murray Beadles.

XI. Acetaldehyde, 2-(2-ethoxyethoxy)ethyl 3,4-(methylenedioxy)phenyl acetate; (Sesamex); courtesy of Morton Beroza.

XII. Propyl 2-propynyl phenylphosphonate; courtesy of FMC Corp.

RESULTS AND DISCUSSION

Table I reports the results obtained when the test materials were applied topically to pupae of the stable fly. The development of pupal-adult intermediates is shown as the final criterion, but there was actually a gradation in the morphogenetic effects. Srivastava and Gilbert (1969) and Wright (1970) demonstrated that the age of the assay Dipteran pupa is of utmost importance because older pupae require a greater amount of material to arrest development during the pharate adult stage. Therefore, the pupal-adult intermediate may reflect arrest during either the early or late stages of this period. The abdomen usually is typically pupal (Figure 1), but may also be partially to fully developed. Moreover, it is not uncommon, especially at the lower doses, to find apparently fully developed dead adults within the puparia, and these dead adults have also been scored as pupal-adult intermediates. In an earlier paper, Wright (1970) reported that the pupal-adult intermediate is a true juvenile hormone effect by histological examination. However, we have not observed a supernumerary molt as reported in larval *Galleria* spp. (Dahm *et al.*, 1967; Röller and Dahm, 1968; Sehnal and Meyer, 1968). However, those that underwent pupal-adult ecdysis and then died were scored as having eclosed successfully, though they may also have been affected by the treatment.

Recently, Bowers (1969) reported that the 3,4-methylenedioxyphenyl ethers of 6,7-epoxy geraniol were active at the ng

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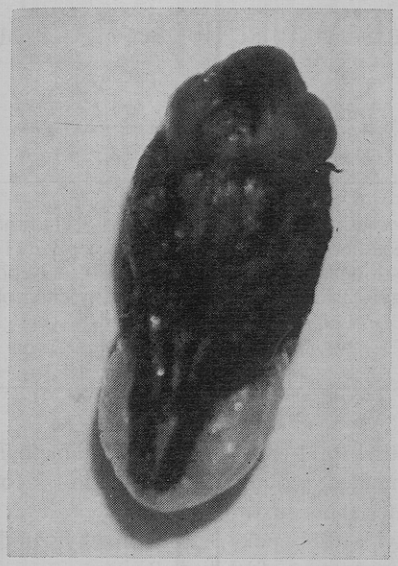

Figure 1. Pupal-adult intermediate of the stable fly, *Stomoxys calcitrans*. The head and thorax are characteristically adult; the abdomen is typically pupal

Table I. Juvenile Hormonal Activity (Three Replicates of Ten Pupae per Replicate) Based on the Suppression of Pupal-Adult Ecdysis in the Stable Fly

	Juvenile Hormone Activity[a] after Indicated Dose (μg/pupa)					
Compound	10	1	0.1	0.01	0.001	0.0001
I		4	2	1	0	0
II		4	2	1	0	0
III		4	4	4	4	4
IV	4	4	4	4	4	3
V		4	3	2	2	1
VI	4	4	4	4	4	3
VII		0	0	0	0	0
VIII		4	0	0	0	0
IX	4	4	3	3	3	
X	4	3	0	0	0	
XI	4	4	3	2	2	
XII	4	4	3	3	2	

[a] 4 = 100–80% suppression; 3 = 79–40% suppression; 2 = 39–20% suppression; 1 = 18–1% suppression; and 0 = 0% suppression.

level against the yellow mealworm, *Tenebrio molitor* L., and in the large milkweed bug, *Oncopeltus fasciatus* (Dallas). Also, our data for similar aromatic terpenoid ethers showed these compounds to have morphogenetic activity when we assayed them against the stable fly pupae at a similar level.

The epoxy C_{17} ethyl ester caused developmental arrest in *Sarcophaga bullata* Parker at the 0.10-μg level (Srivastava and Gilbert, 1969), and our data also showed that this compound is active in stable fly pupae at similar levels. Also, epoxy, methyl farnesoate and piperonyl butoxide had like activity in both Dipteran species. Juvenilizing effects have been observed in mosquitoes with a derivative of farnesoic acid (Spielman and Skaff, 1967), and Bryant and Sang (1968) showed that material resulting from treatment of farnesoic acid with ethanolic HCl reported to be active against *Sarcophaga bullata* (Srivastava and Gilbert, 1969) was also active against Drosophilia.

These results demonstrate that some test materials with juvenile hormone activity can affect the developmental processes of injurious Diptera when they are used at the ngm level and that similar compounds active against species of other orders are also active on the stable fly. The stable fly bioassay will therefore be used in a continuing search for compounds that will give a maximum disruption of development. However, for economic use, successful candidates will have to meet the following criteria: rapid penetration; slow rate of detoxification; optimum activity at the receptor sites of the target organism; and stability. Those active compounds tested in this study obviously meet these criteria in the laboratory. Their economic feasibility should now be investigated in field tests.

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