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STUDIES WITH JUVENILE HORMONE-TYPE COMPOUNDS AGAINST MOSQUITO LARVAE[1]

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Among alternate methods being investigated as substitutes for the conventional chemical control methods against arthropod vectors, the use of insect hormones or chemically-related substances is an exciting possibility. Recent reviews of the history, structure and action of insect hormones have been published (Roller and Dahm, 1968; Berkoff, 1969). The activity of early synthetic juvenile hormone (JH) mimics against mosquito larvae was investigated (Lewallen, 1964; Nair, 1967; Spielman and Williams, 1966). As part of its continuing search for new, safe methods to control insects of public health importance, this laboratory has evaluated synthetic compounds having hormone-like activity against mosquito larvae. The results obtained in laboratory tests with 12 compounds are presented.

MATERIALS AND METHODS. All tests were conducted in 600-ml. glass beakers containing 250 ml. of well water. Twenty-five third instars were introduced not less than ½ hour after treatment with the compound. Food was initially provided within 2 hours thereafter, with additional amounts provided daily until approximately half the specimens had pupated. The number of dead larvae and pupae re-

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moved daily was recorded. Live pupae were removed daily and placed in untreated water in paper cups for observation of subsequent emergence. Mortality was based on the number of "presumed normal" adults emerged in relation to the number of larvae per beaker. Partially emerged adults or those found completely emerged but dead or dying on the water surface were not counted as emerged specimens. No further observations of normality, such as proper rotation of the male genitalia or ability to successfully mate, feed or oviposit, were made. All tests were conducted at 76–80° F. with approximately 40–60 percent R.H. and a 12:12 photoperiod.

The compounds [2] tested and their chemical compositions are shown in Table 1. Stock solutions were prepared and serially diluted with reagent-grade acetone so that the addition of 1 ml. of solution gave the desired concentration in the water. Control beakers were similarly treated with solvent only.

The species tested were *Aedes aegypti* (DDT resistant), *Culex pipiens quinquefasciatus* (DDT-dieldrin resistant), *Anopheles albimanus* (dieldrin resistant), and *Anopheles stephensi* (susceptible).

RESULTS. The data (Table 2) indicate that several compounds (*e.g.*, Compounds III, VII, IX, and XI) at concentrations of 0.1 ppm or less gave at least 95 percent reductions in adult emergence (LC-95) of one or more of the four test species.

Compounds II and III were each equally effective against the test species. Compound IX was markedly more effective against *C. p. quinquefasciatus,* producing 95 percent or higher mortality at 0.0025 ppm, than the other species. Compound XII was notably less effective against anophelines as compared to culicines. In general, *C. p. quinquefasciatus* was the species most responsive to the treatments and thus appears to be a promising test organism for such evaluations.

All compounds were characterized by their inhibition of adult emergence, i.e., development proceeded normally (comparable to controls) to pupation, but emergence of imagoes was adversely affected. Typically, some pupae in emergence cups

[2] Furnished through the courtesy of Zoecon Corporation, Palo Alto, California. The company requested that their code designations not be used to identify compounds. Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education, and Welfare.

TABLE 1.—Chemical composition of experimental compounds.

Compound	Chemical Composition
I	1-[3′,7′-dimethyloct-6′-enyl]-3,4-methylenedioxyphenyl ether
II	(—)-1-[3′,7′-dimethyl–6′,7′-epoxyoctanyl]-3,4-methylenedioxyphenyl ether
III	(+)-1-[3′,7′-dimethyl-6′,7′-epoxyoctanyl]-3,4-methylenedioxyphenyl ether
IV	*Trans* 1-[3′,7′-dimethyl octa-2′,6′-dienyloxy]-3,4-methylenedioxy benzene
V	(dl)-*trans, trans, cis* methyl 3,11-dimethyl-7-ethyl-10,11-epoxytrideca-2,6-dienoate (synthetic Cecropia juvenile hormone)
VI	1-[3′,7′-dimethyl-7′-ethoxyoct-2′-enyl]-3,4-methylenedioxyphenyl ether
VII	1-[3′,7′-dimethyl-6′,7′-epoxyoct-2-enyloxy]-3,4-methylenedioxy benzene
VIII	1-[3′-methyl-7′-ethylnona-2′,6′-dienyl]-3,4-methylenedioxyphenyl ether
IX	1-[3′-methyl-7′-ethyl-6′,7′-epoxynon-2′-enyl]-3,4-methylenedioxyphenyl ether
X	Ethyl 11-chloro-3,7,11-trimethyldodec-2-enoate
XI	Ethyl 11-ethoxy-3,7,11-trimethyldodec-2 (*trans*)-enoate
XII	Ethyl 11-methoxy-3,7,11-trimethyldodec-2 (*trans*)-enoate

TABLE 2.—Concentration (ppm) of juvenile hormone-type compounds required for 95 percent mortality[1] of mosquito species exposed as third instars.

Compound	*Ae. aegypti*	*C. p. quinq.*	*A. albimanus*	*A. stephensi*
I	0.5	0.25	0.5	0.25
II	0.25	0.25	0.25	0.25
III	0.1	0.1	0.1	0.1
IV	0.25	>0.5	>0.25	>0.25
V	1.0	0.5	0.5	1.0
VI	>1.0	0.1	0.25	0.25
VII	0.25	0.1	0.1	>0.25
VIII	0.5	0.25	>0.25	0.25
IX	0.05	0.0025	0.25	0.1
X	1.0	0.25	1.0	>1.0
XI	0.5	0.05	0.1	1.0
XII	0.5	0.25	>2.5	>2.5

[1] Mortality based on number of "normal" adults emerged in relation to the number of larvae used.

die and settle on the bottom, while others elongate and remain at the surface. At concentrations approaching the LC-95 level many adults may partially emerge, whereas others may completely emerge from the pupal skin but are usually found dead on the surface. No marked prolongation of developmental times or toxicity to larvae was attributable to any concentrations of the test materials.

Only Compound IX produced notable activity at the larval-pupal differentiation stage. Such activity was evident with approximately 25 to 33 percent of the anopheline test specimens but was relatively absent with culicines.

With many of the compounds tested a small percentage of the larvae gave rise to anomalous pupal forms (Fig. 1). Such aberrations were more frequently found with *A. albimanus* than with the other test species. Such larval-pupae or adultoid pupae were predicted by Snodgrass (1959). The exact definition of these anomalies is beyond the scope of this report, but such specimens were observed to emerge completely from the last larval skin. They usually remained relatively unmelanized and survived for considerable periods (sometimes as long as 24 hours), usually on the water surface in a horizontal position rather than in the vertical position of the normal pupa.

DISCUSSION. Several compounds were shown to be effective at concentrations considered promising in the case of conventional larvicides. Thus, they may ultimately serve as an alternate control method or "third generation pesticide" (Williams, 1967).

The need for standard techniques and criteria becomes apparent with experience in the evaluation of hormone mimics as control agents against mosquito larvae. JH-like materials have generally been evaluated against last stage immature forms. Spielman and Williams (1966) found differences in response to a crude synthetic farnesoic acid derivative between specific developmental phases of fourth instar *Ae. aegypti.* Many materials with JH-

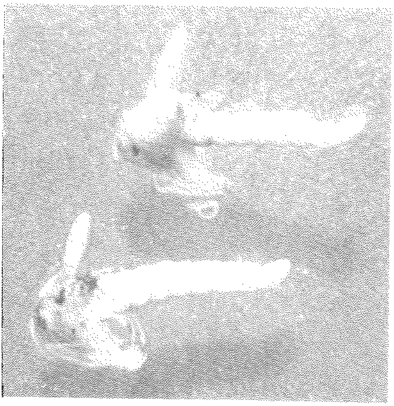

FIG. 1.—Anomalous *A. albimanus* pupae obtained from exposure of third instars to 0.1 ppm Compound VII.

like activity are effective against all larval instars but, in contrast to many conventional pesticides, late instars may be as susceptible as the earlier instars (Sacher, 1971). In evaluating these compounds from an applied standpoint, the use of third instars is an arbitrary compromise. However, in the field, many or most larvae may be at this stage of development when control measures are applied. Similarly, adding food within 2 hours of introducing larvae into the treated medium attempts to simulate natural conditions. The use of apparently "normal" emerged adults as a criterion of effectiveness is based on the fact that the imago is the stage that affects man through annoyance or disease transmission. The relative numbers of male and female adult survivors at LC-95 concentrations indicated that the males frequently were more readily affected than the females.

With the conventional pesticides applied for larval control, the effectiveness generally is measured by the percent kill or reduction in larval densities 24 hours later.

Since JH-like materials do not produce a marked reduction of the number of larvae, their practical use would require marked adjustments in the methods employed to evaluate the effect of such larvicidal agents. The question of their possible effect on nontarget organisms at present remains undefined.

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