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Susceptibility of Stored-Product Insects to Pyridyl Ether Analogues of Juvenile Hormone

Karl J. Kramer,* Harrison E. McGregor, and Kenji Mori

Four pyridyl ether analogues of juvenile hormones were each mixed separately with whole wheat or ground wheat medium and tested for activity against stored product insects. (*E*)-5{[5-(3-butyl-3-methyloxiranyl)-3-methyl-2-pentenyl]oxy}-2-ethylpyridine was effective at 2 and 0.1 ppm in preventing larval and adult development, respectively, from eggs of *Tribolium confusum* Jacquelin duVal. The ID_{95} doses of 0.04–50 ppm also suppressed the development of adult progeny of *Plodia interpunctella* (Hubner), *Ephestia cautella* (Walker), *Sitotroga cerealella* (Olivier), *Rhyzopertha dominica* (F.), *Oryzaephilus surinamensis* (L.), and *Sitophilus oryzae* (L.). Other compounds were less effective. Addition of a propyl substituent on the epoxide terminus of (*E*)-5-{[5-(3,3-dimethyloxiranyl)-3-methyl-2-pentenyl]oxy}-2-ethylpyridine increased activity about tenfold.

One of the most active insect growth regulators (IGR) against stored-product insects is the pyridyl ether analogue of juvenile hormone, (*E*)-5-{[5-(3,3-dimethyloxiranyl)-3-methyl-2-pentenyl]oxy}-2-ethylpyridine (Figure 1, compound III; Solli et al., 1976; Kramer and McGregor, 1978). Although compound III was highly effective as a progeny suppressant against external grain feeders (ID_{95} < 100 ppm), such as the confused flour beetle, *Tribolium confusum* Jacquelin duVal, red flour beetle, *T. castaneum* (Herbst), sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), Indian meal moth, *Plodia interpunctella* (Hubner), and almond moth, *Ephestia cautella* (Walker), it was much less effective against most of the internal grain feeders (ID_{95} > 100 ppm) such as the rice weevil *Sitophilus oryzae* (L.) and Angoumois grain moth, *Sitotroga cerealella* (Olivier).

In order to improve the activity of the pyridyl ether juvenile hormone mimic, we lengthened the molecular chain from 15 to 18 atoms by synthesizing analogues extended by a propyl substituent at the epoxide terminus. A length of 17–18 atoms is usually optimal for IGR activity, depending on the particular derivative being studied (Kiguchi et al., 1974; Mori et al., 1975). The activities of two 18-atom analogues against seven species of stored product insects is reported here.

EXPERIMENTAL SECTION

Chemicals. The chemicals (Figure 1) evaluated were (I) 5-{[5-(3-butyl-3-methyloxiranyl)-3-methyl-2-pentenyl]oxy}-2-ethylpyridine, (II) (*E,E*)-5-[(3,7-dimethyl-2,6-undecadienyl)oxy]-2-ethylpyridine, (III) (*E*)-5-{[(3,3-dimethyloxiranyl)-3-methyl-2-pentenyl]oxy}-2-ethylpyridine (93%, AI3-70644, Stauffer Chemical Co.), (IV) (*E*)-5-[(3,7-dimethyl-2,6-octadienyl)oxy]-2-ethylpyridine (91.5%, AI3-70643, Stauffer). The synthesis of compounds I and II will be described in a separate paper.

Screening Procedure. All insects were obtained from cultures maintained at the U.S. Grain Marketing Research Laboratory. "Chanute" wheat was used in all tests and was obtained from a commercial source. Kernels were cleaned and tempered to a moisture of 12.5 ± 0.5% as determined by a Motomco moisture meter (Motomco, Inc., Electronics Division, Clark, NJ).

The insects were exposed to juvenile hormone analogues admixed with diet. Appropriate stock solutions of chemicals were prepared in water to provide 0.01–100 ppm dosages of insect growth regulator (w/w) when applied to whole wheat or to ground wheat moth medium (Kinsinger, 1975). The kernels were treated as described by McGregor and Kramer (1975, 1976). The chemicals were applied to the grain by uniformly pipetting 5 mL of the appropriate stock solution onto the inside surface of a rotating jar containing 100 g of medium. Then the jar was rotated for 20 min on a mechanical tumbler operating at 40 rpm. The treated diet was allowed to equilibrate for at least 24 h before insects were added. The whole wheat or ground wheat moth medium (100 g) was infested with 50 adult Coleoptera or 50 lepidopteran eggs. Activity was evaluated on the basis of the degree of acute toxicity after 21 days of exposure or the inhibition of progeny development after 9 weeks. All experiments were conducted at 27 °C and 60% RH. The average number of insects found in four replicate samples (minus the number of parent insects when appropriate) was determined. When the numbers of progeny were reduced significantly, the samples were held an additional 6–12 weeks, examined every 2 weeks, and progeny were recorded. The ID_{95} was then expressed as the ppm per weight of grain necessary to obtain 95% inhibition of progeny development when compared with solvent only treated samples. Probit analyses of the data were conducted according to Finney (1952).

RESULTS AND DISCUSSION

Table I shows the biological activity of the pyridyl ether juvenile hormone mimics when they were homogeneously mixed with diet and exposed to stored-product Lepi-

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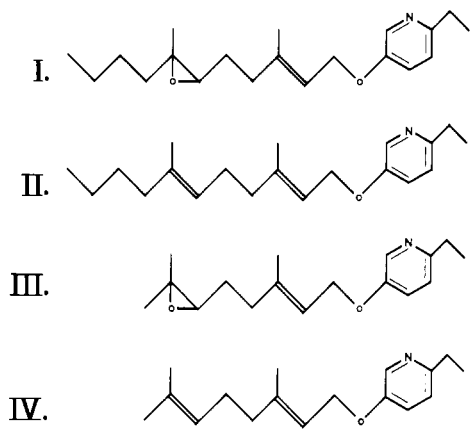

Figure 1. Pyridyl ether analogues of juvenile hormone: (I) 5-{[5-(3-butyl-3-methyloxiranyl)-3-methyl-2-pentenyl]oxy}-2-ethylpyridine, (II) (*E,E*)-5-[(3,7-dimethyl-2,6-undecadienyl)-oxy]-2-ethylpyridine, (III) (*E*)-5-{[(3,3-dimethyloxiranyl)-3-methyl-2-pentenyl]oxy}-2-ethylpyridine, and (IV) (*E*)-5-[(3,7-dimethyl-2,6-octadienyl)oxy]-2-ethylpyridine.

doptera. Eggs of the Indian meal moth and almond moth were placed in coarsely ground wheat medium; those of the Angoumois grain moth were deposited in whole kernel wheat. Compound I was the most effective inhibitor of moth development; ID_{95} values were in the parts per billion range for all three species (30–40 ppb). As might be expected, the external kernal feeders, *Plodia* and *Ephestia*, were slightly more susceptible to these compounds in our bioassay than the internal feeder, *Sitotroga*. However, it is not known what role, if any, the feeding mode of the insect plays in determining the observed differences in susceptibility.

When one compares the activities of several analogues with that of I, it can be seen that addition of an alkyl group and an epoxide function to the terpene backbone of these JH mimics leads to substantial changes in insect growth regulating activity. Epoxidation of the terminal double bond of compound IV increased activity toward the Indian meal moth more than 100-fold. Addition of the propyl substituent to the terminal carbon of the epoxide (III) enhanced the activities toward *Plodia* and *Ephestia* about 30-fold. When the diene analogue (IV) was extended by three carbon atoms, activity was improved by more than two orders of magnitude. However, epoxidation of the propyl terpenyl analogue (II) increased activity only about 7.5 times.

Moth species died while attempting pupation (higher doses) or during adult ecdysis (lower doses). Extra large Indian meal moth larvae were obtained from eggs exposed to 0.8 and 4 ppm compound I, but only normal larvae developed in diet treated at 80 ppb. No adults emerged in any of these tests. In contrast, the almond moth eggs did not produce oversized larvae at any dose of IGR tested. Apparently the species and the concentration of test compound determine whether death occurs at pupal or adult ecdysis and also whether giant larvae develop.

The insect growth regulating activity of the JH mimics toward stored-product beetles is also shown in Table I. None of the compounds exhibited any acute toxic or knockdown action, even after adult insects remained in treated grain for 3 weeks. Also, when these insects were subsequently transferred to untreated grain for egg deposition, there was no latent effect on their progeny. However, the progeny derived from eggs laid in treated grain were adversely affected.

Again, compound I was the most active compound against progeny of all four species of Coleoptera tested.

Table I. Activity of JH Mimics against Development of Insects Exposed from Eggs in Wheat or in Coarsely Ground Wheat Medium

species	compd no.	no. of insects in untreated sample	ID_{95}, ppm[a]
		Lepidoptera	
Indian meal moth	I	48	0.04 (0.03-0.27)
	II	43	0.3 (0.2-0.7)
	III	42	~1
	IV	45	>100
almond moth	I	43	0.03 (0.01-1.17)
	III	32	~100
Angoumois grain moth	I	33	~0.04
	III	31	>100
		Coleoptera	
lesser grain borer	I	312	0.8 (0.4-1.9)
	III	1532	60.4 (40.6-103.7)
confused flour beetle	I	518	2.2 (1.6-3.3)
	II	356	20.3 (10.8-51.8)
	III	266	~5
	IV	496	~10
sawtoothed grain beetle	I	426	~10
	III	945	~100
rice weevil	I	1280	49.3 (30.5-96.1)
	II	773	>100
	III	1588	>100
	IV	1028	>100

[a] The level of JH mimic admixed with diet inhibiting 95% of adult development. Ninety-five percent confidence limits given in parentheses.

It was highly active against the lesser grain borer (ID_{95} of 800 ppb). This result was somewhat unexpected because *Rhyzopertha* is an internal larval feeder and two of the other three species are not. Nevertheless, the most tolerant beetle was the other internal feeding species, the rice weevil. Addition of the propyl substituent to the epoxyterpenyl pyridine compound III enhanced activity 20- and 75-fold against the lesser grain borer and rice weevil, respectively. Only compound I exhibited good activity against *Sitophilus* (ID_{95} about 50 ppm in wheat), a member of the Curculionid family of beetles which is relatively insensitive to insect growth regulators of the JH type (Staal, 1975). The activity of compound I is comparable to those of methoprene and hydroprene, two highly active dodecadienoate JH analogues that have been developed commercially as IGRs (Henrick et al., 1973; Strong and Diekman, 1973; McGregor and Kramer, 1975).

The confused flour beetle was also highly susceptible to the pyridyl JH mimics. At 0.1 and 2 ppm compound I, less than 5% of the *Tribolium* eggs developed into adults or into larvae, respectively. The alkyl extension of the epoxide increased activity against *Tribolium* about 200 times and at the higher concentration the compound had ovicidal activity.

JH mimics generally have two common characteristics. They consist of an unsaturated lipophilic backbone with polar substituents at both ends. The hormone itself contains a methyl ester at one terminus and an epoxide at the other. The compounds tested here have a pyri-

dyloxy function replacing the methyl ester. In addition, the most active analogue has a propyl substituent on the epoxide terminus. The lipophilic extension no doubt decreased the polarity and probably also protected the epoxide from hydration. It also increased the molecular chain length from 15 to 18 atoms. JH mimics of similar structure but containing a phenoxy substituent instead of a pyridyloxy one were found to possess optimal activity against the silkworm, *Bombyx mori* (L.), when their chain length was lengthened to 17 or 18 atoms (Kiguchi et al., 1974). The three-carbon addition to the 15-atom-long pyridyl ether compound III increased the insect growth regulating activity against stored-product insects by more than an order of magnitude.

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Biologically Active Components of Anise: Toxicity and Interactions with Insecticides in Insects

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The biological activity of components of anise tops was studied with insects. *trans*-Anethole was found to be the major insecticidal agent present in anise oil (56% by weight) derived from anise tops, with an LD_{50} of 75 μg/fly when topically applied to houseflies. The toxicity of nine other anise components (anisaldehyde, estragole, anisyl alcohol, anisic acid, *p*-cresol, *p*-creosol, eugenol, hydroquinone, and acetaldehyde) to houseflies was also studied. Anethole and anisaldehyde were both found to increase the toxicity to houseflies when applied simultaneously with parathion, paraoxon, carbaryl, carbofuran, DDT, or pyrethrum. Also, anethole fed to houseflies as 0.5% of their diet resulted in increased insect mortalities due to topically applied parathion or paraoxon in comparison to flies fed a diet without anethole. Further experiments with houseflies which had been fed with anethole as part of their diet indicated that the increased toxicity of paraoxon resulted apparently from an increased penetration of the insecticide into the insect body and a retardation of its degradation to nontoxic, water-soluble metabolites.

The existence of naturally occurring insecticidal plant components has been known for centuries. However, relatively few of these compounds are actually used in crop protection today. Increasing problems concerning the use of modern synthetic insecticides (Hayes, 1975), including insect resistance, persistence of residues, effects on nontarget organisms and human health hazards, has produced renewed interest in these naturally occurring compounds. Since these compounds are often less toxic and less persistent than their synthetic counterparts, and are in some instances already a component of mammalian diets, they are assumed to be environmentally more acceptable and less hazardous to humans. Of special interest, however, are those biologically active compounds which are natural components of food plants. Thus, insecticidal compounds were isolated in our laboratory from turnips (Lichtenstein et al., 1962), parsnips (Lichtenstein and Casida, 1963), and from dill plants (Lichtenstein et al., 1974).

A survey conducted in this laboratory in 1965 indicated that organic solvent extracts of anise plants (*Pimpinella anisum* L.), a widely used spice and flavoring agent, were toxic to fruit flies (*Drosophila melanogaster* M.). Water extracts of anise were also shown to have insecticidal activity when tested with mosquito larvae (*Aedes aegypti* L.). Additional work (Carter, 1976) suggested that two anise compounds, anethole and anisaldehyde, were toxic to fruit flies. The present study was conducted to further investigate the biological activity of anise plants.

MATERIALS

Chemicals. Analytical grade parathion and paraoxon were obtained through the courtesy of Fabenfabriken-Bayer, Leverkusen, West Germany. [^{14}C]Parathion labeled in the 2,6-phenyl positions (sp act., 2.2 mCi/mmol) was

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