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Comparative Juvenile Hormone Activity of Some Terpenoid Ethers and Esters on Selected Coleoptera

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Bioassays of 26 terpenoid ethers and esters applied topically to pupae or prepupae of the beetles *Tenebrio molitor* L., *Leptinotarsa decimlineata* (Say), and *Epilachna varivestis* Mulsant demonstrated many similarities and some striking differences between the species in relative juvenile hormone activity of the compounds. Several polyalkoxy ethers of (2*E*)-6,7-epoxygeraniol were highly ovicidal to young eggs of *E. varivestis* and had high juvenile hormone activity on *E. varivestis* and *T. molitor*, but low activity on *L. decimlineata*. In general, the *m*- and *p*-alkyl and *p*-halogen substituted phenoxy ethers of (2*E*)-6,7-epoxygeraniol were more active than the unsubstituted phenoxy analog. There was a significant overall correlation between the relative topical ovicidal and juvenile hormone activity on *E. varivestis;* however, some notable exceptions occurred. *E. varivestis* eggs were usually more sensitive than prepupae-pupae to vapor action.

Numerous terpenoid ethers and esters have been reported to have significant juvenile hormone activity (Bowers, 1971b; Slama, 1971), and their relative activity on different insects often varies greatly (Redfern *et al.*, 1971; Slama *et al.*, 1970). However, in most studies of structure–activity relationships, only one species of a group of phylogenetically related insects was tested, which leaves unanswered the question of whether the responses obtained are representative of related insects.

Topical or vapor treatment of the newly oviposited eggs of some species of Coleoptera [*e.g.*, the Mexican bean beetle, *Epilachna varivestis* Mulsant; the cigarette beetle, *Lasioderma serricorne* (F.) (Walker and Bowers, 1970); and the convergent lady beetle, *Hippodamia convergens* Guerin-Meneville (Walker, 1970)] with small doses of certain juvenile hormone mimics prevents larval emergence, presumably by inhibiting normal embryonic development. However, little information is available concerning the degree of quantitative correlation between ovicidal and morphogenetic potency of structurally diverse juvenile hormone mimics.

In the present study, we compared the juvenile hormone activity of various terpenoid ethers and esters applied topically to three species of Coleoptera representing three families: the yellow mealworm, *Tenebrio molitor* L. (Tenebrionidae); the Colorado potato beetle, *Leptinotarsa decimlineata* (Say) (Chrysomelidae); and the Mexican bean beetle (Coccinellidae). In addition, the ovicidal activity of these compounds on young *E. varivestis* eggs and the comparative vapor activity of selected compounds to *E. varivestis* prepupae-pupae and eggs were determined. Most of the compounds assayed have previously been reported to have juvenile hormone activity on *T. molitor* or other insects (Bowers, 1968, 1969, 1971a; Bowers *et al.*, 1965; Redfern *et al.*, 1971; Roller and Dahm, 1968).

MATERIALS AND METHODS

Synthesis of Compounds. Compounds **1–23** (Figure 1) were synthesized by the methods of Bowers (1969). Data in support of the given structures for the newer compounds in this group are provided in Bowers (1971a). Compound **24** was obtained from Shulton Inc., Fine

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Chemicals Division, N. Y. Compounds **25** and **26** were synthesized by the methods of Findlay *et al.* (1970). Only the 2*E* isomers of compounds **1–23** and the 2*E*,6*E* isomers of compounds **25** and **26** were assayed.

Topical Assays. The compounds were applied at 10× dilution intervals in 1 μl of acetone to the ventral abdomen of 0 to 4-hr-old *T. molitor* pupae and the middorsal abdomen of 0 to 12-hr-old *L. decimlineata* pupae and 36 to 48-hr-old *E. varivestis* prepupae. Twenty insects were treated with each dose and held at 24°. Adult developmental abnormalities typical of those caused by juvenile hormone compounds on the various species were noted; however, the results were expressed as the lowest dose which reduced the emergence of normal-appearing adults of *T. molitor* by 80% or more and of *L. decimlineata* and *E. varivestis* by 90% or more. In most instances, the range between 0 and 80 or 90–100% effectiveness occurred within a 10× dilution interval. The average pupal weights were: *T. molitor*, 150 mg; *L. decimlineata*, 110 mg; and *E. varivestis*, 45 mg.

In the *E. varivestis* ovicidal assay, each compound was tested at 10× dilution intervals, beginning with a 1% solution. Ten egg masses, 0 to 1 day old, per treatment were dipped for 5 sec in acetone–water (3:1) solutions of the compounds and incubated at 24° in Petri dishes with moist dental rolls. Ovicidal activity was expressed as the lowest concentration causing a 90% or greater reduction in egg hatch. Both solvent-treated controls and untreated egg masses averaged about 80% hatch.

Vapor Assays. The vapor activities of compounds **1–6, 22,** and **26** on eggs and prepupae of *E. varivestis* were determined. Ten 0 to 1-day-old egg masses per dose were held in separate treated Petri dishes for 10 days or until the eggs hatched, as previously described (Walker and Bowers, 1970). Twenty prepupae per dose, 0–12 hr after becoming immobile, were similarly treated and held for 10 days or until adult eclosion. The effective dose (ED_{50}'s) for eggs and prepupae-pupae was determined by computerized log-probit analysis (Daum, 1970) of the results of four to five doses per compound.

RESULTS AND DISCUSSION

The results of the topical bioassays are shown in Table I. Compounds **1–6**, polyalkoxy ethers of 6,7-epoxygeraniol

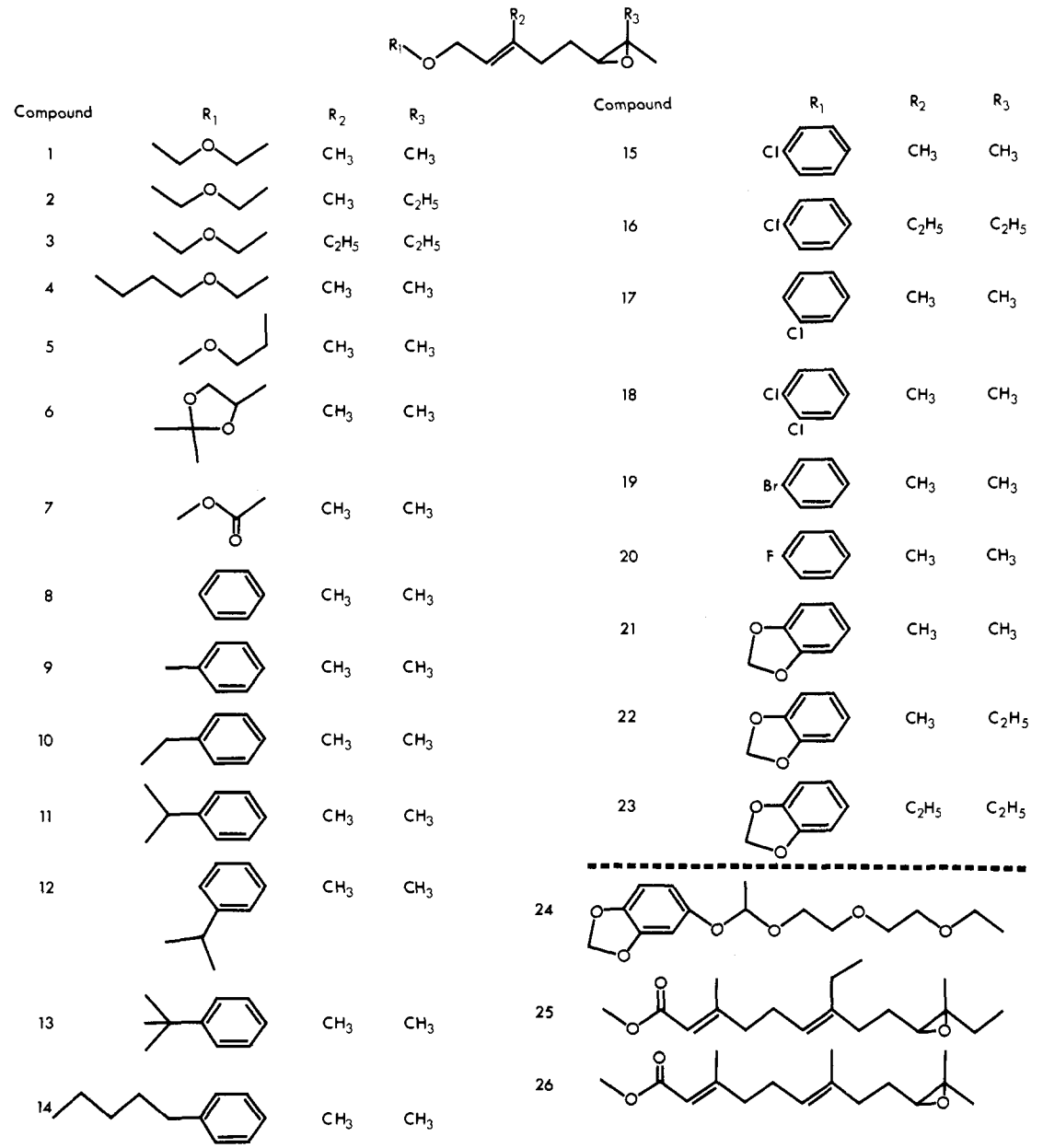

Figure 1. Terpenoid ethers and esters tested for juvenile hormone activity on *Tenebrio molitor, Leptinotarsa decimlineata,* and *Epilachna varivestis.*

Table I. Juvenile Hormone Activity of Test Compounds Applied Topically to Three Species of Coleoptera

Compound[a]	Lowest dose (μg or ppm) applied to indicated stage which prevented adult emergence or egg hatch[b]: *T. molitor* pupae, μg	*L. decimlineata* pupae, μg	*E. varivestis* prepupae, μg	*E. varivestis* eggs, ppm
1	0.1	100.	0.1	1
2	0.1	100.	0.1	10
3	0.1	100.	0.01	1
4	0.1	100.	0.1	10
5	10.	10.+	1.0	1
6	0.1	10.	0.1	10
7	10.	100.+	100.+	100
8	10.	1.	10.	10,000
9	0.1	10.	10.	1,000
10	0.01	0.01	1.	10
11	0.01	1.	1.	100
12	0.1	1.	1.	10
13	1.	1.	1.	1,000
14	1.	1.	1.	1,000
15	0.1	0.1	0.01	10
16	0.01	0.1	0.01	10
17	10.	10.	1.	100
18	0.1	10.	1.	1,000
19	0.1	0.1	0.01	10
20	1.0	1.	0.1	10
21	0.01	0.01	0.01	10
22	0.001	0.1	0.01	10
23	0.0001	0.1	0.1	10
24	1.	1.	1.	1,000
25	0.1	10.	10.	1,000
26	0.1	10.	10.	10,000

[a] See Figure 1. [b] Lowest dose (μg) reducing the emergence of normal-appearing adults of *T. molitor* by at least 80% and *L. decimlineata* and *E. varivestis* by 90%; lowest dose (ppm) reducing *E. varivestis* egg hatch by 90% or more.

and related homologs, were all very active on *E. varivestis* eggs, and all except compound **5** had high activity on *T. molitor* pupae and *E. varivestis* prepupae. In contrast, these compounds had only marginal activity on *L. decimlineata* pupae. Compound **7** exhibited either low or no activity in all pupal and prepupal assays, but at 10 ppm it reduced the egg hatch of *E. varivestis* by 75%. Embryonic development in affected eggs proceeded to the extent that melanized mandibles were visible through the chorion. Also, 5-day-old eggs treated with doses as high as 1000 ppm of compound **7** hatched normally. These characteristics suggest that the ovicidal action of compound **7** is similar to that of compounds which exhibit juvenile hormone activity on *E. varivestis* prepupae (Walker and Bowers, 1970).

Compounds **9–14**, *m*- or *p*-alkyl substituted phenoxy ethers of 6,7-epoxygeraniol, were more active on *T. molitor* pupae and *E. varivestis* eggs than the unsubstituted homolog (compound **8**), with ethyl (compound **10**) and isopropyl (compounds **11** and **12**) substituents being the most effective. *L. decimlineata* pupae and *E. varivestis* prepupae showed much less variability in sensitivity among this series of compounds, except that the *p*-ethyl analog (compound **10**) had high activity on *L. decimlineata*.

Compounds **15–20** are *m*- or *p*-halogenated analogs of compound **8** or closely related homologs. In general, there was good agreement among the various assays as to the relative activities of these compounds. The *m,p*-diCl analog (compound **18**) was an exception: it was as active as the *p*-Cl analog (compound **15**) in the *T. molitor* assay, but in the other assays it was decidedly less active. Unlike the *m*- and *p*-isopropyl isomers (compounds **11** and **12**), which lacked a distinctive pattern of relative activity, the *p*-Cl isomer (compound **15**) was decidedly more active than the *m*-Cl isomer (compound **17**) in all assays. The *p*-Cl and *p*-Br analogs (compounds **15** and **19**) had about equal activity in all assays, and the *p*-F analog (compound **20**) was generally about one-tenth as active.

Ethyl substitution in the R_3 (compound **22**) or R_2 and R_3 (compound **23**) positions of the methylenedioxyphenoxy ether of 6,7-epoxygeraniol (compound **21**) caused a progressive increase in activity on *T. molitor*. This did not occur in the other assays.

Compound **25**, the major active component of adult *Hyalophora cecropia* (L.) and the homologous compound **26**, the juvenile hormone of *Manduca sexta* (L.) (Siddall, 1972), had considerably more activity in the *T. molitor* assay than in the other assays.

The vapor activity of selected compounds on *E. varivestis* prepupae-pupae and eggs is shown in Table II. The

Table II. Juvenile Hormone Activity of Selected Compounds Applied as Vapor to *E. varivestis* Prepupae-Pupae and Eggs

Compound[a]	Eggs ED_{50},[b] μg	Prepupae-pupae ED_{50},[b] μg	Prepupae-pupae ED_{50} / Egg ED_{50}
1	.09	7.0	78.
2	.10	.69	6.9
3	.17	1.1	6.4
4	.66	.24	0.36
5	.10	1.0	10.0
6	.26	1.7	6.5
22	.89	7.9	8.9
26	48.	21.	0.44

[a] See Figure 1. [b] The dose per Petri dish that reduced egg hatch or emergence of normal-appearing adults by 50%.

vapor from as little as 90 ng per Petri dish of compound **1** reduced egg hatch by 50%. Eggs were generally more sensitive than prepupae-pupae, although the ratios of prepupae-pupae ED_{50}'s to the egg ED_{50}'s varied from 0.36 to 78, a difference of 217-fold. However, five of the eight compounds had ratios in close agreement.

In summary, many similarities were found in the juvenile hormone activities of selected terpenoid ethers and esters on three unrelated species of Coleoptera; however, some striking differences were also observed. Thus, in making generalizations about structure–activity relations in regard to juvenile hormone activity, reliance upon one assay species as being representative of other insects in the same order is inadequate. The high ovicidal acitivity of compounds **1–3** and **5** on *E. varivestis* would not have been predicted by the results on either *T. molitor* or *L. decimlineata* pupae. Future studies are necessary to determine if the virtual inactivity of the polyalkoxy ethers (compounds **1–6**) on *L. decimlineata* was caused by a sensitivity spectrum at the active site(s) distinct from the other species tested or whether other differences in the compound–organism interactions were of primary importance. Both the topical and vapor assays indicated a general correlation between the ovicidal and juvenile hormone activity on *E. varivestis*, again with some notable exceptions. There was an overall coefficient of +0.72 (significant at the 5% level) between the lowest dose causing a 90% or greater reduction in egg hatch and a 90% or greater reduction in adult emergence of *E. varivestis*. This finding supports the conclusion of Slama (1971) from results on the bug, *Pyrrhocoris apterus*, that a general correlation exists between the ovicidal and morphogenetic potency of a given juvenile hormone compound. It remains to be determined to what extent the deviations from this generalization are caused by differences between the stages in penetrability or metabolic stability rather than by differences in potency at the site(s) of action.

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Uptake and Metabolism of DDT by Six Species of Marine Algae

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The uptake and metabolism of DDT by six species of marine phytoplankton was studied. Uptake of DDT by the various species increased linearly with an increasing concentration of DDT but nonlinearly with an increasing concentration of cells. The species with the higher numbers of cells per unit of mass took up greater amounts of DDT per unit weight than species with lower numbers of cells. All species concentrated DDT to levels many times higher than the original concentration in the medium. DDT was accumulated by the six species in the following order: *Skeletonema costatum* > *Cyclotella nana* > *Isochrysis galbana* > *Olisthodiscus luteus* > *Amphidinium carteri* > *Tetraselmis chuii*. All of the species converted small amounts of DDT to DDE. After 24 days of treatment, the amount of DDE produced by different species ranged from 0.03 to 12% of the total DDT in the cells. Maximum conversion of DDT to DDE was observed in cultures of *Tetraselmis*.

In recent years there has been a great deal of concern about the effects of organochlorine pesticides on the environment. Biological magnification of chlorinated hydrocarbon pesticides like 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane (DDT) has been well documented and is of great concern. Since algae represent the first link in the pelagic food chain, it becomes important to examine the interaction between DDT and algae. It has been shown that some species of algae possess a marked capacity to concentrate DDT from the surrounding medium (Gregory *et al.*, 1969; Kiel and Priester, 1969; Sodergren, 1968; Vance and Drummond, 1969; Ware *et al.*, 1968). The degree of DDT accumulation varied with the concentration of the pesticide in the medium and with the algal species. Following an exposure of algae to a medium containing 0.1–5 ppm of DDT, the concentration of the pesticide in the organisms was 200–1000 times that in the medium. On the other hand, Cox (1970a) reported a concentration factor of 25,000–80,000 in three species of marine algae which were exposed to DDT concentrations ranging from 1 to 3 parts per trillion (ppt). Working in the laboratory with *Chlorella*, Sodergren (1968) found uptake to be rapid (15 sec) and permanent. He concluded that the uptake was passive since killed cells absorbed DDT as effectively as live cells. DeKoning and Mortimer (1971) found that uptake by *Euglena* was very rapid and that DDT was held without being desorbed.

Knowledge concerning the accumulation and metabolism of DDT by marine algae is limited. The DDT accu-

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