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Lawrence G. Harshman
University of Nebraska - Lincoln, lharshman1@unl.edu

Ki-Duck Song
Kosin College and Korea Theological Seminary

Josephina Casas
RUBAM Institut de Química Avançada de Catalunya

A. Schuurmans
University of Nebraska - Lincoln

Eichii Kuwano
Kyushu University

See next page for additional authors

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Authors

Lawrence G. Harshman, Ki-Duck Song, Josephina Casas, A. Schuurmans, Eichii Kuwano, Stephen D. Kachman, Lynn M. Riddiford, and Bruce D. Hammock

Bioassays of Compounds with Potential Juvenoid Activity on *Drosophila melanogaster*: Juvenile Hormone III, Bisepoxide Juvenile Hormone III, and Methyl Farnesoates

Lawrence G. Harshman,[1] Ki-Duck Song,[2] Josephina Casas,[3]

A. Schuurmans,[1] Eichii Kuwano,[4] Stephen D. Kachman,[5]

Lynn M. Riddiford,[6] and Bruce D. Hammock[7]

1. School of Biological Sciences, University of Nebraska–Lincoln, Lincoln, Nebraska 68588, United States
2. Department of Biology, Kosin College and Korea Theological Seminary, Pusan, Republic of Korea
3. RUBAM Institut de Química Avançada de Catalunya, CSIC, Jordi Girona 18, 08034 Barcelona, Spain
4. Department of Agricultural Chemistry, Kyushu University, Fukuoka, Japan
5. Department of Statistics, University of Nebraska–Lincoln, Lincoln, NE 68583, United States
6. Department of Biology, University of Washington, Seattle, Washington 98195-1800, United States
7. Department of Entomology, University of California, Davis, California, United States

Corresponding author – Bruce D. Hammock, telephone +1-530-752-7519, email bdhammock@ucdavis.edu

Note: Present address of Lynn M. Riddiford is Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA 20147, United States.

Abstract
Metabolites of the 6,7,10,11 bisepoxide juvenile hormone III (JHB$_3$), and other potential juvenoids, were tested for juvenile hormone activity using early instar or early stage pupae of *Drosophila melanogaster*. Importantly, methyl farnesoates were tested as they might have JH-like activity on Dipteran juveniles. Larvae were exposed to compounds in medium, or the compounds were applied to white

puparia. In the assays employed in the present study, there was no indication for JH activity associated with the metabolites of JHB₃. The activity of methyl farnesoate (MF) was higher than that of JH III and far greater than bisepoxide JH III. As opposed to the two endogenous juvenile hormones, methyl farnesoate has weak activity in the white puparial bioassay. When fluorinated forms of methyl farnesoate, which is unlikely to be converted to JH, were applied to *Drosophila* medium to which fly eggs were introduced, there was a high degree of larval mortality, but no evidence of subsequent mortality at the pupal stage. One possible explanation for the results is that methyl farnesoate is active as a hormone in larval stages, but has little activity at the pupal stage where only juvenile hormone has a major effect.

Keywords: bisepoxide juvenile hormone, juvenile hormone, methyl farnesoate, *Drosophila*, bioassay

1. Introduction

The identification of the basis of all the sources of JH activity in *Drosophila melanogaster* has proven to be a difficult problem. The titers of JH III are relatively low in this insect (Sliter et al., 1987; Bownes and Rembold, 1987). Low levels of juvenile hormone are present in all larval stages. JH III is undetectable at mid-pupal stages but rises sharply at the time of eclosion in both sexes. Another issue is the role of JH in larval *D. melanogaster*. Ashburner (1970) described atypical effects of topical administration of juvenile hormone to third instar (last instar) *D. melanogaster* larvae of the wild type Canton S strain. The observed effects consisted of perturbations of bristle morphology but not retention of juvenile characteristics in adults, nor blockage of pupation, as would be expected from such an experiment. To further test the effect of JH on metamorphosis of *D. melanogaster*, Riddiford and Ashburner (1991) continuously exposed Canton S larvae to a JH analog in the food medium and the results were quite similar. There was very little suppression of pupariation in spite of the fact that high concentrations of potent analogs were used in the study. However, there was evidence that exposure of early stage larvae to high concentrations caused a failure to differentiate adult structures. Administration of the analogs to white puparia resulted in a concentration-dependent suppression of eclosion and of normal adult development of the abdomen as previously observed by Ashburner (1970) and Postlethwait (1974).

Early on it was assumed that the JH of *D. melanogaster* was JH III, but Richard et al. (1989a) documented that the juvenile hormone bisepoxide III (JHB₃) is the predominant product of an *in vitro* JH biosynthesis assay. As a control, labeled JH III was included in the incubation medium and very little was converted to JHB₃. Treatment of white puparia with test compounds, and subsequent determination of eclosion failure, is a standard dipteran JH bioassay (Postlethwait, 1974). Using this assay, the activity of JHB₃ on *D. melanogaster* was observed to be about 10% of that of JH III. JHB₃ is synthesized by higher Diptera, but not by mosquitoes, and it has been shown to have JH activity in a range of bioassays (Richard et al., 1989a, 1990). Casas et al. (1991) demonstrated that homogenates of *D. melanogaster* convert JHB₃ *in vitro* to the epoxydiol, *cis-* and *trans-*tetrahydrofuran diols and tetraol metabolites. The question arose, as to whether these compounds have juvenile hormone activity.

    The goal of the present study was to test metabolites of JHB$_3$ and methyl farnesoates for JH activity using the standard white puparial assay and an assay based on continuous exposure of larvae. Generally speaking, it is difficult to extrapolate from the bioassays to *in vivo* activities. Nevertheless, it is worthwhile to test the hormonal activity of compounds of interest with the understanding that positive results would motivate further research. A range of other relevant compounds was employed in the present study for comparison.

2. Methods

2.1. Test compounds

Some of the compounds assayed were provided by commercial sources and others were donated. S-methoprene was obtained as a gift from Zoecon Corporation and S-31183 (pyriproxifen) was provided by Sumitomo Chemical Company. In the present study, these potent JH analogs served as positive controls for JH activity in the assays on *D. melanogaster*. Farnesol (2E, 6E) was purchased from Sigma Chemical Company ("mixed isomers, approximately 90%"). The tetraol metabolite of JHB$_3$ (Messeguer et al., 1991) was derived enzymatically from incubation of racemic bisepoxide juvenile hormone III with cytosolic mammalian epoxide hydrolase (Casas et al., 1991) and thus consists of a diasteromeric mixture. Mono-fluorinated methyl farnesoate, methyl (2E, 6E) 10-fluorofarnesoate, and trifluorinated methyl farnesoate, methyl 12,12,12-trifluoromethylfarnesoate, were kindly provided by A. Messeguer (Camps et al., 1988a,b). The trifluoromethyl farnesoate was a mixture of (9:1) of methyl (2E, 6E, 10Z)- and (2E, 6E, 10E)-12,12,12-trifluorofarnesoate. The halogenated forms of methyl farnesoate (2E, 6E) are very refractory to epoxidation, which is required to form juvenile hormone III.

    The remainder of the test compounds were synthesized for this study. All of the compounds tested are shown in figure 1. The described NMR spectra (90 MHz) were determined with a Varian EM-390 spectrophotometer with Me$_4$Si as an internal standard (CDCL$_3$ was used a solvent). Gas chromatographic analysis was performed on a Hewlett Packard (Model 5890) equipped with a hydrogen flame ionization detector.
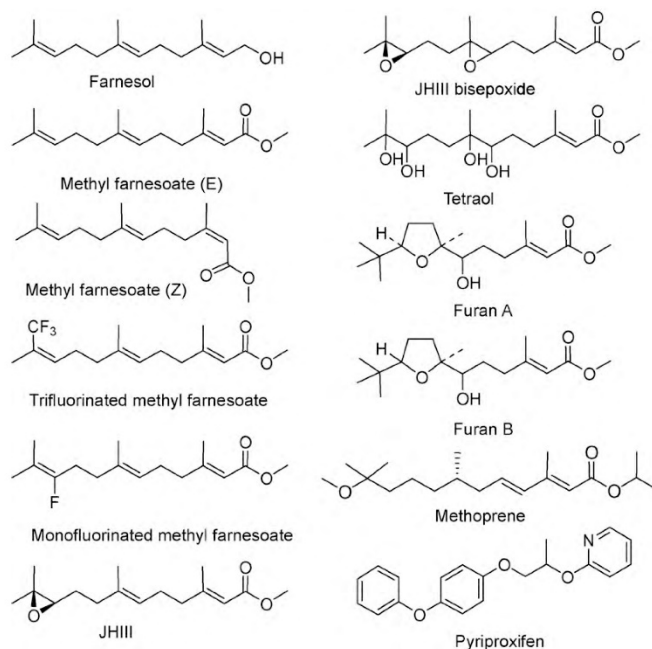
Figure 1. Chemical structures of tested compounds.

2.2. Synthesis of methyl farnesoates

The synthesis of methyl (2E, 6E) farnesoate (methyl farnesoate) was initiated by 8.2 g of trimethyl phosphonoacetate to a suspension of 1.8 g of NaH (60% in oil) in 40 µl of dimethylformamide under N_2 atmosphere at 25–30°C. After stirring for 1 h at room temperature, a solution of 8.7 g of (E)-geranylacetone (Fluka, 99.8% purity by GC) in 10 ml of dimethylformamide was added dropwise at 0–5°C. The mixture was stirred for 20 h at room temperature and was then poured into water. The product was extracted with ether and the ether solution was washed with brine and dried over Na_2SO_4. After removal of the solvent, the residue was chromatographed on silica gel and eluted with hexane-ether (100:1) and (50:1). The isomers of methyl farnesoate were identified by comparison of the ^{1}H NMR data reported by Burrell et al. (1966).

Methyl (2Z, 6E)-farnesoate (1) was obtained from the hexane-ether (100:1) eluate. Concentration of the eluate under reduced pressure afforded 0.9 g (8%) of (1). NMR δ: 1.60 (6H, s), 1.66 (3H, s), 1.88 (3H, d, *J* = 1.5 Hz), 1.7–2.4 (6H, m), 2.5–2.8 (2H, m), 3.67 (3H, s), 4.9–5.3 (2H, m), 5.70 (1H, broad s). In tables 1 and 3, this compound is referred to as methyl farnesoate (Z).

Methyl (2E, 6E)-farnesoate (2) was eluted after (1) with hexane-ether (50:1). After concentration of the eluate under reduced pressure, the residue was chromatographed on silica gel and eluted with hexane-ether (100:1) and (50:1). Concentration of the hexane-ether (50:1) eluate under reduced pressure afforded 2.8 g (25%) of (2). GC analysis showed compound (2) was contaminated with 1.3% of compound 1. NMR δ: 1.60 (6H, s), 1.70 (3H, s), 1.8–2.4 (8H, m), 2.18 (3H, d, *J* = 1.5 Hz), 3.70 (3H, s), 4.9–5.2 (2H, m), 5.70 (1H, broad s). In tables 1 and 3, this compound is referred to as methyl farnesoate (E).

2.3. Synthesis of (+)-methyl 10,11-epoxy-(2E, 6E)-farnesoate (JH III)

To a cooled (ice bath) solution of 7.3 g of (2) in 80 ml tetrahydrofuran and 25 ml of water was added drop wise (over a 30 min. period) a solution of 5.8 g of N-bromosuccinimide in 10 ml of tetrahydrofuran. After a further 4 h stirring at 0–5°C, the solution was concentrated under reduced pressure and the product was extracted with ether. The ether solution was washed with 5% $NaHCO_3$ solution and brine and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure afforded 9.0 g of crude bromohydrin. To a solution of this crude bromohydrin I 70 ml of dry methanol was added 16.1 g of anhydrous K_2CO_3 under an atmosphere of N_2. After stirring vigorously for 30 min at room temperature, the solid was filtered off and washed with methanol. After concentration of the combined filtrates, the residue was dissolved in ether and the ether solution was washed with brine and dried over Na_2SO_4. After removal of the solvent, the residue was chromatographed on silica gel and eluted with hexane-ether (30:1). Concentration of the eluate under reduced pressure afforded 4.4 g (56.6% of JH III. NMR δ: 1.26 (3H, s), 1.63 (3H, s), 1.63 (3H, s), 1.5–1.8 (4H, m), 2.0–2.2 (4H, ml), 2.18 (3H, d, *J* = 1.5 Hz), 2.72 (1H, t, *J* = 6 Hz) 3.70 (3H, s) 5.0–5.3 (1H, m), 5.73 (1H, broad s). GC analysis showed that the purity of JH III was 96.4%. The product of synthesis was a racemic mixture of 10R- and 10S-JH III which was identified by comparison of the ^{1}H NMR data with those reported by Van Tamelen and McCormick (1970).

2.4. Synthesis of methyl 6,7,10,11-diepoxy-(2E)-farnesoate (bisepoxide JH III)

To cooled (ice bath) solution of 2.9 g of JH III in 40 ml of dichloromethane was added 4.8 g of 85% m-chloroperbenzoic acid. After the stirring for 1 h at 0–5°C and then for 20 h at room temperature, dichloromethane solution was washed with 5% $NaHCO_3$ solution and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was chromatographed on silica gel and eluted with hexane–ethyl acetate (5:1) and (2:1). Concentration of the hexane–ethyl acetate (2:1) eluate under reduced pressure afforded 2.3 g (70.3%) of (3). NMR δ: 1.26 (6H, s), 1.30 (3H, s) 1.5–1.9 (6H, m), 2.20 (3H, s), 2.1–2.5 (2H, m), 2.6–2.9 (2H, m), 3.70 (3H, s), 5.76 (1H, broad s). The product of synthesis was obtained as a diastereomeric mixture which was identified by comparison to the ^{1}H NMR data reported by Ichinose et al. (1978).

2.5. Synthesis of cyclic compounds from methyl 6,7,10,11-diepoxy-(2E)-farnesoate (tetrahydrofuran diols)

To a solution of 1.8 g of bisepoxide in 25 ml of tetrahydrofuran was added 10 ml of 2.5% $HClO_4$ solution at room temperature. After stirring for 4 h at the same temperature, the product was extracted with ether. The ether solution was washed with 5% $NaHCO_3$ and brine and dried over Na_2SO_4. TLC of the products using hexane-ethyl acetate (1:1) showed two distinct spots (A: Rf = 0.27, B: Rf = 0.22). These two compounds were separated by flash chromatography on silica gel using hexane-ethyl acetate (2:1) and (1:1). Compound A was identified as the *cis*-isomer, and compound B as the *trans*-isomer, by comparison of the ^{3}H NMR data with those reported by Messeguer et al. (1991).

Compound A was obtained from the hexane-ethyl acetate (2:1) eluate. Yield 0.21 g. NMR δ: 1.16 (6H, s), 1.28 (3H, s), 1.4–2.7 (9H, m, (1H) D20 exchangeable), 2.20 (3H, s), 3.40

(1H, broad s, D₂O exchangeable), 3.58 (IH, dd, Ja = 3 Hz, Jb = 9 Hz), 3.70 (3H, s), 3.83 (1H, t, *J* = 6 Hz), 5.75 (1H, broad S).

Compound B was obtained from hexane-ethyl acetate (1:1) eluate. Yield 0.31 g. NMR *δ*: 1.13 (6H, s), 1.20 (3H, s), 1.4–2.6 (9H, m, (1H) D20 exchangeable), 2.20 (3H, s), 2.63 (1H, broad s, D20 exchangeable), 3.5–3.7 (1H, m), 3.72 (3H, s), 3.7–4.0 (1H, overlapped), 5.80 (1H, broad s).

2.6. Bioassays

The Canton S strain of *D. melanogaster* was used for all assays. This stock was maintained by placing 10 females and 10 males in an 8 dram vial containing 10 ml of standard *Drosophila* medium (cornmeal, yeast, sugar, agar) for 2 days at 22°C. After 2 days these flies were transferred to a new set of vials for egg laying. The flies used for stock maintenance were 4–16 days old.

In one set of bioassays, larvae were continuously exposed to the test compounds which were applied to the surface of the diet beforehand. Any one dose of a test compound was dissolved in 100 μl of ethanol (EtOH). This solution was added to the surface of *Drosophila* medium in an 8 dram vial. The solution was swirled across the surface for even distribution and allowed to dry for 24 h. EtOH alone was added to vials as a control and some vials received no solvent to assess any effect of EtOH on larval survival. All vials were subjected to an overnight drying period regardless of whether they received solvent. The purpose was to be sure there was little solvent in the medium at the start of the bioassays.

A standard number of eggs were transferred to the bioassay vials. As a source of eggs, approximately 100 inseminated females were added to "cut bottles" for overnight egg laying. The top half of the bottle was removed and 40 eggs were transferred from the surface of the bottle medium to the surface of the vials using a flat toothpick. The vials with eggs were held at room temperature (approximately 22°C) until all adults eclosed. The number of survivors to the pupariation stage, and the number to the adult stage, was determined.

The white puparial assay, as described in Riddiford and Ashburner (1991), was used as a standard JH bioassay in the present study. For this assay, white puparia were collected from the inside glass wall of culture vials. This early stage was identified when the larvae first become immobile, rounded, and have everted their spiracles. The white puparia were placed ventral side down on one side of double-stick tape affixed to a microscope slide. The test compounds were administered in 0.2 μl of acetone with a repeating Hamilton syringe. The compounds, or the same volume of acetone as a control, were administered within 15 min after pupariation. Slides with treated and control puparia were placed in separate Petri plates with a moist Kimwipe to maintain humidity. The plates were placed at 25°C until all adult eclosion was completed. The number eclosed was tabulated and the uneclosed puparia were scored as described in Riddiford and Ashburner (1991). One set of these assays was conducted at the University of Washington at Seattle (table 3) and another set was conducted at the University of Nebraska–Lincoln (table 4).

3. Results

Table 1 presents the bioassay results based on continuous exposure of larvae. Compared to vials with nothing added, the survival to the pupariation and adult stages was not significantly affected by the addition of 100 µl of EtOH. To facilitate comparison of all the compounds tested, the same doses were used for all compounds except the potent juvenile hormone analogs (table 1). There is a five-fold difference between each dose in a dose series.

Table 1. Exposure of larvae of *Drosophila melanogaster* to compounds applied in 100 µl of ethanol (EtOH) onto the surface of fresh fly medium

Treatment	Number of puparia ± SD	Number of adults ± SD	Number of vials
Nothing added	36.2 (3.4)	36.0 (3.4)	40
EtOH only	35.6 (3.8)	35.4 (3.6)	36
Farnesol			
1×10^{-7} mol	35.4 (1.5)	35.4 (1.5)	5
5×10^{-7} mol	34.8 (5.6)	34.5 (6.1)	6
2.5×10^{-6} mol	36.2 (3.5)	35.2 (2.3)	6
1.25×10^{-5} mol	0.2 (0.4)	0.2 (0.4)	6
Methyl farnesoate (E)			
1×10^{-7} mol	35.2 (4.8)	35.0 (5.0)	9
5×10^{-7} mol	26.4 (6.5)	22.9 (7.2)	9
2.5×10^{-6} mol	14.6 (9.1)	3.1 (2.5)	8
1.25×10^{-5} mol	2.4 (5.7)	0.2 (0.6)	9
Methyl farnesoate (Z)			
1×10^{-7} mol	32.0 (7.7)	31.8 (7.5)	6
5×10^{-7} mol	36.0 (2.1)	35.6 (2.2)	6
2.5×10^{-6} mol	32.0 (9.3)	31.0 (9.3)	6
1.25×10^{-5} mol	2.0 (2.9)	2.0 (2.9)	6
JH III			
1×10^{-7} mol	34.6 (5.8)	34.6 (5.8)	9
5×10^{-7} mol	30.2 (2.6)	28.6 (6.1)	8
2.5×10^{-6} mol	29.6 (3.9)	23.0 (5.8)	8
1.25×10^{-5} mol	12.0 (6.1)	4.7 (3.1)	9
JH III bisepoxide			
1×10^{-7} mol	35.4 (6.1)	35.0 (6.5)	8
5×10^{-7} mol	38.0 (3.5)	37.7 (3.5)	7
2.5×10^{-6} mol	31.0 (14.5)	27.0 (12.6)	5
1.25×10^{-5} mol	38.9 (2.2)	13.1 (6.0)	7
Tetrahydrofuran diol (A)			
1×10^{-7} mol	36.7 (1.5)	36.0 (1.0)	3
5×10^{-7} mol	38.3 (1.1)	38.0 (1.0)	3
2.5×10^{-6} mol	38.0 (1.0)	38.0 (1.0)	3
1.25×10^{-5} mol	38.3 (1.5)	38.0 (1.7)	3

Tetrahydrofuran diol (B)

1×10^{-7} mol	34.7 (1.5)	34.7 (1.5)	3
5×10^{-7} mol	36.0 (1.7)	35.7 (1.5)	3
2.5×10^{-6} mol	32.0 (1.7)	32.0 (1.7)	3
1.25×10^{-5} mol	33.7 (2.1)	31.3 (4.0)	3

Methoprene

4×10^{-9} mol	31.0 (3.0)	28.7 (2.1)	3
2×10^{-8} mol	31.7 (3.2)	0.3 (0.6)	3
1×10^{-7} mol	27.7 (3.8)	0.0 (0.0)	3
5×10^{-7} mol	24.0 (1.7)	0.0 (0.0)	3

Pyriproxyfen

3.2×10^{-11} mol	32.3 (3.8)	31.7 (3.0)	3
1.6×10^{-10} mol	33.3 (3.5)	29.7 (3.0)	3
$8 \times 10^{-}$ mol	29.3 (5.5)	3.7 (2.1)	3
4×10^{-9} mol	32.0 (3.0)	0.0 (0.0)	3

Forty eggs were added to each vial and the mean survival (± standard deviation, SD) to the pupariation and adult stages are shown. Furan A refers to the *cis*-isomer of tetrahydrofuran diol metabolite of JHB$_3$ and furan B refers to the *trans*-isomer (Methods). The synthesis of the E (active) and Z (inactive) forms of methyl farnesoate are also described in the Methods section.

There was an overall effect of dose in the experiment for both larval survival and survival after pupariation formation ($p < 0.0001$) (tables 1 and 2). The compounds that showed a significant effect of dose on pupae are farnesol ($p < 0.0001$), JH III ($p < 0.0001$), JH3bisepoxide ($p < 0.0001$), both forms of methyl farnesoate (E and Z) ($p < 0.0001$), both florinated forms of methyl farnesoate ($p < 0.0001$), and methoprene ($p < 0.0039$). A similar set of compounds has a dose-dependent effect on survival after pupariation formation: farnesol ($p < 0.0001$), JH III ($p < 0.0001$), JH3bisepoxide ($p < 0.0001$), both forms of methyl farnesoate (E and Z) ($p < 0.0001$), both florinated forms of methyl farnesoate ($p < 0.0001$), methoprene ($p < 0.0039$), and pyrproxyfen ($p < 0.0001$). In general, there was evidence for a high degree of biological activity of the endogenous form of methyl farnesoate (MF E) and no activity of the JHB$_3$ metabolites.

Table 2. Mean survival (± standard deviation, SD) of *Drosophila melanogaster* to the pupariation and adult stages after larval exposure to mono-fluorinated methyl farnesoate (MFMF) and tri-fluorinated methyl farnesoate (TFMF) on medium

Treatment	Number of puparia ± SD	Number of adults ± SD	Number of vials
MFMF			
5×10^{-7} mol	31.8 (1.1)	31.8 (1.1)	6
2.5×10^{-6} mol	14.3 (13.6)	13.3 (12.8)	6
TFMF			
5×10^{-7} mol	31.8 (1.1)	31.4 (3.6)	6
2.5×10^{-6} mol	0.7 (1.0)	0.0 (0.0)	6

Forty eggs were added to each vial.

3.1. Biological activity of methyl farnesoates

The results of exposure to MF are as follows. For the biologically active isomer (E), there was dose-dependent larval mortality and a relatively high level of mortality following larval exposure to higher doses of the compound. The effect of exposure to the 2.5×10^{-6} mol of methyl farnesoate (E) was greater than that observed after exposure to the same level of JH III (table 1). In both cases, there is significant mortality to larvae and after puparial formation. Exposure to methyl farnesoate (Z) produced a similar pattern of mortality to that observed for farnesol which has a potentially toxic effect at the highest dose (table 1). Thus, the 2Z isomer was much less potent than the 2E isomer of methyl farnesoate.

JHB$_3$ caused little larval mortality but mortality after puparium formation at the highest doses (table 1). High pupal mortality with relatively little larval mortality was also observed for the two juvenile hormone analogs: methoprene and pyriproxifen (table 1).

The indication of significant JH activity associated with exposure to methyl farnesoate (E) (table 1) motivated an experiment to test the hypotheses that the biological activity of methyl farnesoate (E) resulted from the conversion of methyl farnesoate to juvenile hormone. The mono-fluorinated and trifluorinated synthetic derivatives of the biologically active form of methyl farnesoate are refractory to conversion to juvenile hormone. These compounds were applied to medium at the two intermediate doses tested for methyl farnesoate (E) and methyl farnesoate (Z). Only two doses were tested due to a limited abundance of each compound. The standard number of eggs (40) was added to the fly medium, and the number of surviving pupae and adults recorded (table 2). Marked larval mortality, but no significant mortality after pupariation formation was observed for the highest level of the mono-fluorinated compound. Exposure to the lower level of this compound resulted in a little larval mortality and no added pupal mortality. The trifluorinated compound resulted in the same pattern of mortality at the lower level, but the higher dose caused nearly 100% larval mortality.

In the case of exposure to farnesol, at the three lower doses there was no significant mortality after pupariation as indicated by the fact that the number of puparia and the number of adults were very similar. At the highest dose of farnesol, there was very low survival to pupariation formation.

3.2. White puparial assays

The results of the white puparial assays conducted in two laboratories are shown in tables 3 and 4 based on the results from each laboratory. The JHB$_3$ metabolites [furan A and furan B (table 3), tetraol (table 4)], did not prevent eclosion. The topical administration of methyl farnesoate (E) to white puparia was associated with eclosion failure, but the effect was not strong (tables 3 and 4). The administration of JHB$_3$ in doses ranging from 0.5 to 4 nmol resulted in a dose-dependent increase in eclosion failure (tables 3 and 4) and an increased incidence of abnormal abdominal bristles in adults (table 3).

Table 3. White puparial assay for a JH effect after exposure to methyl farnesoate (E), JH III bisepoxide, and the tetrahydrofuran diols (Furan A and Furan B).

Treatment	Number tested	Eclosed	Non-eclosed phenotypes		
			Normal pharate	Abnormal abdominal bristles	Pupa*
Methyl farnesoate (E)					
0.05 nmol	20	17 (85%)	1	0	2
0.14 nmol	19	12 (63%)	5	0	2
0.44 nmol	20	17 (85%)	0	0	
1.4 nmol	20	17 (85%)	3	0	0
4 nmol	20	19 (95%)	1	0	0
JH III bisepoxide					
1 nmol	19	7 (37%)	0	11	1
2 nmol	20	4 (20%)	0	16	0
4 nmol	10	1 (10%)	0	19	0
Tetrahydrofuran diol (A)					
10 nmol	20	20 (100%)	0	0	0
20 nmol	19	19 (100%)	0	0	0
Tetrahydrofuran diol (B)					
10 nmol	20	19 (95%)	1	0	0
20 nmol	20	19 (95%)	1	0	0
Control (acetone)	43	42 (98%)	1	0	0

* Death after head eversion but before eye pigmentation.

Table 4. White puparial assay for a JH effect after exposure to JHB_3, the tetraol metabolite of JHB_3, methyl farnesoate (E and Z), and methoprene

Treatment	Number tested	Number eclosed
JH III bisepoxide (JHB_3)		
0.25 nmol	30	26 (87%)
0.50 nmol	30	20 (67%)
1.00 nmol	30	9 (30%)
Tetraol metabolite (JHB_3)		
0.25 nmol	80	73 (91%)
0.50 nmol	80	75 (94%)
1.00 nmol	60	56 (93%)
Methyl farnesoate (E)		
0.25 nmol	30	26 (87%)
0.50 nmol	30	20 (67%)
1.00 nmol	30	28 (93%)
Methyl farnesoate (Z)		
0.25 nmol	30	24 (80%)
0.50 nmol	30	26 (87%)
1.00 nmol	30	24 (80%)
Methoprene		
5 pmol	40	34 (85%)
20 pmol	40	28 (93%)
50 pmol	40	22 (55%)
80 pmol	40	13 (32%)
Control (acetone)	360	338 (94%)

4. Discussion

In the present study, a focal outcome was the biological activity of methyl farnesoate which is active on larvae and pupae. This activity has implications for an endogenous JH-like role for this compound.

4.1. Activity of methyl farnesoates

The activity of the methyl farnesoates is intriguing (tables 1–4). Methyl farnesoate (E) appeared to have "JH activity" when larvae were exposed to the compound on medium. The effect is greater than that observed for larval exposure to the same amount of JH III. It kills larvae and has a residual mortality effect after pupariation formation (table 1). The effect of the JH analogs is that they act at the pupal stage but have low activity on larvae, which is not the same pattern as the active form of methyl farnesoate. The monofluorinated methyl farnesoate exhibited the same mortality effect on larvae as observed for methyl farnesoate (E) at 2.5×10^{-6} mol but had no residual effect on survival after pupariation

formation (tables 1 and 2). Exposure to the trifluorinated form of methyl farnesoate resulted in even greater larval mortality at 2.5×10^{-6} mol but no mortality beyond the larval stages. This implies that methyl farnesoate must be converted to JH III to be active at the pupal stage. The JH activity of methyl farnesoate (E) in the white puparial assay was much less than that of JHB_3 (tables 3 and 4). One hypothesis to account for these findings is that methyl farnesoate is active as a JH during larval life but not during the pupal stage. At the latter stage, it must be converted to JH III to be active. This is possible for MF (E) (table 1) but not for the fluorinated forms of methyl farnesoates, thus explaining their lack of effect on pupal survival (table 2). Perhaps, the JH activity of methyl farnesoate during the larval stages of *D. melanogaster* explains the low levels of JH III found in larvae (Bownes and Rembold, 1987; Sliter et al., 1987). Interestingly, Jones et al. (2010) found that feeding farnesol, but not JH III and/or methyl farnesoate, could rescue larval mortality in larvae expressing 3-hydroxy-3-methylglutaryl CoA reductase (HMGCR) RNAi in the corpora allata, which they suggest may indicate that the proper ratio of the farnesoid derivatives is necessary for normal larval development.

There is evidence that methyl farnesoate plays a juvenile hormone-like role in *D. melanogaster* and other higher Diptera (reviewed in Jones and Jones, 2007). The level of methyl farnesoate produced by the larval ring gland (Richard et al., 1989b) is higher than that produced by the corpora allata of adult females (Tu et al., 2005). Also, Ultraspiracle binds methyl farnesoate at nanomolar levels (150-fold higher affinity than for JH III) (Jones et al., 2006; Jones and Jones, 2007).

4.2. Activity of JHB$_3$ and metabolites

There was negligible mortality at the pre-pupal and pupal stages after exposure of larvae to the tetrahydrofuran diols (Furan A, Furan B, table 1). JHB_3 has been shown to interfere with normal abdominal bristle development (Richard et al., 1989b) as also indicated by the results of the present study (table 3). However, the *in vivo* role of JHB_3 is not clear. One reason for questioning the importance of JHB_3 is that its biological activity is relatively low in spite of the fact that its polarity is only slightly higher than JH III. The biological activity of JHB_3 is substantially lower than JH III in the white puparial assay, inhibition of ecdysteroid biosynthesis, stimulation of yolk protein production and interference with the rotation of male genitalia (Richard et al., 1989a,b, 1990). However, JHB_3 is relatively effective in breaking diapause by stimulating vitellogenesis (Saunders et al., 1990). In *Phormia regina*, JHB_3 is at least as effective as JH III in restoring oogenesis in allatectomized flies (Yin et al., 1995). In the present study, the high doses of JH III and JHB_3 have a similar effect on mortality after pupariation formation when larvae are exposed to the compounds applied to medium (table 1). Establishing the potency of JHB_3 thus may require testing in a broad range of bioassays. As another consideration, the relatively low activity of JHB_3 might be due to the fact that we tested a diastereometric mixture. In order to further investigate the activity of JHB_3, it would be informative to test natural enantiomers and test hormones in combination with each other. There is evidence that a blend of JHB_3, JH III, and methyl farnesoate is especially biologically active in *Phormia* adults (Yin et al., 1995).

In summary, there are basic science outcomes and an applied science suggestion from the results of the present study. Methyl farnesoate might be a significant source of juvenile

hormone activity in *D. melanogaster* juvenile stages. Thus, there is potential for an advance in understanding the endocrinology of this important model for genetics research. Also, methyl farnesoate appears to be active at the larval stage but not the pupal period tested. The applied science suggestion is based on the high level of mortality after exposure of larvae to methyl farnesoate. Specifically, there is the possibility that plants might be engineered to produce methyl farnesoate as an anti-dipteran, or other invertebrate, control agent. Methyl farnesoate does occur in some wild plant species. All plant make the main compounds in the terpene pathway. Plants are known to epoxidize double bonds. Lemon grass is known to make juvenile hormone III. It might be straightforward to synthesize methyl farnesoate in crop species based on expression of one, or a few, transgenically derived enzymes. It can block development of insect embryos and act to kill juvenile insects. Methyl fanesoate and juvenile hormone in plants has the potential to be a valuable invertebrate pest control agent.

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