Zoological Studies **33**(4): 237-245 (1994)

Zoological Studies

Review Article

Juvenile Hormone III Bisepoxide: New Member of the Insect Juvenile Hormone Family

Chih-Ming Yin

Department of Entomology, University of Massachusetts, Amherst, Massachusetts, 01003-2410, USA

CONTENTS

ABSTRACT

Chih-Ming Yin (1994) Juvenile hormone III bisepoxide: New member of the insect juvenile hormone family. *Zoological Studies* **33**(4): 237-245. The insect juvenile hormone family has recently been enlarged by the addition of its 6th member, juvenile hormone III bisepoxide (JHB_3). JHB_3 is first discovered as an unknown juvenoid biosynthesized, in vitro, by the adult corpus allatum (CA) of the female black blow fly, *Phormia regina* (Liu 1985, Liu et al. 1988). A similar substance is produced by the isolated larval ring gland of *Drosophila melanogaster*; its chemical structure is identified as methyl (2*E*,6*E*)-(10*R*,11*S*)-6,7;10,11-bisepoxy-3,7,11-trimethyl-2-dodecenoate or methyl-6,7;10,11-bisepoxyfarnesoate (Richard et al. 1989a). This substance is structurally identical to juvenile hormone III, except for the 6,7-epoxy group. Bioassays of synthetic JHB_3 demonstrate its biological activities in the regulation of development, metamorphosis and oögenesis in *Drosophila melanogaster* and *Phormia regina* (Richard et al. 1989a,b 1990, Yin et al. submitted). JHB_3 also has been found to be a product of either the ring glands or the CA in all the higher dipterans (i.e., cyclorrhaphous flies) studied so far. Its production by the mosquito male accessory gland and the tick synganglion, in vitro, has also been reported (Borovsky et al. 1994, Roe et al. 1993). The occurrence of JHB_3 calls for future research aiming at the discovery of JHB_0, JHB_1, and JHB_2. Its presence also makes clear that the conventional wisdom of thinking that all non-lepidopterans can produce only JH III is far from reality.

Key words: *Phormia*, Corpus allatum, Diptera, JH bisepoxide, JH biosynthesis.

INTRODUCTION

The interest in insect juvenile hormone (JH) begins with the studies of JH-producing glands: insect corpora allata (CA), which also have been called paired posterior visceral ganglia, ganglia allata, or corpora incertae by earlier investigators (see Cassier 1990). The endocrine nature of the

CA is first suggested by Nabert (1913) and first experimentally proven by Wigglesworth (1935). Since then the CA and the JH (i.e., the glandular secretion from the CA) have been amongst the most researched subject of insect sciences.

The CA arise early in embryonic development as a pair of ectodermal infoldings positioned at the mandibular or maxillary somites. Subsequently, the CA migrate dorso-mesially from their original sites. The degree of migration appears to reflect the evolutionary status of an insect (Cassier 1990). Thus, in Thysanura, the CA are positioned at ventral sites. In Odonata, the CA are positioned more dorsally but still occupy relatively ventral positions. In Hemiptera, the CA are found immediately ventrad to the aorta. In Holometabola, the CA are usually located slightly ventrad to the aorta on the sides of esophagus. Finally, in higher dipterans, the CA are located dorsad to the aorta (Dai et al. 1987). According to Cazal (1948), the CA of various insects can be categorized into five morphologic types: the lateralized, the distally lateralized, the semicentralized, the centralized, and the annular types.

Histological observations lead to the identification of four types: the pseudolymphoid, the small-cell, the macrocell, and the vesicular types (Cazal 1948). In a lepidopteran, *Mamestra configurata* (Noctuidae), Luo and Bodnaryk (1987) are able to identify a capsular type common to other lepidopterans and a special isolated-cell type of CA in adults. This isolated-cell type of CA is also present in the adult Loreyi leafworm, *Leucania loreyi*, but not in larvae whose CA remain the capsular type (Kou et al. submitted).

Ultrastructural studies have shown that each corpus allatum cell is surrounded by a plasma membrane that delineates even the cells with the most complex forms. The plasma membrane, which makes contact with the basal lamina, projects numerous digitations. These digitations occur even when glandular crypts are formed by invaginations of the plasma membrane (Odhiambo 1966, Papillon et al. 1976). The plasma membranes of an active gland are, in general, less convoluted and their glandular crypts, if present, are far fewer in number. In addition to being held by the basal lamina and the interdigitation of the plasma membrane projections of the adjacent cells, hemidesmosomes, desmosomes (of the septate, zonula, and macula adherens types) as well as gap junctions are all found to aid to cellullar cohesion of the CA. The nucleus is usually small and spherical (or oval) in shape in cells of either the pseudolymphoid or the microcellular CA type, whereas nucleus is large and lobed (or branched, or star-shaped) in the macrocellular CA type. The numbers and sizes of nucleoli are of special interest because they apparently indicate the level of cellullar activity. In inactive glands, the nucleoli are large and compact; in active glands, they are fragmented or annular in configuration (Baehr et al. 1973, Panov and Bassurmanova 1970). Numbers as well as morphology of mitochondria can also be used to assess the CA activity. When inactive, the numbers of mitochondria are relatively fewer, and their shape is mostly globular or rod-like. When active, numbers and sizes increase considerably and shape is changed to dumbbell, cup, or ring-formed. Mitochondria of an active cell also tend to show signs of division. According to the presence and absence of smooth and rough endoplasmic reticula, Cassier (1990) has separated insect CA into five groups. However, these groupings do not coincide with the taxonomic Orders of the Insecta. Thus CAs of hemipterans are assigned to three of the five groups. By studying the abundance and the morphology of the smooth endoplasmic reticulum, rough endoplasmic reticulum or both, one can estimate the CA activity.

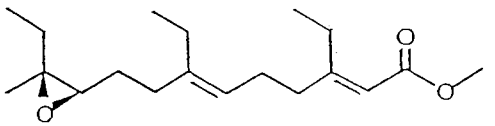
Despite the diversity in CA morphology, histology and ultrastructure, six different JHs have been identified so far (Fig. 1). All six are sesquiterpenoids, with five possessing a 10,11-epoxy group and one possessing a 6,7;10,11-bisepoxy structure. According to the number of carbon atoms in each hormone, these JHs have been named JH I (C_{18} JH), JH II (C_{17} JH), JH III (C_{16} JH), JH 0 (C_{19} JH), iso-JH 0 (4-methyl JH I, C_{19} JH), or JH III bisepoxide (JHB_3, C_{16} JH).

DISCOVERY AND IDENTIFICATION OF JUVENILE HORMONES WITH ONE EPOXY GROUP

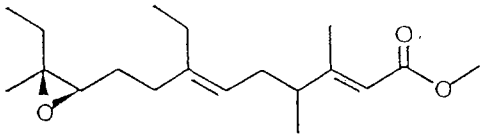
Comprehensive reviews are available on the discovery and identification of the JHs with a single epoxy group (Trost 1972, Dahm et al. 1976, Schooley and Baker 1985, Baker 1990). Only a brief account of these JHs will be given here.

A. Juvenile hormone I

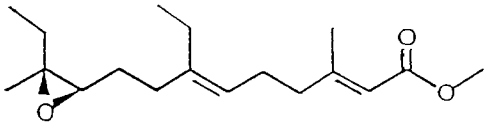
The first rich source of natural JH (i.e., the abdomens of male cecropia moths, *Hyalophora cecropia*, has been discovered by Williams (1956). Using this source, Williams and Law (1965) purify



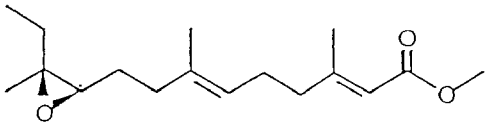
Juvenile hormone 0 (JH 0)



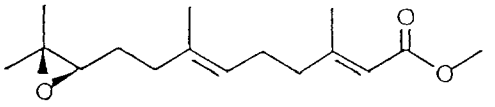
Iso-JH 0 or 4-methyl-JH I



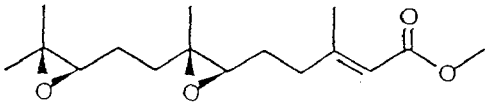
JH I



JH II



JH III



JH III bisepoxide or JHB_3

Fig. 1. Structure of insect juvenile hormones.

a substance and proposed its structure as methyl 9,10-epoxyhexadecanoate. However, synthetic isomers of this substance failed to show any biological activity. This same source also leads to the purification of a compound with high JH activity (Röller and Bjerke 1965), and to the identification of JH I (Fig. 1). Mass spectrometry indicated that this compound has a $C_{18}H_{30}O_3$ molecular formula. Elaborated chemical analyses including catalytic hydrogenation, mass spectrum of a model substance, and NMR data established its structure as methyl (2*E*,6*E*)-(10*R*-11*S*)-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate (Röller et al. 1967).

B. Juvenile hormone II

A year later, a second JH, JH II [methyl (2*E*,6*E*)-(10*R*-11*S*)-10,11-epoxy-3,7,11-trimethyl-2,6-tridecadienoate] (Fig. 1), is identified as a minor active substance from the same *Hyalophora cecropia* source using spectral techniques similar to those described above (Meyer et al. 1968 1970). The ratio between JH I and JH II varies in each preparation from 4:1 to 10:1. Synthetic JH II also shows good juvenile hormone activity.

C. Juvenile hormone III

JH III [methyl (2*E*,6*E*)-(10*R*-11*S*)-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate] (Fig. 1), the third JH, is first identified from several insects including *Schistocerca vaga, Manduca sexta*, and *Tenebrio molitor* (Judy et al. 1973a,b 1975), and *Periplaneta americana* (Müller et al. 1974). Judy et al. (1973a) are the first to chemically identify JH III while developing an in vitro technique to incubate corpus cardiacum-corpus allatum complexes from adult female *Manduca sexta* using Grace's medium supplemented with *L*-[methyl-^{14}C] methionine. They harvested some ethyl acetate-extractable, labeled substances that share the same R_f as cecropia JHs. GC analysis of pooled samples revealed two materials. One substance is identified as JH II, and the other as JH III because the latter shows exactly the same characteristics as a synthetic C_{16} JH on GC. Three new records, e.g., in vitro incubation of CA, production of JHs in vitro, and identification of JH III are made in this one study.

D. Juvenile hormone 0 and iso-juvenile hormone 0

JH 0, [methyl (2*E*,6*E*)-(10*R*-11*S*)-10,11-epoxy-3,7-diethyl-11-methyl-2,6-tridecadienoate], the fourth JH, is identified from *Manduca sexta* eggs 6 to 29 hr before hatching (Bergot et al. 1980). It is the first JH identified from insect eggs. About 200 ng of the trihomosesquiterpenoid JH 0 is used for its structure elucidation. Catalytic hydrogenation yields a tetrahydro derivative which is identical to the product obtained from a similar catalytic hydrogenation of a sample of synthetic 10*R*-11*S*-JH 0. Comparative mass-spectrometry analysis of the tetrahydro derivative of JH III and JH 0 strongly suggests

of an extra methylene at the C-3 of the latter. Capillary GC of natural JH 0 shows its retention time identical to that of synthetic (2*E*,6*E*,10*R*)-JH 0 but different to that of synthetic (2*E*,6*E*,10*S*)-JH 0.

A year later, a second C-19 JH is identified as iso-JH 0 (or 4-methyl JH I) from both wild-type and black-mutant *Manduca sexta* embryos (Bergot et al. 1981). GC, and MS (including both electron impact and chemical ionization) analyses of iso-JH 0 derivative (d_3-methoxyhydrin) and comparisons to 4-methyl JH I as well as synthetic model compounds [tetrahydroepoxymethyl ester and (2*E*,6*E*,10*R*-11*S*)-4-methyl JH I] have confirmed the 4-methyl substitution in the natural iso-JH 0.

DISCOVERY AND IDENTIFICATION OF JUVENILE HORMONE WITH TWO EPOXY GROUPS

For many years, no new JH has been found until 1989 when Richard et al. (1989a) report the identification of methyl-6,7; 10,11-bisepoxyfarnesoate or juvenile hormone III bisepoxide (JHB_3) as a product found in the medium when larval ring glands of *Drosophila melanogaster* are incubated in vitro. The clue leading to the identification of JHB_3 has been discovered a few years ago in a thesis on the biosynthesis of JH in vitro by the CA of adult female *Phormia regina* (Liu 1985). Now, JHB_3 has been identified from several higher and lower dipterans and in the American dog tick, *Dermacentor variabilis*.

A. *Phormia regina*

JH biosynthesis has been studied in *Phormia regina*, with respect to dietary regulated endocrine activities, using a radiochemical assay in vitro (Liu 1985, Liu et al. 1988, Zou et al. 1989, Yin et al. 1989 1993 1994, Yin and Stoffolano 1990 1994). As early as 1985, evidence emerges from *Phomira regina* (i.e., a dipteran), contrary to the general believe at that time, that JH III is not the predominant radiolabeled juvenoid produced by the CA in vitro. An unknown substance can contain more than 10 times the radioactivity of the JH III (Fig. 2). This predominant unknown is more polar than JH III as judged from its mobility on TLC and HPLC. It is produced by male and female adult CA alike in all the stages examined (Zou et al. 1989, Yin et al. submitted).

A substance similar in polarity to the above mentioned unknown has been found in the medium

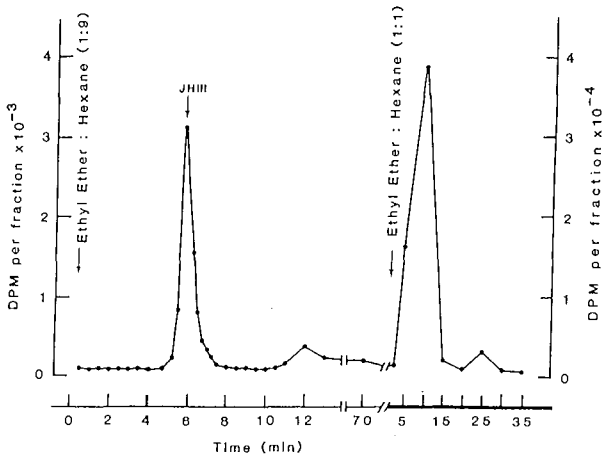


Fig. 2. Radioactivity profile of biosynthates from CA in vitro of *Phormia regina* separated by HPLC (Varian 5000 instrument, Lichrosorb Diol Column of 10 um, 250 mm X 4 mm). JH III was eluted around 6 min by ethyl ether:hexane (1:9) at 1 ml/min. JHB_3 was eluted around 10 min after the solvent was changed to ethyl ether:hexane (1:1) also at 1 ml/min (Yin, Stoffolano and Zou, unpublished).

after *Drosophila melanogaster* larval ring glands are incubated in vitro. This substance has been chemically identified as methyl-6,7; 10,11-bisepoxyfarnesoate or juvenile hormone III bisepoxide (JHB_3) by Richard et al. (1989a). Richard et al. (1989a) suggest that the unknown produced by the CA of adult *Phormia regina* may also be JHB_3.

Our latest results (Yin et al. submitted) show that the unknown in *Phormia regina* is indeed JHB_3. It is the major product in vitro by the CA of both male and female adults. We also show that JH III and methyl farnesoate (MF, a JH III precursor) are also released into the incubation medium (Fig. 3). The quality and quantity of juvenoids produced by the CA in vitro are dependent on the sex and the dietary status of the fly. The CA from sugar-fed flies always produces much less total juvenoids than their liver-fed counterparts regardless of the sex. On the other hand, regardless of dietary status, the CA of male flies produces relatively more JHB_3 than females do (Fig. 3). Bioassay of synthetic JHB_3 confirms its biological activity because this juvenoid can restore oögenesis in allatectomized, liver-fed females (Table 1). Biological activity of JHB_3 in males remains unconfirmed, although our preliminary observations suggest that it may regulate mating behavior.

We are presently synthesizing quantities of JHB_3 and MF using established procedures (Yin et al. submitted) to facilitate further biological testing

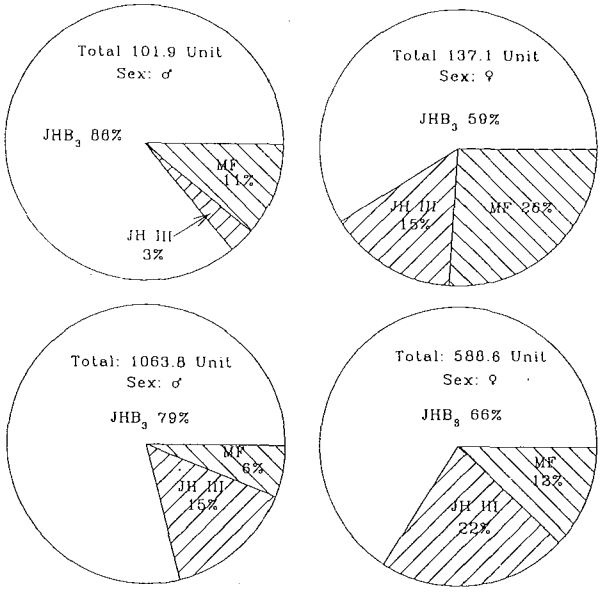


Fig. 3. Pie charts showing the percentage compositions of radioactively labeled JHB_3, JH III, and methyl farnesoate produced by the adult CA of male and female *Phormia regina* in vitro. Results from sugar-fed and liver-fed flies are diagramed as top and bottom pies, respectively. CA of liver-fed flies produced much more total juvenoids than that of sugar-fed flies. CA of male flies produced relatively more JHB_3 than females regardless of the dietary status. Each unit = 1 fmol/hr/pair of CA.

Table 1. Oögenesis restored in allatectomized, liver-fed flies by topical applications of JHB_3

Dosage	Fillicle stages (No. of flies in stages) 1-3	4-6	7-8	9-10	% Mature eggs
50 ug, twice	0	5	7	7	37
25 ug, twice	0	5	4	3	25
Acetone, (2 ul, twice)	7	6	1	0	0
Sham	0	0	4	16	80

Sham = sham operated, liver-fed flies which were not treated with JHB_3 or acetone. Descriptive statistics were omitted for brevity.

of JHB_3 in males and females. We would also like to test some juvenoid blends (i.e., mixtures of JHB_3, JH III, and MF in different proportions that approximate to the natural proportions present in the medium when CA are incubated). These blends will be tested for their ability to restore oögenesis in allatectomized females and to initiate mating behavior in allatectomized males. We hypothesize that certain juvenoid blends may be more effective in exerting their function than any of the above three individual juvenoids applied alone.

B. *Drosophila melanogaster*

As mentioned above, JHB_3 is first identified in *Drosophila melanogaster* by Richard et al. (1989a). The same authors also report the presence of a JHB_3-like substance in *Musca domestica, Sarcophaga bullata*, and *Calliphora vicina*, because when larval ring glands from these three cyclorrhaphous dipterans are incubated, in vitro, chromatographic profiles of JHB_3 are observed.

In *Drosophila melanogaster*, radiochemical assay shows that JH III is only a minor product of the larval ring gland, while 95% of the radioactivity is associated with JHB_3 (Richard et al. 1989a). Further, addition of farnesoic acid (a precursor) can increase the JHB_3 production. The most effective concentration is 20 uM. Since whole-body homogenates of higher dipterans can metabolize exogenous JH I to it bisepoxide (Ajami and Riddiford 1973, Yu and Terriere 1978), it is conceivable that JHB_3 is a metabolite rather than an active hormone. Several lines circumstantial evidence, argue in favor of the hormonal status of JHB_3. Richard et al. (1989a) have found that in larval *Drosophila melanogaster*, no tissue other than the ring gland produces JHB_3, in vitro. Also, only the CA portion of the larval ring gland from *Musca domestica* is capable of producing JHB_3, in vitro. They have also identified several biological functions for JHB_3 when it is applied topically. These functions include the blockage of adult eclosion, reduction of the number of adult bristles and evocation of abnormal rotation of the male genitalia. Data also show that JHB_3 biosynthesis is developmentally regulated in the third instar larval *Drosophila melanogaster* (Richard et al. 1989b). The highest level of biosynthesis occurs during the post-feeding stage and synthesis declines after pupariation. More direct evidence may emerge, when the receptor of JHB_3 and/or the binding of the JHB_3-receptor complex to the genomic DNA are established.

When the JHB_3 production by the brain-ventral ganglion-ring gland complexes is compared to that of the isolated ring glands, the complexes clearly produce less of this hormone. This indicates that there may be a brain-centered allatostatic mechanism that down-regulates JHB_3 biosynthesis (Richard et al. 1990). Brain extracts from third instar larvae of both *Sarcophaga bullata* and *Dro-*

sophila melanogaster can cause a dose-dependent reduction of JHB_3 biosynthesis by isolated larval ring glands of *Drosophila melanogaster*. Since JHB_3 production is reduced when nervous connections between the brain and the ring gland are intact (i.e., in the brain-ventral ganglion-ring gland complexes), it appears that the brain allatostatic factor(s) may be neurally transmitted to ring gland, in situ. The same report indicates as well that the agents 3-isobutyl-1-methylxanthine, forskolin, and 8-benzyoyl cAMP, which act to increase the intracellular levels of cAMP, all decrease JHB_3 biosynthesis. This inhibitory effect on the level of cAMP can be overcome by adding farnesoic acid to the incubation medium. Extracellular Ca^{2+} is also important for JHB_3 biosynthesis. Presence of a Ca^{2+} channel blocker, or absence of Ca^{2+}, in the medium decreases JHB_3 biosynthesis. This inhibition can also be reversed by adding farnesoic acid to the medium. However, the Ca^{2+} ionophore, A 23187, which should increase the intracellular level of Ca^{2+}, also reduces JHB_3 production. This reduction can not be completely restored by adding farnesoic acid. It appears that optimal JHB_3 biosynthesis occurs within a fixed range of intracellular Ca^{2+} concentrations in *Drosophila* larval ring glands.

C. *Calliphora vomitoria*

Recently, Cusson et al. (1991) and Duve et al. (1992), using HPLC and a synthetic JHB_3 standard, have identified JHB_3 production, in vitro, by the female adult CA of another blow fly, *Calliphora vomitoria*. A new radioactive unknown with an intermediate HPLC retention time between JH III and JHB_3 is discovered. The chemical structure of this unknown remains to be determined (Cusson et al. 1991). As is true in the case of larval *Drosophila*, partially purified adult brain extracts from *Calliphora vomitoria* contain allatostatic factors which can inhibit JHB_3 production by the adult CA of the same species, in vitro (Duve et al. 1992). These extracts can even inhibit JH III biosynthesis by the CA of a cockroach, *Diploptera punctata*, although the cockroach's allatostatins 1 to 4 (at 10^{-4} to 10^{-7} M) have no effect on JHB_3 biosynthesis by the CA of *Calliphora vomitoria*.

In sharp contrast to the case of the *Drosophila* ring gland, forskolin (5×10^{-5} M) and 8-bromo-cAMP (10^{-4} M) have no inhibitory effect on blow fly JHB_3 biosynthesis, indicating that cAMP may be not involved in JHB_3 production by the CA of the adult blow fly. Another difference between the larval *Drosophila* ring gland and the adult CA of *Calliphora* is that addition of farnesoic acid to the incubation medium of the latter does not stimulate JHB_3 production (Duve et al. 1992). Thus we know that, even among closely related higher dipterans, different mechanisms may have been evolved to regulate juvenoid biosynthesis by the CA.

D. *Lucilia cuprina*

JHB_3 biosynthesis in vitro by the larval ring glands and adult CA of the Australian sheep blow fly, *Lucilia cuprina* is demonstrated by Lefevere et al. (1993). Because the previous conclusions on the kind of JHs can be produced by higher dipterans, have been contradictorily changed first from all JH I, II, and III are produced, to none is produced, then to only JH III is produced, and finally to JHB_3 is the main product, Lefevere et al. (1993) have tested these conclusions using larval and adult *Lucilia cuprina*. Their GC-MS analyses of the biosynthates from larval ring glands and adult CA, identify JHB_3 as the main product, accompanied by only a very small quantity of JH III and methyl farnesoate. Since methyl farnesoate is an immediate precursor of JH III, and JH III is an immediate precursor of JHB_3, Lefevere et al. (1993) conclude that JHB_3 is the only JH released by the CA of higher dipterans. This conclusion is not consistent with our own results from examining juvenoid production by the adult CA of *Phormia regina*. We find that the predominance of JHB_3 over JH III and methyl farnesoate is more variable and less overwhelming in *Phormia regina* (Yin et al. submitted).

E. Others

More recently, a study of the de novo biosynthesis of JH by the male accessory gland of *Aedes aegypti* (Borovsky et al. 1994) has shown that, if the gland is incubated with [^{14}C]-acetate, radioactively labeled JH III, JHB_3, and methyl farnesoate are produced. Similarly, when incubated with *L*-[methyl-^{3}H]-methionine, JH III, JHB_3, and methyl farnesoate (and possibly JH I) are produced. Further, when male accessory glands of *Culex nigripalpus, Anopheles rangeli*, or *Anopheles trinkae* are incubated with *L*-[methyl-^{3}H]-methionine, radioactively labeled JHB_3, JH III and methyl farnesoate are produced.

Interestingly, the CA of male adult *Aedes aegypti* can synthesize JH III only when incubated with *L*-[methyl-^{3}H]-methionine (Borovsky et al.

1994). Likewise, the isolated larval CA (from the last larval instar) of the mosquito, *Toxorhynchites brevipalpis* can synthesis only JH III (Richard et al. 1989a). Therefore, the observation by Borovsky et al. (1994) suggests that JHB_3 biosynthesis is not a pathway restricted to the CA. In certain species, tissues or glands other than the CA are the site of JHB_3 biosynthesis.

Such a conjecture is supported by another study on juvenoid biosynthesis, in vitro, by the synganglion of the American dog tick, *Dermacentor variabilis*. Roe et al. (1993) report production of JH III and JHB_3 by the tick's synganglion, in vitro. Since the identity of JH III and JHB_3 is determined only by using HPLC, the presence of JH III and JHB_3 in the tick may need further, more vigorous, confirmation. Nevertheless, the tick study provides another case in which JHB_3 is produced by tissues other than the insect CA.

CONCLUSION

We have just begun to understand the occurrence and the function of JHB_3 in dipteran insects and in ticks, if the presence of JHB_3 in the tick, *Dermacentor variabilis* or some other ticks can be independently confirmed. The distribution of JHB_3 also may be much wider across the spectrum of Insecta than it appears presently. Mecopterans, being closely related to dipterans may be natural candidates for future investigations on the occurrence of JHB_3.

The discovery of JHB_3 (Liu 1985, Liu et al. 1988) and the identification of its chemical structure (Richard et al. 1989a) are significant and important in several contexts that are not immediately obvious. Before the discovery and the identification of JHB_3, it was widely believed that lepidopterous species produce JH 0, iso-JH 0, JH I, JH II, and JH III, whereas non-lepidopterous insects produce JH III only. Since JHs have been chemically identified in only a relatively small number of insect species, generalization like this must be made with caution (Baker 1990). The occurrence of JHB_3 in dipterans makes clear how far from reality the conventional generalization may be.

The discovery and the identification of JHB_3 also calls for future efforts to search for other possible JH bisepoxides, such as JHB_0, JHB_1, and JHB_2. It is also possible that other isomers of JHs, such as methyl (2*E*,6*E*)-(10*R*-11*S*)-10,11-epoxy-3-ethyl-7,11-dimethyl-2,6-tridecadienoate (iso-JH I), methyl (2*E*,6*E*)-(10*R*-11*S*)-10,11-epoxy-3,7-diethyl-11-methyl-2,6-dodecadienoate (another iso-JH I), methyl (2*E*,6*E*)-(10*R*-11*S*)-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-dodecadienoate (iso-JH II) and methyl (2*E*,6*E*)-(10*R*-11*S*)-10,11-epoxy-3-ethyl-7,11-dimethyl-2,6-dodecadienoate (another iso-JH II) may exist.

The endocrine nature of JHB_3 is established mostly by bioassay in *Drosophila melanogaster* (Richard et al. 1989a) and in *Phormia regina* (Yin et al. submitted). Functions, in any of the other JHB_3-producing insect species, are not yet tested. Thus, two possibilities prevail. Several laboratories consider JHB_3 to be a hormone (Richard et al. 1989a,b 1990, Cusson et al. 1991, Duve et al. 1992, Lefevere et al. 1993, Yin et al. submitted), whilst others consider JH bisepoxide to be a metabolite (Ajami and Riddiford 1973, Yu and Terriere 1978, Borovsky et al. 1994). Both of these possibilities may prove to be right because JHB_3 may vary in role between hormone and metabolite depending on the species in which it is found. Within any one species, however, the hormonal properties of JHB_3 may not be fully established until we can demonstrate the detailed molecular mechanism through which JHB_3 exerts its effect. Obviously, more studies are needed if we are going to have a better understanding of the functional significance of JHB_3.

Acknowledgements: This work was supported by the National Science Foundation (DCB-9104757 and IBN-9306650), and the Massachusetts Agricultural Experiment Station (Hatch 660). The author also received support from the National Science Council, Republic of China while writing this article during a sabbatical visiting National Taiwan University. The author wishes to thank JG Stoffolano Jr., M-A Liu, GL Jones, B-X Zou, W Qin, TL Potter for their assistance in the discovery and identification of JHB_3 in *Phormia regina*. This paper is published as Contribution No. 3138 from the Mass. Agri. Exp. Stn.

REFERENCES

Ajami AM, LM Riddiford. 1973. Comparative metabolism of the *Cecropia* juvenile hormone. J. Insect Physiol. **19:** 635-645.

Baehr JC, P Cassier, MA Fain-Maurel. 1973. Contribution expérimentale et infrastructurale a l'étude de la dynamique de corpus allatum de *Rhodnius prolixus* Stal: influence de la nutrition, de l'activité ovarienne, de la pars intercerebralis et de ses connexions. Arch. Zool. Exp. Gen. **114:** 611-626.

Baker FC. 1990. Techniques for identification and quantifica-

tion of juvenile hormones and related compounds in arthropods. *In* Morphogenetic Hormones of Arthropods, Discovery, Synthesis, Metabolism, Evolution, Mode of Action, and Techniques, ed. AP Gupta. New Brunswick: Rutgers Univ. Press, pp. 153-161.

Bergot BJ, FC Baker, DC Cerf, GC Jamieson, DA Schooley. 1981. Qualitative and quantitative aspects of juvenile hormone titers in developing embryos of several insect species: Discovery of a new JH-like substance extracted from eggs of *Manduca sexta*. *In* Juvenile Hormone Biochemistry, action, agonism and antagonism, eds. GE Pratt, GT Brooks. Amsterdam: Elsevier/North-Holland Biomedical Press, pp. 33-45.

Bergot BJ, GC Jamieson, MA Ratcliff, DA Schooley. 1980. JH zero: New naturally occurring insect juvenile hormone from developing embryos of the tobacco hornworm. Science **210:** 336-338.

Borovsky D, DA Carlson, RG Hancock, H Rembold, E Van Handel. 1994. *De novo* biosynthesis of juvenile hormone III and I by the accessory glands of the male mosquito. Insect Biochem. Molec. Biol. **24:** 437-444.

Cassier P. 1990. Morphology, histology and ultrastructure of JH-producing glands in insects. *In* Morphogenetic Hormones of Arthropods, Vol. 1, ed. AP Gupta. New Brunswick and London: Rutgers Univ. Press, pp. 84-194.

Cazal P. 1948. Les glandes endocrines retrocerebrales des Insectes. Bull. Biol. Fr. Belg. Suppl. **32:** 1-227.

Cusson M, KJ Yagi, Q Ding, H Duve, A Thorpe, JN McNeil, SS Tobe. 1991. Biosynthesis and release of juvenile hormone and its precursors in insects and crustaceans: The search for a unifying arthropod endocrinology. Insect Biochem. **21:** 1-6.

Dahm KH, G Bhaskaran, MG Peter, PD Shirk, KR Seshan, H Röller. 1976. On the identity of the juvenile hormone in insects. *In* The Juvenile Hormone, ed. LI Gilbert. New York: Plenum Press, pp. 19-47.

Dai JD, JG Stoffolano Jr., C-M Yin. 1987. Median neurosecretory cells and associated nerves of the adult black blow fly, *Phormia regina* (Diptera: Calliphoridae). Ann. Entomol. Soc. Am. **80:** 339-345.

Duve H, A Thorpe, KJ Yagi, CG Yu, SS Tobe. 1992. Factors affecting the biosynthesis and release of juvenile hormone bisepoxide in the adult blowfly *Calliphora vomitoria*. J. Insect Physiol. **38:** 575-585.

Judy KJ, DA Schooley, LL Dunham, MS Hall, BJ Bergot, JB Sidall. 1973a. Isolation, structure, and absolute configuration of a new natural insect juvenile hormone from *Manduca sexta*. Proc. Nat. Acad. Sci. USA **70:** 1509-1513.

Judy KJ, DA Schooley, MS Hall, BJ Bergot, JB Sidall. 1973b. Chemical structure and absolute configuration of a juvenile hormone from grasshopper corpora allata in vitro. Life Sci. **13:** 1511-1516.

Judy KJ, DA Schooley, RG Troetschler, RC Jennings, BJ Bergot, MS Hall. 1975. Juvenile hormone production by corpora allata of *Tenebrio molitor* in vitro. Life Sci. **16:** 1059-1066.

Kou R, MP Tu, C-M Yin. The isolated cell type corpora allata in adults of the Loreyi leafworm, *Leucania loreyi* Duponchel (Lepidoptera: Noctuidae). (Submitted).

Lefevere KS, MJ Lacey, PH Smith, B Roberts. 1993. Identification and quantification of juvenile hormone biosynthesized by larval and adult Australian sheep blowfly *Lucilia cuprina* (Diptera: Calliphoridae). Insect Biochem. Molec. Biol. **23:** 713-720.

Liu M-A. 1985. Development and evaluation of an in vitro radiochemical assay for juvenile hormone biosynthesis in the black blowfly, *Phormia regina* (Meigen). Master's thesis, University of Massachusetts, Amherst, MA.

Liu M-A, GL Jones, JG Stoffolano Jr., C-M Yin. 1988. Conditions for estimation of corpus allatum activity in the blowfly, *Phormia regina*, in vitro. Physiol. Ent. **13:** 69-79.

Luo M, RP Bodnaryk. 1987. Morphology and ultrastructure of an isolated cell type corpora allata in adults of the bertha armyworm, *Mamestra configurata* Wlk. (Lepidoptera: Noctuidae). Int. J. Insect Morphol. & Embryol. **16:** 143-151.

Meyer AS, E Hanzmann, HA Schneiderman. 1970. The isolation and identification of the two juvenile hormones from the *Cecropia* silk moth. Arch. Biochem. Biophys. **137:** 190-213.

Meyer AS, HA Schneiderman, E Hanzmann, JH Ko. 1968. The two juvenile hormones from the Cecropia silk moth. Proc. Natl. Acad. Sci. USA **60:** 853-860.

Müller PJ, P Masner, KH Trautmann, M Suchy, HK Wipf. 1974. The isolation and identification of juvenile hormone from cockroach corpora allata in vitro. Life Sci. **15:** 915-921.

Nabert A. 1913. Die Corpora allata der Insekten. Z. Wiss. Zool. **104:** 181-359.

Odhiambo TR. 1966. Morphometric changes and the hormonal activity of the corpus allatum in the adult male of the desert locust. J. Insect Physiol. **12:** 655-664.

Panov AA, OK Bassurmanova. 1970. Fine structure of the gland cells in inactive and active corpus allatum of the bug *Eurygaster integriceps*. J. Insect Physiol. **16:** 1265-1281.

Papillon M, P Cassier, J Girardie, M Lafon. 1976. Influence de la température d'élévage sur l'actvité endocrine de *Schistocerca gregaria*. Arch. Biol., Bruxelles **87:** 103-127.

Richard DS, SW Applebaum, LI Gilbert. 1989b. Developmental regulation of juvenile hormone biosynthesis by the ring gland of *Drosophila melanogaster*. J. Comp. Physiol. B. **159:** 383-387.

Richard DS, SW Applebaum, LI Gilbert. 1990. Allatostatic regulation of juvenile hormone production in vitro by the ring gland of *Drosophila melanogaster*. Molec. Cellu. Endocrinol. **68:** 153-161.

Richard DS, SW Applebaum, TJ Sliter, FC Baker, DA Schooley, CC Reuter, VC Henrich, LI Gilbert. 1989a. Juvenile hormone bisepoxide biosynthesis in vitro by the ring gland of *Drosophila melanogaster*: A putative juvenile hormone in the higher Diptera. Proc. Natl. Acad. Sci. USA **86:** 1421-1425.

Roe RM, VL Kallapur, C Majumder, MT Lassiter, CS Apperson, DE Sonenshine, BS Winder. 1993. Biochemical evidence for the presence of a juvenoid in ticks. *In* Host Regulated Developmental Mechanisms in Vector Arthropods: Proceedings of the Third Symposium, Vero Beach, Florida, eds. D Borovsky, A Spielman. pp. 110-120.

Röller H, JS Bjerke. 1965. Purification and isolation of juvenile hormone and its action in lepidopteran larvae. Life Sci. **4:** 1617-1624.

Röller H, KH Dahm, CC Sweeley, BM Trost. 1967. The structure of juvenile hormone. Angew. Chem. **6:** 179-180.

Schooley DA, FC Baker. 1985. Juvenile hormone biosynthesis. *In* Comprehensive Insect Physiology Biochemistry and Pharmacology, eds. GA Kerkut, LI Gilbert. New York: Pergamon Press, Vol. 8, pp. 386-383.

Trost BM. 1972. The origin of juvenile hormone chemistry. *In* Insect Juvenile Hormones, Chemistry and Action, eds. JJ Menn, M Beroza. New York: Acad. Press, pp. 217-235.

Wigglesworth VB. 1935. Functions of the corpora allata of insects. Nature, Lond. **136:** 338-339.

Williams CM. 1956. The juvenile hormone of insects. Nature, Lond. **178:** 212-213.

Williams CM, JH Law. 1965. The juvenile hormone. IV. Its extraction, assay, and purification. J. Insect Physiol. **11:** 569-580.

Yin C-M, H Duan, JG Stoffolano Jr. 1993. Hormonal stimulation of the brain for its control of oögenesis in the black blowfly, *Phormia regina* (Meigen). J. Insect Physiol. **39:** 165-171.

Yin C-M, JG Stoffolano Jr. 1990. The interactions among nutrition, endocrines and physiology on the reproductive development of the black blowfly, *Phormia regina* (Meigen). Bull. Inst. Zool., Acad. Sinica, Monograph **15:** 87-108.

Yin C-M, JG Stoffolano Jr. 1994. Endocrinology of vitellogenesis in blow flies. *In* Perspectives in Comparative Endocrinology, eds. KG Davey, RE Peter, SS Tobe. Ottawa: Natl. Res. Council of Canada, pp. 291-298.

Yin C-M, B-X Zou, M Jiang, M-F Li, W Qin, TL Potter, JG Stoffolano Jr. Biosynthesis of JH III bisepoxide (JHB_3), JH III and methyl farnesoate by the corpus allatum of *Phormia regina* (Meigen), in vitro and function of JHB_3. (Submitted).

Yin C-M, B-X Zou, M-F Li, JG Stoffolano Jr. 1994. Discovery of a midgut peptide hormone which activates the endocrine cascade leading to oögenesis in *Phormia regina* (Meigen). J. Insect Physiol. **40:** 283-292.

Yin C-M, B-X Zou, JG Stoffolano Jr. 1989. Precocene II treatment inhibits terminal oöcyte development but not vitellogenin synthesis and release in the black blowfly, *Phormia regina* Meigen. J. Insect Physiol. **35:** 465-474.

Yin C-M, B-X Zou, S-X Yi, JG Stoffolano Jr. 1990. Ecdysteroid activity during oögenesis in the black blowfly, *Phormia regina* (Meigen). J. Insect Physiol. **36:** 375-382.

Yu SJ, LC Terriere. 1978. Metabolism of juvenile hormone by microsomal oxidase, esterase and epoxide hydrase of *Musca domestica* and some comparisons with *Phormia regina* and *Sarcophaga bullata*. Pestic. Biochem. Physiol. **9:** 237-246.

Zou B-X, C-M Yin, JG Stoffolano Jr., SS Tobe. 1989. Juvenile hormone biosynthesis and release during oöcyte development in *Phormia regina* Meigen. Physiol. Ent. **14:** 233-239.

雙環氧基保幼激素 III：昆蟲保幼激素家族之新成員

殷 之 銘[1]

昆蟲保幼激素(又稱青春激素)家族最近又添了一名成員，那就是雙環氧基保幼激素III。此成員的化學構造，除了在碳六與碳七之間多了一個環氧基之外，和保幼激素III完全相同。其構造是爲 methyl (2*E*, 6*E*)-(10*R*, 11*S*)-6, 7; 10, 11-bisepoxy-3, 7, 11-trimethyl-2-dodecenoate，簡稱 JHB_3。JHB_3 最初是在做體外培養伏蠅（*Phormia regina*）雌成蟲咽側體時發現的(Liu 1985, Liu et al. 1988)。當時只知其可能是保幼激素一類，而未鑑定其化學結構，1989 年由果蠅幼蟲中發現了一個相同的物質，並鑑定爲 JHB_3。JHB_3 對果蠅及伏蠅都有明顯的生物效應，能影響他們的生長，變態及卵巢發育(Richard et al. 1989a 1989b 1990, Yin et al. submitted)。目前所有研究過的環裂亞目的蠅類都分泌 JHB_3。另外數種雄蚊的生殖副腺及美洲犬壁蝨的綜合神經球亦能分泌 JHB_3。JHB_3 的發現使非鱗翅目昆蟲只能分泌保幼激素III的說法，不攻自破。目前仍有人認爲 JHB_3 不是一種新的保幼激素，而只是保幼激素III的代謝物。JHB_3 的身份，大概要在研究其作用機制後證實了其確有專一的受體，而此受體與 JHB_3 結合後既能專一的與特別的 DNA 順序親和時，才能完全確定。

關鍵詞：Phormia, 咽側體, 雙翅目, 保幼激素的合成。

[1] Department of Entomology, University of Massachusetts, Amherst, Massachusetts 01003-2410, USA