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

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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 (JHB₃). JHB₃ 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 (2E,6E)-(1R,11S)-6,7,10,11-bisepoxy-3,7,11-trimethyl-2-dodecenoate or methyl-s,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 JHB₃ demonstrate its biological activities in the regulation of development, metamorphosis and oogenesis in Drosophila melanogaster and Phormia regina (Richard et al. 1989a,b 1990, Yin et al. submitted). JHB₃ 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 JHB₃ calls for future research aiming at the discovery of JHB₀, JHB₁, and JHB₂. 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 interdigitiation 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 cellular 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 cellular 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
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 (2E,6E)-(10R-11S)-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 (2E,6E)-(10R-11S)-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 (2E,6E)-(10R-11S)-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-14C] 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 (2E,6E)-(10R-11S)-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 10R-11S-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 (2E,6E,10R)-JH 0 but different to that of synthetic (2E,6E,10S)-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 (d3-methoxyhydrin) and comparisons to 4-methyl JH I as well as synthetic model compounds [tetrahydroepoxymethyl ester and (2E,6E,10R,10S)-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 (JHB3) 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 JHB3 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, JHB3 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 Phormia 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 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 (JHB3) by Richard et al. (1989a). Richard et al. (1989a) suggest that the unknown produced by the CA of adult Phormia regina may also be JHB3.

Our latest results (Yin et al. submitted) show that the unknown in Phormia regina is indeed JHB3. 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 JHB3 than females do (Fig. 3). Bioassay of synthetic JHB3 confirms its biological activity because this juvenoid can restore oogenesis in allatectomized, liver-fed females (Table 1). Biological activity of JHB3 in males remains unconfirmed, although our preliminary observations suggest that it may regulate mating behavior.

We are presently synthesizing quantities of JHB3 and MF using established procedures (Yin et al. submitted) to facilitate further biological testing.
of JHB₃ in males and females. We would also like to test some juvenoid blends (i.e., mixtures of JHB₃, 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 oogenesis 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, JHB₃ is first identified in Drosophila melanogaster by Richard et al. (1989a). The same authors also report the presence of a JHB₃-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 JHB₃ 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 JHB₃ (Richard et al. 1989a). Further, addition of farnesoic acid (a precursor) can increase the JHB₃ production. The most effective concentration is 20 uM. Since whole-body homogenates of higher dipterans can metabolize exogenous JH I to its bisepoxide (Ajami and Riddiford 1973, Yu and Terriere 1978), it is conceivable that JHB₃ is a metabolite rather than an active hormone. Several lines circumstantial evidence, argue in favor of the hormonal status of JHB₃. Richard et al. (1989a) have found that in larval Drosophila melanogaster, no tissue other than the ring gland produces JHB₃, in vitro. Also, only the CA portion of the larval ring gland from Musca domestica is capable of producing JHB₃, in vitro. They have also identified several biological functions for JHB₃ 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 JHB₃ 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 JHB₃ and/or the binding of the JHB₃-receptor complex to the genomic DNA are established.

When the JHB₃ 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 JHB₃ 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 JHB₃ biosynthesis by isolated larval ring glands of Drosophila melanogaster. Since JHB₃ 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-benzoyl cAMP, which act to increase the intracellular levels of cAMP, all decrease JHB₃ biosynthesis. This inhibitory effect on the level of cAMP can be overcome by adding farnesoic acid to the incubation medium. Extracellular Ca²⁺ is also important for JHB₃ biosynthesis. Presence of a Ca²⁺ channel blocker, or absence of Ca²⁺, in the medium decreases JHB₃ biosynthesis. This inhibition can also be reversed by adding farnesoic acid to the medium. However, the Ca²⁺ ionophore, A 23187, which should increase the intracellular level of Ca²⁺, also reduces JHB₃ production. This reduction can not be completely restored by adding farnesoic acid. It appears that optimal JHB₃ biosynthesis occurs within a fixed range of intracellular Ca²⁺ concentrations in Drosophila larval ring glands.

C. Calliphora vomitoria

Recently, Cusson et al. (1991) and Duve et al. (1992), using HPLC and a synthetic JHB₃ standard, have identified JHB₃ 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 JHB₃ 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 JHB₃ production by the adult CA of the same species, in vitro (Duve et al. 1992). These extracts can even inhibit JHB₃ 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 JHB₃ biosynthesis by the CA of Calliphora vomitoria.

In sharp contrast to the case of the Drosophila ring gland, forskolin (5 x 10⁻⁵ M) and 8-bromo-cAMP (10⁻⁴ M) have no inhibitory effect on blow fly JHB₃ biosynthesis, indicating that cAMP may be not involved in JHB₃ 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 JHB₃ 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

JHB₃ 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 JHB₃ 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 JHB₃ 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 JHB₃, Lefevere et al. (1993) conclude that JHB₃ 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 JHB₃ 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 [¹⁴C]-acetate, radioactively labeled JH III, JHB₃, and methyl farnesoate are produced. Similarly, when incubated with L-[methyl-³H]-methionine, JH III, JHB₃, 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-³H]-methionine, radioactively labeled JHB₃, 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-³H]-methionine (Borovsky et al.
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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 JHB3 biosynthesis is not a pathway restricted to the CA. In certain species, tissues or glands other than the CA are the site of JHB3 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 JHB3 by the tick’s synganglion, in vitro. Since the identity of JH III and JHB3 is determined only by using HPLC, the presence of JH III and JHB3 in the tick may need further, more vigorous, confirmation. Nevertheless, the tick study provides another case in which JHB3 is produced by tissues other than the insect CA.

CONCLUSION

We have just begun to understand the occurrence and the function of JHB3 in dipteran insects and in ticks, if the presence of JHB3 in the tick, Dermacentor variabilis or some other ticks can be independently confirmed. The distribution of JHB3 also may be much wider across the spectrum of Insecta than it appears presently. Mecoptera, being closely related to dipterans may be natural candidates for future investigations on the occurrence of JHB3.

The discovery of JHB3 (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 JHB3, 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 JHB3 in dipterans makes clear how far from reality the conventional generalization may be.

The discovery and the identification of JHB3 also calls for future efforts to search for other possible JH bisepoxides, such as JHB0, JHB1, and JHB2. It is also possible that other isomers of JHs, such as methyl (2E,6E)-(10R-11S)-10,11-epoxy-3-ethyl-7,11-dimethyl-2,6-tridecadienoate (iso-JH I), methyl (2E,6E)-(10R-11S)-10,11-epoxy-3,7-diethyl-11-methyl-2,6-dodecadienoate (another iso-JH I), methyl (2E,6E)-(10R-11S)-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-dodecadienoate (iso-JH II) and methyl (2E,6E)-(10R-11S)-10,11-epoxy-3-ethyl-7,11-dimethyl-2,6-dodecadienoate (another iso-JH II) may exist.

The endocrine nature of JHB3 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 JHB3-producing insect species, are not yet tested. Thus, two possibilities prevail. Several laboratories consider JHB3 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 JHB3 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 JHB3 may not be fully established until we can demonstrate the detailed molecular mechanism through which JHB3 exerts its effect. Obviously, more studies are needed if we are going to have a better understanding of the functional significance of JHB3.

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**Yin — JHB3: New Insect Juvenile Hormone**


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