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# Insect Juvenile Hormone III in the Sedge, *Cyperus iria* L.: Distribution and Possible Biological Significance

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*Abstract:* Insect juvenile hormones (JHs) are involved in the regulation of physiological processes such as metamorphosis and reproduction in most insect species. In 1988, juvenile hormone III (JH III) and its biosynthetic precursor (insects), methyl farnesoate, were isolated from the sedges *Cyperus iria* L. and *C. aromaticus* (Ridley) Mattf and Kük (ref. 1). In *C. iria* roots, concentrations of 27.2 ± 3.3 μg of JH III per gram fresh weight was extracted; this is approximately 37 times the amount of JH detected in whole body extracts of the cockroach, *Diploptera punctata* (ref. 2, ref. 3). The extremely high concentration found in the plant and its presence throughout development suggests that JH III plays an important biological role(s) in the plant, perhaps through plant-insect, plant-plant or other interactions.

## INTRODUCTION

Many plants defend themselves against insect herbivory through the production of secondary metabolites which interfere with insect physiological functions (ref. 4 and references therein). These may include compounds which mimic JHs or interfere with their biosynthesis. Insect JHs are a group of structurally related sesquiterpenoids which are involved in the regulation of metamorphosis and reproduction (adult females) in most insect species. The JH analogue juvabione (the "paper factor"), the methyl ester of todomatuic acid, was isolated from the pulp wood of the balsam fir, *Abies balsamea* (L.) Miller (ref. 5). Application of this compound to fifth instar larvae of susceptible insects in the family Pyrrhocoridae such as the European linden bug, *Pyrrhocoris apterus*, results in a supernumary moult with the ensuing giant immature larvae which eventually die without reaching the adult stage (ref. 6). Other JH mimics such as juvadecene and the juvocimenes have been isolated from dried roots of the pepper-tree, *Macropiper excelsum* Miq, and leaves of sweet basil, *Ocimum basilicum* L., respectively (ref. 7, ref. 8). Topical application of these compounds to final larval instars of susceptible insect species also result in supernumary moults. Other phytochemicals such as the precocenes I and II, isolated from *Ageratum houstonianum* Mill. (ref. 9), interfere with JH biosynthesis. In the corpora allata (CA)

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of susceptible insect species, these compounds are epoxidated to form highly reactive 3,4-epoxy intermediates which alkylate cellular proteins. This results in the atrophy of the CA and inhibition of JH biosynthesis (ref. 10). As a consequence, the larval insect undergoes precocious metamorphosis whereas the adult female insect will be infertile.

In 1988, insect juvenile hormone III (JH III), methyl-10*R*,11-epoxy-3,7,11-trimethyl 2*E*,6*E*-dodecadienoate, and its biosynthetic precursor (insects), methyl farnesoate (MF), methyl-3,7,11-trimethyl 2*E*,6*E*,10*E*-dodecatrienoate, were isolated from the sedges *Cyperus iria* L. and *C. aromaticus* (Ridley) Mattf and Kük (ref. 1). This is the first and only example to date of the production of JH III in a plant. The concentrations of these compounds in mature *C. iria* plants were reported to be 151 μg JH III/g fresh weight (FW) and 14 μg MF/g FW. This is approximately 200 times the maximal JH III concentration found in whole body extracts of the cockroach, *Diploptera punctata* (mated adult female, day 5) (ref. 2, ref. 3).

## INSECT JUVENILE HORMONES

Insect JHs share the common structural features of a sesquiterpenoid skeleton with a methyl ester group on C-1 and an epoxide function. Of these compounds, JH III appears to be ubiquitous and has been detected in most insect species studied. In the Lepidoptera (moths and butterflies), four other homologs have also been identified. Juvenile hormone I, methyl-10*R*,11-epoxy-7-ethyl-3,11-dimethyl 2*E*,6*E*-tridecadienoate, and JH II, methyl-10*R*,11-epoxy-3,7,11-trimethyl 2*E*,6*E*-tridecadienoate and their corresponding acids predominate in these insects (ref. 11, ref. 12, ref. 13). Two additional homologs, JH 0, 10*R*,11-epoxy-3,7-diethyl-11-methyl-2*E*,6*E*-tridecadienoate, and 4-methyl JH I have been isolated from *Manduca* embryos and JH 0 has also been isolated from *Hylophora cecropia* males (ref. 14, ref. 15). In most of the higher Diptera (flies), the JH bisepoxide, 6,7,10,11-bisepoxy-3,7,11-trimethyl 2*E*-dodecadienoate, has been isolated and may be the principle JH (ref. 16).

In the juvenile stages, the presence of JH in the insect haemolymph prior to the moult is necessary for the maintenance of juvenile characteristics (ref. 17, ref. 18). In adult females of most insect species, JH is a major regulator of ovarian maturation. In the cockroach, *D. punctata*, high haemolymph titre corresponds to times of rapid oocyte development and vitellogenin synthesis (ref. 2, ref. 3). In this species, lower biosynthetic rates were observed during pre- and post-vitellogenic periods. In some insect species, JHs may also be involved in other physiological processes such as diapause (ref. 19).

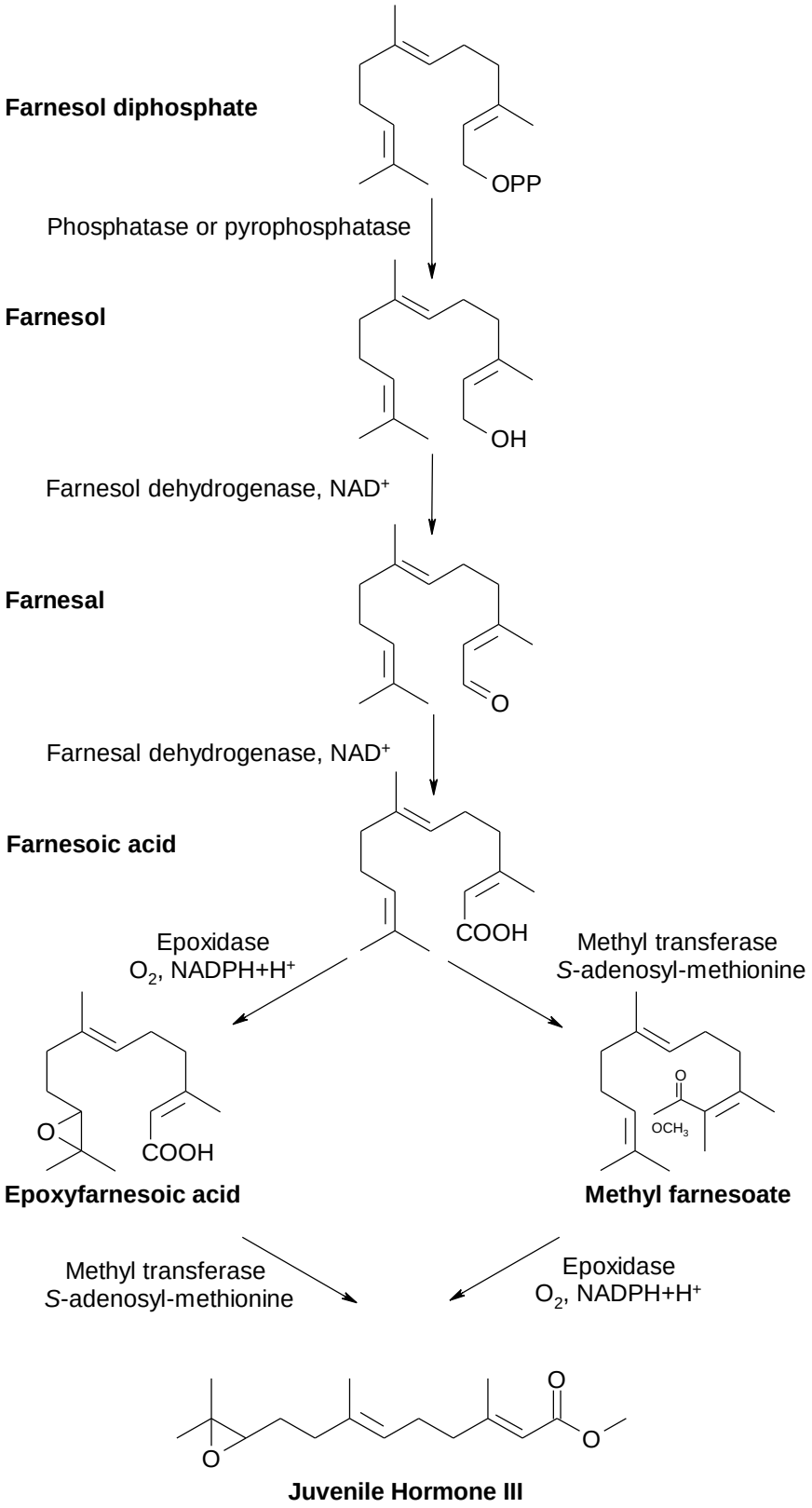
### Biosynthesis of Insect Juvenile Hormone III

Juvenile hormones are synthesized in the insect corpora allata, a small retrocerebral endocrine organ. In insects, these sesquiterpenoids are derived biosynthetically from acetate via the intermediates 3-hydroxy-3-methylglutaryl-CoA, mevalonate and farnesyl diphosphate. Mevalonate is formed through the condensation of three acetyl Co-A molecules. Phosphorylation of mevalonate generates the important intermediate isopentenyl diphosphate (IDP), which is isomerized to dimethylallyl diphophate (DMADP). Chain elongation through covalent linkages of DMADP and IDP generates prenyl diphosphates, the acyclic precursors to the various terpenoid classes, such as monoterpenes (C-10), sesquiterpenes (C-15), diterpenes (C-20) and steroids. Insects lack the enzymes to synthesize the higher terpenoids and the JHs are synthesized from the

$C_{15}$ farnesyl diphosphate (FDP). The ethyl branches, found in the higher JH homologs of the Lepidoptera, are derived from the metabolism of isoleucine and valine which have been converted into propionate and incorporated in the early steps of biosynthesis (ref. 20).

In the insect, JH III is biosynthesized from FDP in four steps (Fig. 1). A phosphatase or pyrophosphatase catalyzes the removal of the pyrophosphate from FDP, producing farnesol. This is followed by the oxidation of this alcohol to a carboxylic acid via an aldehyde intermediate (farnesal). These steps are catalyzed by one or two $NAD^+$-dependent dehydrogenase(s) (ref. 21). Following the synthesis of farnesoic acid, two steps, a methylation and an epoxidation, are required to generate JH III. The sequence of these steps is controversial; it is generally thought that the left-hand branch of the pathway predominates in the Lepidoptera whereas the right-hand or both branches are found in other insect species (ref. 22, ref. 23). In the locust, *Locusta migratoria*, and in the cockroach, *D. punctata*, the *o*-methyltransferase is a cytosolic enzyme (ref. 24, ref. 25). In *L. migratoria*, the enzyme which catalyzes the epoxidation, methyl farnesoate epoxidase, has been characterized as a cytochrome $P_{450}$ mixed function oxidase and is associated with the microsomal fraction (ref. 24).

**Figure 1.** Proposed biosynthetic pathway of insect juvenile hormone III. It is generally thought that after the synthesis of farnesoic acid, the left-hand branch occurs in the Lepidoptera and the right-hand or both branches occur in other insect species (ref. 22, ref. 23).



**Distribution in the plant**

We have investigated the distribution of JH III in *C. iria* plants (seeds obtained from Y.C. Toong, Penang, Malaysia) using a radioimmunoassay (RIA) which was developed to determine JH III titres in insect haemolymph (ref. 26) and optimized for use in the plant system. Plant tissues were divided into the fruits, inflorescence and bracts (modified leaves which subtend the inflorescence), the culm (stem), leaves and roots. In seven month-old mature plants, the roots contained the highest concentration of JH III (27.2 ± 3.3 μg/g FW); approximately 500 and 300 times the amount found in either the inflorescence (fruits, inflorescence, bracts) or leave tissues, respectively. This profile is maintained over the course of development in immature (not yet flowering) and mature plants, with the highest concentrations of JH III being found in the root tissue and substantial quantities in the leaf tissue (data not shown). This suggests that the roots may be the site of JH III biosynthesis in *C. iria*; alternatively, it may be synthesized in the leaf tissue and transported to the roots. The total JH III concentration in these seven month-old *C. iria* plants is approximately eleven times less than that originally reported (ref. 1). This discrepancy is probably attributed to the different cultivars and environmental conditions under which the plants were grown.

**Biological role(s)**

The high concentration of JH III in the root and leaf tissues throughout development of *C. iria* suggests that it plays an important biological role(s) in the plant. Possible interactions which are immediately apparent are plant-insect, plant-plant (allelopathic), plant-nematode and plant-fungal. There have been few direct investigations of the ecology of *C. iria* and associated insect species. Rather, the studies have focused on the use of *Cyperus* species as an alternative host plant either for insects which feed on economically important crops such as rice, or for insects which are vectors of disease in these crop plants. At present, the literature on the interactions between *C. iria* and insects is vague and contradictory. In laboratory studies, third stadium instars of the grasshopper, *Melanoplus sanguinipes*, were reared on either wheat seedlings or C. iria; no difference in growth was observed. Following the moult to an adult, 90% of those fed exclusively on *C. iria* exhibited deformed wings as well as colour changes characteristic of development in the presence of excess JH (ref. 1). Adult female grasshoppers reared on *C. iria* were found to be infertile and their ovaries contained immature oocytes (ref. 1). Under field conditions, eggs of the Dipteran leafminer, *Hydrellia* sp., did not hatch when laid on *C. iria* leaves (ref. 27). However, nymphs of the planthoppers, *Nisia strovenosa* and *N. nervosa*, were raised successfully on both *C. iria* and *C. rotundus* L., although more nymphs reached adult stages on *C. rotundus* (ref. 28). Also, in a feeding preference study, the rice stink bug, *Oebalus pugnax*, was observed to feed on *C. iria* though the mean number was less than those found on the Vasey grass, *Paspalum urvillei* Steud. (ref. 29).

*Cyperus iria* is an extremely invasive weed, responsible for the reduced yield of economically important crops particularly rice (ref. 30). There is some evidence that two of the biosynthetic precursors of JH III (insects) may act as allelopathic agents, reducing plant competition by inhibiting the germination and growth of other plants in the immediate vicinity. The essential oil fraction extracted from tubers of the related sedge *C. serotinus* Rottb. inhibited

the germination of lettuce seeds and the growth of lettuce and rice seedlings as well as that of *C. serotinus,* itself (ref. 31). Further identification of the agents responsible for this activity led to the isolation of β-selinene, MF, farnesyl acetone and farnesol. At concentrations of 1 mM, both MF and farnesol inhibited the growth of rice and lettuce seedlings, but stimulated root elongation of these plants. Similar results were obtained in other studies on the allelopathic activity of farnesol. Application of farnesol (83.3 μM) to seeds at root emergence was found to enhance root elongation in barley, but slightly inhibited root growth in mustard, tomato, spinach and wheat (ref. 32). No effect was observed on lettuce, carrot or cabbage roots at this concentration. However, at higher concentrations (516.6 μM), farnesol was found to inhibit root elongation in barley. These results reflect the common observation that allelopathic agents often exhibit opposing biological activities depending on their concentration. The extremely high concentrations of JH III present in the roots, coupled with the above evidence of allelopathic activities of two biosynthetic precursors of JH III, farnesol and MF (ref. 31), raise the possibility that MF and JH III accumulate in the roots from which they may subsequently diffuse or be transported out of the tissue and inhibit the germination and growth of neighbouring plants.

Protection against nematode attack may be another possible function of JH III. Davey has discussed the physiological effects of topically applied JH on nematode development (ref. 33). Application of JH III affects larval development and egg production in adults of the rodent hookworm, *Nippostrongylus brasiliensis* (ref. 34). An extremely high concentration of JH III (3.4 mM) was required to cause a 50% reduction in egg production of *N. brasiliensis* females. The root tissue of mature *C. iria* plants contain 102.3 μmoles JH III/g FW (amount/root 324.1 μg). If JH III is localized in oil bodies in the root, concentrations may be sufficient to arrest nematode development. In the same paper, application of JH III showed no effect on egg hatching and larval development of the phytoparasitic soybean cyst nematode, *Heterodera glycines.* Aqueous extracts of root tissue from related plant species, *C. esculentus* and *C. rotundus*, inhibited egg hatching and reduced larval survival of the rootknot nematode, *Meloidogyne incognite* (ref. 35). At present, there is no evidence to suggest that these two sedges produce JH III and if such is the case, it is unlikely that this effect could be attributed to the lipophilic JH III because an aqueous extract was employed.

There are reports of interactions between *C. iria* and fungal endophytes such as *Phoma cyperi* (ref. 36). However, at present, there is no evidence to suggest that JH III plays a role in any plant-pathogen (fungal or bacteria) defense. The antifungal and/or antibacterial activities of other sesquiterpenoids are well established. Phytoalexins are low molecular weight antimicrobial compounds which structurally may be phenolic, polyacetylene or isoprenoid (ref. 37). For example, the sesquiterpene rishitin isolated from potato (*Solanum tuberosum* L.) infected with the late blight fungus, *Phytophora infestans,* or the bacteria, *Erwinia carotovora*, is implicated in plant defense against the fungal pathogen (ref. 38) and exhibits bactericidal activity *in vitro* (ref. 39). However, phytoalexins are synthesized *de novo* in response to microbial attack, whereas JH III is present in relatively high concentrations in the plant throughout development.

Although JH III has only been isolated from *C. iria* and *C. aromaticus* (ref. 1), structurally similar linear sesquiterpenoids have been isolated from the roots of *C. iria* and a number of related *Cyperus* species (ref. 31, ref. 40-44). For example, MF, the immediate biosynthetic precursor of JH III in insects, has also been identified in extracts from *C. iria*, *C. monophyllus, C. pilosus* and *C. serotinus* (ref. 31, ref. 43, ref. 44). Methyl farnesoate has also been isolated from grape skins of the Traminer variety (ref. 45) and from the bark of *Polyalthia viridis* Craib (ref. 46). The linear

sesquiterpenoid methyl (*E,E*)-10, 11-dihydroxy-3,7,11-trimethyl-2,6-dodecadienoate has been identified in dried roots of a canopy tree native to Guyana, *Hortia regia* Sandwith., and the root bark of a rain forest tree from west Africa, *Cleistopholis patens* (Benth.) Engl. and Diels, and the stem bark of *Cl. staudtii* (Engl. & Pierre) (ref. 47-49). This last compound is structurally identical to JH III except that the epoxide has been modified into a *vicinal* diol at C10 and C11, a reaction which readily occurs in the presence of acid or base. This indirect evidence suggests that JH III, MF and structurally similar compounds may be present in a diverse range of plant species. Further work is necessary to determine the function of these compounds in plants.

## CONCLUSION

The value of JH as a potential 'insecticide' has long been promoted. However, its use has been confined to specific environments in which pest species can be directly targeted (*e.g.* cockroaches, fleas, stable flies); this no doubt derives from the ubiquitous nature of JH among insect species and its profound disruptive effects following topical application on both metamorphosis and reproduction throughout insect orders. Hence, the value of JH as an 'insecticide' is limited and its use can only be envisaged if its application is restricted to pest species. This is particularly important in crops where the inadvertent exposure of non-target species is likely. The production of JH III in target crop plants may confer some degree of protection specifically against pest attack. This could be effected in at least two ways; through the development of transgenic plants expressing enzymes from the JH III biosynthetic pathway or through the enhancement or overproduction of JH III in an already existing pathway.

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