NEW INSECTICIDES WITH ECDYSTEROIDAL AND JUVENILE HORMONE ACTIVITY

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ABSTRACT

Agrochemical research over the last two decades has resulted in the discovery of chemically novel insecticides that mimic the action of the two insect growth and developmental hormones, the steroidal 20-hydroxyecdysone (20E) and the sesquiterpenoid juvenile hormone (JH). Bisacylhydrazines are non-steroidal agonists of 20E and exhibit their insecticidal activity via interaction with the ecdysteroid receptor proteins. Interestingly, two of the bisacylhydrazine (tebufenozide and RH-2485) insecticides are very selectively toxic to lepidopteran pests. These insecticides are safe to beneficial insects and have a benign ecotoxicological profile. Aromatic non-terpenoidal insecticides (fenoxycarb and pyriproxyfen) mimic the action of JHs. However, like the JHs, their exact mode of action is not well understood. These insecticides are toxic to a broad spectrum of insects during their embryonic, last larval, or reproductive stages. The insecticidal, ecotoxicological properties and the mode of action of the two groups of insecticides are reviewed in this article.

INTRODUCTION

After Williams’ (134) suggestion that compounds that mimic the action of juvenile hormones (JHs) could be used as safe insecticides, numerous JH analogs (JHAs) were discovered, and some, like methoprene, have been used as commercial insecticides (96, 120). The agricultural use of these earlier JHAs has been limited, however, because of their lack of outdoor stability, their limited...
insect control spectrum, and their slow toxic action. Insecticides with an insect molting hormone mode of action have also been pursued without much success (132), largely owing to their structural complexity and the relative chemical and metabolic instability of the steroid nucleus.

Early research in the JH and ecdysteroid fields produced candidate insecticides, either naturally occurring or synthetic, with strong structural resemblance to the natural hormones. More recently, persistent efforts by the agrochemical industry have led to the discovery of several new and much more chemically diverse insecticidal agents with JH or ecdysteroid mode of action. Such compounds, which are the subject of this review, have much greater metabolic and environmental stability than earlier analogs and are much better suited to agriculture. In addition, some display remarkable target pest selectivity.

Here we review two classes of chemistry, namely, the bisacylhydrazines, which are novel non-steroidal ecdysteroid agonists, and the aromatic non-terpenoidal JHAs. A brief overview of the endocrine regulation of growth and development in insects has also been included as background information. For greater detail on this subject, the reader should refer to Nijhout (85) and recent reviews (100, 102, 142).

ENDOCRINE REGULATION OF INSECT GROWTH AND DEVELOPMENT

Growth and development in insects, which are punctuated by periods of molting, are regulated by the steroid 20-hydroxyecdysone (20E; molting hormone; ecdysterone) and the sesquiterpenoid JHs. In the adult stage, both of these hormones are also involved in the regulation of reproductive maturation.

Molting and metamorphosis have been extensively studied in several representative insects (reviewed in 85, 100, 102). The molting process is initiated by an increase in the titer of 20E and is completed following the decline of 20E titer and the release of eclosion hormone. As a larva prepares to undergo a larval molt, it stops feeding. With increasing 20E titer, the epidermis separates from the old cuticle (apopysis), and the ecdysial space that results is filled with molting fluid that contains inactive chitinolytic enzymes to be used later for digestion of the old cuticle. Meanwhile, the epidermal cells reorganize for massive protein synthesis, as well as secretion of the new epicuticle and cuticle. Once 20E titer begins to decline, enzymes in the molting fluid are activated to start digestion of the procuticle. After this process is completed, the molting fluid is resorbed and pre-ecdysial tanning of the new cuticle takes place (99). Finally, when the 20E titer has declined to a basal level, escape from the old cuticle (ecdysis) is initiated by the release of the peptides, eclosion hormone and ecdysis-triggering hormone, which together act on a number of targets to
ensure the successful completion of a molt (reviewed in 50). Feeding then resumes and endocuticular deposition continues during the intermolt period.

During the last larval instar, the commitment to undergo a pupal molt requires the action of ecdysteroids in the absence of JH. This commitment usually happens during the intermolt period. The larval-pupal molt is then induced with molting levels of ecdysteroids in the presence of JH. Juvenile hormone is required to prevent precocious development of the imaginal disks in holometabolous insects. Finally, for the pupal-adult transformation, a steady increase of ecdysteroid titers at low levels commits the tissues to adult differentiation, and at higher levels, it elicits that differentiation.

Growth and development in insects are very well orchestrated by 20E, JH, eclosion hormone, and other neurohormones. The morphological and ultrastructural changes that occur in the epidermis during insect growth and development are dependent upon the regulation of gene expression with different titers of 20E in the absence or presence of JH (102). Any interference in the homeostasis of one or more of these hormones with exogenous sources of the hormones or with synthetic analogs (agonists or antagonists) would result in the disruption or abnormal growth and development of the target insect. Similarly, any interference in the various hormone-dependent steps involved in the synthesis and/or resorption of the cuticle would be detrimental to the survival of the affected developmental stage.

**Molecular Basis of 20E Action**

As a result of work by several investigators on ecdysteroid-induced “puffing” patterns of the giant salivary chromosomes of *Chironomus tentans* and *Drosophila melanogaster*, Ashburner and coworkers (3) proposed a model for ecdysteroid action. In this model, ecdysteroid coupled to an ecdysteroid receptor protein acts differentially to regulate several classes of “early” and “late” target genes. The early genes are activated directly by the ecdysteroid-receptor complex, whereas late genes are repressed by it. Proteins encoded by the early genes induce secondary responses to ecdysteroids by repressing their own expression and activating late genes. Several of the early gene products are now known to be transcription factors.

Recent studies indicate that the molecular target for ecdysteroids consists of at least two proteins, the ecdysteroid receptor (EcR) and the product of another gene, *ultraspiracle* (USP; 143). Both EcR and USP are members of the steroid hormone receptor superfamily characterized by signature DNA and ligand-binding domains (71). Yao et al (143) demonstrated that ecdysteroids bind to EcR only when EcR and USP exist as a heterodimeric complex. However, additional factors may also be involved for ligand binding and ecdysteroid-dependent gene regulation.
Molecular Basis for JH Action

The action of JH at the molecular level is not well understood. It appears to act through both membrane and intracellular receptors. The best-characterized system of JH action at the membrane level has been described for the ovarian follicle cells of *Rhodnius prolixus* (56, 108) and *Locusta migratoria* (109) for the uptake of vitellogenin (also see 142 for a review). A membrane receptor-mediated action of JH causes a widening of the inter-follicular spaces in the epithelium. This is due to an activation of the Na$^+$, K$^+$-ATPase and subsequent shrinkage of the cells, which then promotes vitellogenin uptake. Apparently, this action of JH may involve the activation of protein kinase C (108).

Intracellular interaction of JH, presumably with nuclear receptors, has been investigated in the fat bodies of *L. migratoria* (11) and *Leucophaea maderae* (34) and in the epidermis of *Manduca sexta* (see 100, 101). The binding affinities of radiolabeled JHs or JHAs to isolated nuclei or cytosolic or nuclear extracts have been determined. The proteins that bind to photoaffinity analogs of the JHs as well as of JHAs have been characterized electrophoretically (see 102, 142). However, the molecular characteristics of the JH receptor(s) remain unknown.

BISACYLHYDRAZINE ECDYSTEROID AGONISTS AS INSECTICIDES

The first bisacylhydrazine ecdysteroid agonist (Figure 1, structure 1) was discovered serendipitously by Rohm and Haas Company scientists in 1983 (52). Subsequent chemical modification of this early lead soon produced a simpler, slightly more potent analog, RH-5849 (Figure 1), which had commercial-level activity versus a modest range of larval lepidopteran, coleopteran, and dipteran pest species (2). Although RH-5849 was eventually superseded by other more potent and cost-effective bisacylhydrazine analogs, it was the subject of most of the symptomatological (M Thirugnanam, personal communication) and physiological work on this series (137, 140). It was also the first bisacylhydrazine to be extensively tested in the field (2) and has been the subject of a previous review (87).

RH-5992 (tebufenozide; Figure 1) (52), a substituted analog of RH-5849 announced in 1992 (47), is currently marketed worldwide under the tradenames MIMIC®, CONFIRM®, and ROMDAN®. It is significantly more toxic than RH-5849 to lepidopteran larvae but, unlike RH-5849, is generally devoid of toxicity to non-lepidopteran species, including a wide range of important predators and parasitoids.
RH-0345 (halofenozide; Figure 1) has an overall insect control spectrum somewhat similar to that of RH-5849 but with significantly accentuated soil-systemic efficacy against scarabid beetle larvae, cutworms, and webworms (105). This compound has been introduced for use on turfgrass and ornamentals in the United States by a joint venture of Rohm and Haas Company and American Cyanamid Company (RohMid LLC, personal communication) under the tradename MACH 2®.

RH-2485 (proposed name methoxyfenozide; Figure 1), announced in 1996 and the newest member of the bisacylhydrazine class, is under development by Rohm and Haas Company (69). This compound is significantly more active than tebufenozide, acting against a wide range of lepidopteran pests of cotton (58), corn (127), and other major agronomic crops (69), yet it appears to retain tebufenozide’s high degree of safety with respect to non-target organisms.

**Mode of Action**

**Larvicidal Effects** The toxicity and the pest control spectrum of the three leading bisacyldrazines (tebufenozide, halofenozide, and RH-2485) vary,
although the toxic symptomatology induced by these compounds is very similar. Because of the early discovery of RH-5849, most of the initial mode of action and efficacy research has been done with this compound. However, all three compounds have a similar mode of action (53, 69).

The first evidence that RH-5849 acts as an ecdysteroid agonist in intact insects came from Wing et al (140). RH-5849 was 30 to >670 times more effective than 20E in inducing lethal molts in all larval stages of *M. sexta* (140). This effect was not due to endogenous levels of ecdysteroids because it could be produced in ligated larvae lacking prothoracic glands. In fact, in this study and a subsequent study on the beet armyworm, *Spodoptera exigua*, and the Colorado potato beetle, *Leptinotarsa decemlineata* (113), ingestion of RH-5849 actually resulted in a decrease in hemolymph ecdysteroid titers as compared with those of control larvae. Tebufenozide produced this effect in *S. exigua* larvae but not in the larvae of *L. decemlineata* (113), which reflects its lepidopteran specificity. The reduction of ecdysteroid titers in bisacylhydrazine-susceptible insects may be due to induction of enzymes involved in the metabolic inactivation of ecdysteroids, as demonstrated for *M. sexta* with RH-5849 and 20E (135). Growth and development of larvae of the tomato moth, *Lacanobia oleracea*, were not affected following ingestion of diet containing 400 ppm 20E (10). However, in the same study, ingestion of diet containing 1 or 10 ppm tebufenozide or RH-5849, respectively, by larvae resulted in premature lethal molts.

The bisacylhydrazines have been tested in larvae and adults from at least 16 different insect orders (24; GR Carlson, unpublished results). Members of most insect orders are unaffected, but in almost all cases where these compounds have produced lethal effects (mostly lepidopteran, dipteran, and coleopteran larvae), the symptoms have been similar to those expected from a state of ecdysteroid excess, called hyperecdysionism (134). Although a lethal molt can be induced in all larval stages of susceptible insects (127, 140), this effect, in some cases, may be dependent upon when the bisacylhydrazines are ingested during the larval stadium. For instance, in fifth and sixth larval stages of the spruce budworm, *Choristoneura fumiferana*, oral doses of tebufenozide induced lethal molts in 100% of the larvae only when ingested well before the appearance of the endogenous molt-inducing ecdysteroid peak (before day three in the fifth instar and before day four in the sixth instar) (91). When tebufenozide was administered any time later in either of the two stages, over 90% of the larvae underwent a normal molt but followed with another incomplete lethal molt immediately thereafter.

Lepidopteran larvae generally stop feeding within 4–16 h after ingestion of toxic doses of either RH-5849 or tebufenozide (98, 113, 126, 140). By this time the molting process is initiated (98, 116, 126), and, by 24 h, intoxicated larvae prematurely slip their old head capsules in an attempt to ecdyse (Figure 2). However, they are unable to do so, probably because of sustained effective levels
of RH-5849 or tebufenozide in the hemolymph and the epidermis (97, 113; TS Dhadialla & C Thompson, unpublished data). The presence of the effective titers of ecdysteroid agonists in the hemolymph results in the lack of eclosion hormone release (97), which, during a normal molt, occurs only after a decline in 20E titer to near basal level (128).

Examination of the newly cuticularized head and mouth parts under the slipped head capsule of bisacylhydrazine-intoxicated larvae reveals a lack of sclerotization and tanning of the new cuticle (Figure 2; see also 98). In addition, such larvae extrude their hind guts and suffer loss of hemolymph and molting fluid, which results in desiccation and ultimate death. Similar effects have been demonstrated in a number of lepidopteran larvae with RH-5849 and tebufenozide (40, 113, 139, 140) and with RH-5849 in coleopteran larvae (82, 113, 140).

**Figure 2** Scanning electron micrographs of second larval instars of the southern armyworm, *Spodoptera eridania*, taken 24 h after treatment. Panels A and C are lateral and ventral views, respectively, of control-treated larvae. Notice the well-sclerotized cuticle and mouth parts. Panel B is a lateral view of larva treated with 1-ppm tebufenozide, showing slipped head capsule (SHC) and the unsclerotized new cuticle (NC) over the head. Panel D is a ventral view of tebufenozide-treated larva whose slipped head capsule was removed to reveal the unsclerotized mouth parts, which are unsuitable for feeding damage. The white bar at the bottom of each panel represents 100-µm scale.
The disruptive effects of tebufenozide on cuticle formation have been studied at the ultrastructural level in the beet armyworm, *S. exigua* (116), and the spruce budworm, *C. fumiferana* (98). In intoxicated larvae, formation of the new cuticle was incomplete because the new procuticle was either absent or contained a very low number of endocuticular lamellae. In addition, the epidermal cells showed increasing signs of degeneration. A double cuticle, separated by an ecdysial space, was present 6–48 h after treatment. Tateishi et al (126) observed that, in isolated larval abdomens of the common cutworm, *Spodoptera litura*, RH-5849 also induced a double cuticle, which signifies an attempt to molt. However, the cuticle formed with RH-5849 was thinner than that induced with 20E, which suggests a deficiency in deposition of the endocuticular lamellae.

The disruptive effects of bisacylhydrazines on cuticle are produced differently from those produced by benzoylphenylureas, which specifically inhibit chitin synthesis (99). Moreover, unlike the benzoylphenylureas, which during larval development manifest their effects at the time of the natural molt, bisacylhydrazines can induce lethal molt attempts in susceptible insects at any time prior to the natural molt.

Besides having larvicidal effects, RH-5849 and tebufenozide have also been shown to reduce egg production in various target lepidopteran, coleopteran, and dipteran insects (2, 82, 114, 138, 140). Both tebufenozide and RH-2485 also have ovicidal activity against the European corn borer, *Ostrinia nubilalis* (127). Tebufenozide was also shown to disrupt normal spermatogenic processes in several lepidopteran species (15, 37, 41).

**Tissue and Cellular Effects** Experiments using insect tissues/organs or cells in culture demonstrate that bisacylhydrazines act by a similar mode of action as 20E. In holometabolous insects, imaginal disks continue to stay undifferentiated until the metamorphic molt. At this time, they evaginate and develop into wings, legs, and other adult structures, in response to increasing 20E titer. This evagination response in wing disks from lepidopteran and coleopteran larvae could be induced in vitro with both ecdysteroids and bisacylhydrazines but with varying potencies (111, 113, 115). Tebufenozide was much more effective than 20E in inducing evagination of lepidopteran imaginal wing disks.

In spite of the fact that tebufenozide is not toxic to coleopteran insects, evagination of imaginal wing disks from *L. decemlineata* could be induced in vitro when they were incubated in the presence of high concentrations of tebufenozide (115). Interestingly, unlike the bisacylhydrazines, which have a higher potency in lepidopteran wing disk preparations, 20E produced the evagination response in imaginal wing disks from both *L. decemlineata* and *S. exigua* at similar concentrations (113).
RH-5849 has ecdysteroid agonist activity in other tissue culture systems as well. During metamorphosis in holometabolous insects, cellular disintegration and/or reorganization is mediated by lysosomal activity triggered by 20E. RH-5849 stimulated acid phosphatase activity in in vitro cultured fat body from ligated larvae of the rice moth, *Corcyra cephalonica* (4). At a concentration much lower than ecdysone (E) or 20E, RH-5849 elicited a 50-fold increase in ecdysone 20-monooxygenase activity in midguts of head- and thorax-ligated last larval instars of *M. sexta* (62).

Further evidence that RH-5849 (137) and tebufenozide (78) act as ecdysteroid agonists at the cellular and biochemical levels has been demonstrated using *D. melanogaster* Kc cells. The cellular responses consisted of formation of cell processes and inhibition of cell proliferation. At the biochemical level, endogenous acetylcholinesterase activity (137), as well as transient expression of the luciferase gene under an ecdysteroid inducible promoter (78), was inducible with both 20E and bisacylhydrazines. In these experiments, 20E was over 100-fold more active than RH-5849. Effects on cell growth and morphology have also been observed with RH-5849 and tebufenozide in a cell line (IAL-PID2; 70) that was derived from the imaginal wing disks of *P. interpunctella* (70, 111) and in two other cell lines derived from the forest tent caterpillar, *Malacosoma disstria*, and the spruce budworm, *C. fumiferana* (117). In both cases, tebufenozide was about 100-fold more active than RH-5849. Morphological changes in the multicellular vesicular cell line from *C. tentans* that were inducible with 20E have also been observed with RH-5849 (119), as well as with tebufenozide (94).

At the biochemical level, RH-5849 stimulated 14C-N-acetylglucosamine uptake in cells derived from the imaginal wing disks of *P. interpunctella* (IAL-PID2; 70) but at much higher concentrations than 20E (111). Similarly, enhancement of N-acetylglucosamine incorporation into in vitro cultured integuments of *Chilo suppressalis* was obtained both with 20E and diacylhydrazines (88). In contrast, both 20E and RH-5849 inhibited chitin synthesis in *C. tentans* cells (119). RH-5849 also increased acetylcholinesterase activity in these cells (119). Tebufenozide, like 20E, was also shown to induce cell differentiation, arrest cell growth, and inhibit chitin synthesis in *C. tentans* cells, with relative potency of tebufenozide $\gg$ 20E $>$ RH-5849 (94). Furthermore, Retnakaran et al (97) demonstrated that transcripts of ecdysteroid-inducible genes in larval *M. sexta* epidermis could also be expressed with tebufenozide. Similar induction of another 20E-inducible transcription factor (CHR3) was observed in the midgut, fat body, and epidermal tissues of *C. fumiferana* (91). These studies indicate that bisacylhydrazines have the same mode of action as 20E.

In order to conclude that two different compounds have the same mode of action, it is important to demonstrate that their effects are mediated via
interaction with the same molecular target. Both 20E and RH-5849 and tebufenozide competitively displaced tritiated ponasterone A (PoA) bound to cytosolic protein extracts from *D. melanogaster* Kc (53, 78, 137) and *C. tentans* (94, 119) cells, respectively. Generally, tebufenozide displaced tritiated PoA with much greater affinity than 20E, which in turn was a better competitor than RH-5849.

Both RH-5849 and tebufenozide also compete with tritiated PoA for binding to protein extracts from a cell line derived from imaginal wing disks of the lepidopteran, *P. interpunctella* (53, 139). Competitive displacement of radio-labeled PoA has also been used to demonstrate indirectly specific binding of ecdysteroids and bisacylhydrazines to presumed ecdysteroid receptors in intact imaginal wing disks from lepidopteran and coleopteran larvae cultured in vitro (113, 115). The equilibrium dissociation constants (Kd) for PoA, tebufenozide, 20E, and RH-5849 binding in wing disks of *S. exigua* were 2.5, 13.4, 179, and 582 nM, respectively (113).

The relative binding affinity of ecdysteroids and bisacylhydrazines has also been investigated in intact imaginal wing disks from *L. decemlineata* (115). The concentrations of PoA, tebufenozide, 20E, and RH-5849 required to displace 50% of tritiated-PoA in wing disks were 1, 316, 425, and 740 nM, respectively. Although tebufenozide is not toxic to coleopteran larvae (113), it did compete with tritiated PoA and induced evagination of *L. decemlineata* imaginal wing in vitro but at very high concentrations (115).

The binding affinities of PoA, 20E, and bisacylhydrazines to putative ecdysteroid receptor complexes in cytosolic extracts from *D. melanogaster* Kc cells, nuclear extracts from the *P. interpunctella* IAL-PID2 cells, or embryonic cell lines of *L. decemlineata* (30) and cotton boll weevil, *Anthonomus grandis* (123), have been determined (27). Whereas bisacylhydrazines bind to ecdysteroid receptor proteins in cellular extracts from different orders of insects with very different affinities (Kd), PoA binds with similar affinities (Table 1). For example, tebufenozide and RH-2485 bind to proteins in *P. interpunctella* nuclear extracts with affinities similar to or greater than that of PoA, but they bind with much lower affinities to proteins in extracts from non-lepidopteran cell lines. The high binding affinity of tebufenozide and RH-2485 to proteins in nuclear extracts of lepidopteran cell lines correlates very well with their selective toxicity to lepidopteran pests.

The availability of cloned DNA sequences coding for EcRs and USPs from several dipteran and lepidopteran insects (20, 38, 60, 61, 64, 65, 90, 125, 129) has made it possible to determine that tebufenozide competes with tritiated PoA for binding to EcR/USP heterodimers produced by in vitro transcription and translation using rabbit reticulocyte lysates (TS Dhadialla & D Cress, unpublished data). While tritiated PoA bound in vitro produced EcR/USP
### INSECTICIDE INSECT HORMONE MIMICS

#### Table 1

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Drosophila Kc</th>
<th>Plodia</th>
<th>Leptinotarsa</th>
<th>Anthonomus</th>
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<td>60</td>
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<td>40</td>
<td>247</td>
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<td>129</td>
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<td>3</td>
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<td>ND</td>
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*Extrapolated values; ND—not determined.

complexes of the dipterans, *D. melanogaster* and *Aedes aegypti*, and the lepidopteran, *C. fumiferana*, with similar affinity (0.8, 2.8, and 3 nM, respectively), the $K_d$ for tebufenozide for the same heterodimers was 336, 28, and 0.5 nM, respectively.

These results provide compelling evidence that the bisacylhydrazines act as mimics of PoA and the insect molting hormone, 20E, and manifest their effects via interaction with the EcR/USP receptor complex. However, their ability to induce lethal molts in susceptible intoxicated larvae is due to a combination of their ecdysone agonist activity and persistence in tissues. Therefore, although the bisacylhydrazines permit the expression of genes and behavioral events that are dependent upon the presence of 20E, those that are dependent upon the absence of 20E, such as the expression of the dopa-decarboxylase enzyme for tanning of the new cuticle and the ecdysis behavior, are prevented because of the persistence of the bisacylhydrazines in the tissues (97, 98).

### Field Efficacy of the Bisacylhydrazines

Tebufenozide is highly effective in the field versus *Cydia pomonella* and miscellaneous leafrollers in apples (17, 47, 54, 131), *S. exigua* in cotton, *C. fumiferana* and *Lymantria dispar* in forestry, *Lobesia botrana* in grapes (47), *Cnaphalocrocis medinalis* and stemborers in rice (14), various leafrollers in treefruit (47, 122), *Diatraea saccharalis* in sugarcane (104), and *S. exigua*, *Helicoverpa* spp., and numerous other lepidopteran pests in vegetables and ornamentals (47). Use rates typically range between 100 and 300 g of active ingredient/hectare, depending on the target crop and pest (86).

Halofenozide applied at 1.75 to 2.0 pounds of active ingredient/acre, as a surface spray or as broadcasted granules, is highly effective against the soil-dwelling larval stages of scarabaeid beetles such as *Popillia japonica*,...
Phyllophaga spp., Cyclocephala spp., and Hyperodes spp. (48, 93), as well as various soil- or sod-dwelling caterpillars such as cutworms and webworms (105, 110). Halofenozide was effective on all stages of *P. japonica*, but its effects were stage dependent for the European chafer, *Rhizotrogus majalis*, and the oriental beetle, *Anomala orientalis* (22). Generally, the effects were more pronounced when applications were made in the egg or first larval stages. RH-2485 has high field efficacy against caterpillar pests of apple, corn, cotton, grape, rice, treefruit, and vegetables (69).

**Safety of the Bisacylhydrazines to Non-Target Invertebrates**

Tebufenozide is selectively toxic to lepidopteran pests. It has been shown to be essentially non-toxic to a wide range of coleopteran, homopteran, orthopteran, hemipteran, and dipteran pests (86, 112, 113). However, it has very modest toxicity [lethal concentration (LC$_{50}$) = 3–6 ppm; RJ Ramsay, unpublished observation] to certain mosquito larvae. Tebufenozide is safe for pollinators such as the honey bee, *Apis mellifera* (47); various predatory insects/arthropods including lacewings (86), predatory beetles (54), predatory bugs (113), spiders (86), and predatory mites (8, 47, 86, 131); and a number of important hymenopteran parasitoid species (12, 13, 59) and detritus-feeding soil insects (1). This unusual level of safety to non-target arthropods has made tebufenozide a useful tool in lepidopteran-directed IPM programs.

RH-2485 seems to share tebufenozide’s caterpillar-selective attributes. It has low reported toxicity to a large range of non-lepidopteran pests and is safe for *A. mellifera*, as well as several species of predatory mites and hymenopterous parasitoids (69). Less is known about halofenozide, but, based on its reported narrow pest control spectrum, its reported safety for *A. mellifera*, and its structural and mechanistic similarity to the other two bisacylhydrazines, it is expected to have low toxicity to non-target arthropods.

Tebufenozide has been reported to have low acute toxicity to several species of crustaceans and 11 species of aquatic insects (67, 86). Tebufenozide, halofenozide, and RH-2485 have comparably low acute toxicity to *Daphnia magna* (69, 86, 105). All three compounds are essentially non-toxic to earthworms (86, 105).

**Safety of the Bisacylhydrazines to Vertebrates**

The leading bisacylhydrazines have low acute toxicity to mammals, birds, and fish (47, 69, 86, 105). They are non-mutagenic in a variety of standard in vitro and in vivo laboratory assays and cause no developmental and reproductive toxicity in subchronic studies. Tebufenozide was completely non-oncogenic in chronic feeding studies in both mice and rats (86).
NON-TERPENOIDAL JUVENILE HORMONE ANALOGS AS INSECTICIDES

Since the early 1970s, numerous analogs of JH have been tested for insecticidal activity (96). Most of the early analogs resemble JH in their basic terpenoid structure. The most active ones, such as methoprene (Figure 3) and hydroprene (121), however, lack the epoxide function present in JH (Figure 3; 96). More recently, several highly active compounds that have less apparent similarity to juvenile hormones have been synthesized: fenoxycarb (registered as INSEGAR®, LOGIC®, TORUS®, PICTYL®, and VARIKIL®; Roche/Maag; 29), pyriproxyfen (registered as KNACK®, SUMILARV®, and ADMIRAL®; Sumitomo Chemical Co.; 46), and diofenolan (CGA 59205; AWARE®; Ciba Geigy; 107) (Figure 3). Some effects of fenoxycarb and pyriproxyfen have been reviewed (43, 81).

Whole Organism Effects

In holometabolous insects, the commitment for a larval-pupal molt is induced by 20E in the absence of JH (102). Therefore, persistence of JH or JHA during that time results in a supernumerary larva, and, depending upon the dose and time of application, permanent larvae and larval-nymphal, larval-pupal, or larval-adult intermediates that are unable to give rise to normal adults.

![Figure 3](image-url) Chemical structures of the juvenile hormones, terpenoidal (methoprene), and non-terpenoidal (fenoxycarb, pyriproxyfen, and diofenolan) juvenile hormone analogs.
In the European corn borer, *O. nubilalis*, application of fenoxycarb during the second to fourth larval instars had no effect on the duration of these instars. However, the duration of the resulting fifth instars increased significantly (39). Applications of fenoxycarb in the fifth instar produced different effects, depending upon the dose and the timing of application, which resulted in production of supernumerary or permanent larvae, or of larval-pupal intermediates. Treatment of *C. fumiferana* larvae with fenoxycarb resulted in larval-pupal intermediates with precocious evagination of wing disks and production of deformed pupae (49, 83). Related morphogenetic effects have been observed with fenoxycarb for *Heliothis virescens* (73); leafroller, *Adoxophyes orana* (17); and light-brown apple moth, *Epiphyas postvittana* (74).

Hatakoshi et al (46) found that pyriproxyfen was much more potent in inducing supernumerary larvae than methoprene and JH I when injected into last larval-stage *S. litura*. Interestingly, in this study, both pyriproxyfen and methoprene suppressed normal hemolymph ecdysteroid titers and caused the ecdysteroid titers to appear 12–24 h earlier than normal.

Adult emergence was completely prevented in the lesser mealworm, *Alphitobius diaperinus*, when it ingested poultry food containing 0.05-ppm fenoxycarb (32).

### Reproductive and Developmental Effects

Application of fenoxycarb to the fifth instar of the German cockroach, *Blatella germanica*, not only induced morphological deformities but also induced sterility of adults. The sterility seems to have been transferred from treated males mated to untreated females, which suggests effects on sperm (63). Similar sterility effects were also obtained for *C. fumiferana* (49). INSEGAR® 25 (0.1%) affected larval development and adult reproductive ability of the greenhouse whitefly, *Trialeurodes vaporariorum* (84).

Topical application of fenoxycarb suppressed egg production by queens of the red imported fire ant, *Solenopsis invicta* (5), and reduced both egg production and hatching in the California fivespined ips, *Ips paraconfusus* (19). In *S. invicta*, fenoxycarb treatment of adult females suppressed growth of the follicular epithelium and nurse cells, inhibited yolk deposition within the ovaries, and promoted resorption of developing eggs (42).

Application of pyriproxifen to day-0 pupae of the tobacco cutworm, *S. litura*, inhibited oviposition but not development of eggs in adult females (45). Oviposition was inhibited due to the lack of release of an oviposition stimulating factor that was present in the hemolymph of mated untreated females. This illustrates the subtlety with which hormone analogs may disrupt one or more developmental processes in target insects.

Embryonic effects of fenoxycarb have been observed in the eggs of the eastern spruce budworm, *C. fumiferana* (49), and the cat flea, *Ctenocephalides felis*.
(72). Cat flea eggs exposed to fenoxycarb show disruption of the blastoderm with associated cellular and organelle disruption. Fenoxycarb also effected caste differentiation in social insects, which led to an imbalance of the castes in the colonies (reviewed in 43).

Mode of Action

CELLULAR AND BIOCHEMICAL EFFECTS In a number of insects, JHAs, like the natural JHs, can restore physiological and biochemical processes that are dependent upon the presence of JH, such as synthesis of the major egg yolk proteins, vitellogenins, or their uptake by the developing oocytes, which suggests a JH mode of action (see 142). In Locusta spp., in which JH III is the gonadotrophic hormone, high doses of this hormone with simultaneous injection of a JH esterase inhibitor were required to induce vitellogenin synthesis in allatectomized females (141). However, strong induction of vitellogenin synthesis was obtained with a single application of pyriproxyfen, fenoxycarb, and 7S-methoprene with 50% effective dose (ED50) values of 2 µg, 15 µg, and 30 µg, respectively (28, 31, and cited in 142). Topical application of methoprene or pyriproxyfen was 25- and 300-fold more active than JH I in inducing vitellogenin production in the short-day adults of the armyworm, Pseudaletia unipunctata, in which vitellogenin production was completely prevented by decapitation (23).

Although little is known about the molecular characteristics of the receptor proteins by which JHs or JHAs manifest their activity, the available evidence suggests that these compounds may exert their effects via different cellular binding proteins. The action of JH via membrane receptors has been investigated in great detail in the ovarian follicle cells of R. prolixus and L. migratoria. Whereas JH I binds in a specific and saturable manner to follicle cell membrane preparations from R. prolixus, neither JH II nor JH III exhibits biological activity or competes for binding (56). On the other hand, in a similar system from L. migratoria. JH I did not compete for binding with JH III (109). The above results suggest specific structural requirements for different JHs to act through membrane receptors in follicle cells from R. prolixus and L. migratoria. Interestingly, the photolabile methoprene diazoketone binds to a protein in the locust membrane preparations with a much lower molecular weight than that of the one that binds JH II and III (109).

The binding of radiolabeled JH I and a radio-iodinated analog of methoprene, iodovinylmethoprenol (IVMA), to nuclei isolated from the larval epidermis of fifth instars has been tested (103). Although methoprene competed for binding to radiolabeled IVMA, JH I did not. However, excess unlabeled IVMA did not compete for tritiated JH I binding either. These results imply that the JHAs and the natural JHs produce the same effects but through different receptor proteins, which can work independently or as heterodimeric or multimeric complexes.
Several reports (see below) suggest that the cytosolic or nuclear JH or JHA receptor protein(s) may be structurally related to the steroid receptor superfamily of proteins, including the thyroid hormone receptor. In support of this hypothesis, methoprene, hydroprene, and their acid derivatives were shown to interact with vertebrate retinoid X receptor (RXR) to transactivate a reporter gene via RXR response elements (44). Although methoprenic acid competed for binding with 9-cis-retinoic acid, the normal ligand for RXR, methoprene was ineffective, which suggests that the two ligands could be activating similar genes via different receptor proteins. As additional evidence, both farnesol and JH III induced activation of a reporter gene via interaction with an orphan vertebrate nuclear receptor, farnesoid X-activated receptor (FXR), and RXR heterodimer (36). Methoprene had no effect. Finally, thyroid hormones, which have some structural resemblance to fenoxycarb (142), induced JH-like effects (described above) on the follicle cells of L. migratoria in vitro (25). In this study, tri-iodothyronine, the effective vertebrate hormone, had an effect equal to that of JH III.

Insecticidal Efficacy

Unlike the bisacylhydrazines, the JHAs have a relatively broad spectrum of insect toxicity. Different formulations of fenoxycarb have been developed for different groups of pests; fenoxycarb has been used for the control of a number of coleopteran and lepidopteran pests of stored wheat (66) and rice (21). It is also effective against the pear sucker, Cacopsylla pyricola (118); summer fruit tortrix, Adoxophyes orana (cited in 43), fruit tree tortrix, Archips podana, and several other tortricid species (26); tufted apple bud moth, Platynota idaealis (55); codling moths, C. pomonella; and a number of leaf rollers, pear psyllids (16), and diaspidid scales (76). Fenoxycarb is also marketed as LOGIC® for the control of fire ants, as TORUS® for the control of fleas and cockroaches, and as PICTYL® for the control of mosquito larvae (cited in 43).

Pyriproxyfen is active against a number of mosquito species (35, 89) and as a feed-through compound in poultry, cattle, and swine for control of the housefly, Musca domestica, and the face fly, Musca autumnalis (79). It has also been reported to be active against a number of diaspidid and coccid scales (92); sweetpotato whitefly (57); greenhouse whitefly, T. vaporariorum; the green peach aphid, Myzus persicae; and pear psylla, Psylla pyricola (cited in 81).

Diofenolan, the newest aromatic JHA, has good activity against lepidopteran pests in deciduous fruit, citrus, grapes, and olives (124) and scale insects in pome fruit and citrus (107).

Effects on Non-Target Invertebrates and Vertebrates

The ecotoxicological effects of the JHAs have been reviewed (43, 81, 96). Both fenoxycarb and pyriproxyfen have measurable toxicity to a number of dipteran,
coleopteran, and hemipteran predators and parasitoids of the scale insects (75). However, *Aphytis holoxanthus*, the ectoparasite of the California red scale and Florida wax scale, was insensitive to pyriproxyfen (92). Pupation in the predatory coccinellid, *Chilocorus bipustulatus* L., was inhibited when larvae fed on the scale insect, *Chrysomphalus aonidum*, dipped into 0.025% active ingredient fenoxycarb (cited in 43). Fenoxycarb was also toxic to *Colpoclypeus florus*, which is a parasitoid of *A. orana* and *Pandemis heparana* (cited in 43).

Fenoxycarb is harmless to adult bees. However, effects on brood development were observed as a result of workers bees feeding pollen containing fenoxycarb residues to larvae (133).

Last instars of certain aquatic insects are susceptible to JHAs. Fenoxycarb produced various morphogenetic effects in the heteropteran *Notonecta unifasciata* and in the dragonflies *Anax junius* and *Pantala hymenaea* (80). Similar effects were obtained with pyriproxyfen in last instars of the dragonfly *Orthetrum albistylum* and the midge *Chironomus yoshimatsui* (81).

Some information on the vertebrate toxicology of fenoxycarb has been reviewed (43). In general, the JHAs have low acute toxicity to fish, birds, and mammals. For example, the acute 50% lethal dose (LD₅₀) for fenoxycarb in rat is >10 g/kg (oral), >2 g/kg (dermal), and >480 mg/m³ for 4-h exposure (inhalation) (43).

POTENTIAL FOR RESISTANCE TO NON-TERPENOIDAL AND NON-ECDYSTEROIDAL INSECTICIDES

When Williams (134) proposed the use of compounds with insect hormone activity as “third generation insecticides,” insects were believed to be unable to develop resistance to molecules that mimic their own hormones. This presumption has not proved true. Several instances of JHA resistance have been documented. For example, resistance to pyriproxyfen has been observed in the sweetpotato whitefly, *Bamesia tabaci* (51), and resistance to methoprene has been shown to take place at the receptor level in methoprene-resistant mutants of *D. melanogaster* (136).

In a recent report, Sauphanor & Bouvier (106) observed a loss in efficacy of tebufenozide in a laboratory strain of codling moth, *C. pomonella*, originally collected in southeast France and amplified for resistance to the chitin synthesis inhibitor, diflubenzuron. However, in another study with a different codling moth strain resistant to benzoylphenylurea, no such loss in tebufenozide susceptibility was observed (95). Likewise, no loss in tebufenozide susceptibility was found in an organophosphate-resistant strain of the tufted apple bud moth, *P. idaeusalis* (9), and a pyrethroid-resistant strain of the cotton leafworm, *S. littoralis* (58). Moreover, in the latter study, there was no loss in RH-2485 susceptibility in the resistant strain.
Although it is probably inevitable that insects will ultimately develop resistance to any new insecticide, particularly when it is misused, resistance to the newer JHAs (54) and the bisacylhydrazines ought to be delayed by implementation of resistance management programs. Both tebufenozide and RH-2485 should be particularly well suited for use in resistance and integrated pest management programs because of their novel mode of action and high level of safety to predators and parasitoids.

CONCLUSIONS AND FUTURE PROSPECTS

As our knowledge of the precise mode of action of JH and 20E increases, we should expect the discovery of newer chemicals that mimic the action of these hormones. Although the molecular characterization of the JH receptors has been elusive, it nevertheless seems clear that JHs and JHAs could act via different receptor proteins that are conceivably members of dimeric or multimeric receptor complexes. Hence, potential may exist for the discovery of more highly target-selective juvenoids, an outcome that has so far eluded pesticide scientists.

On the other hand, the high target pest specificity of the bisacylhydrazines seems paradoxical because most insects use 20E as the molting hormone. Nevertheless, the fact that this specificity exists suggests that it may be possible to discover ecdysteroid agonists with new chemistries and different target pest specificities. Recently, two additional new non-steroidal ecdysteroid agonists have been discovered. The first is a benzamide, 3,5-di-tert-butyl-4-hydroxy-N-isobutyl-benzamide (DTBHIB; Figure 4) discovered by Sumitomo Company (77). The second is a naturally occurring iridoid glycoside, 8-O-acetylharpagide (Figure 4), isolated from Ajuga reptans by Merck Research Laboratories (33).

Figure 4  Chemical structures of non-steroidal ecdysone agonists, 3,5-di-tert-butyl-4-hydroxy-N-isobutyl-benzamide (DTBHIB) and 8-O-acetylharpagide.
INSECTICIDE INSECT HORMONE MIMICS 563

Both compounds reportedly induce 20E-like morphological changes in Kc cells and competitively displace tritiated PoA from Drosophila ecdysteroid receptors with potencies similar to that of RH-5849. However, the insecticidal properties of these compounds have not yet been described.

A different approach would be to discover chemicals that would disrupt either JH or ecdysteroid biosynthesis or exhibit anti-JH or anti-ecdysteroid effects. Research along these lines has already resulted in the discovery of compounds such as the arylpyridyl-thiosemicarbazones and the 1,5-disubstituted imidazoles, which inhibit JH biosynthesis (6, 68, 130), and analogs of metyrapone, which have anti-ecdysteroid activity in insects (7). Such chemistries may eventually lead to useful insecticidal compounds.

Finally, with the availability of cloned DNA sequences encoding receptors that mediate the action of insect hormones, we should expect greater use of in vitro target site assays in combination with traditional whole insect assays to discover new compounds that mimic hormone action. The significant advances in the use of combinatorial chemistry and high throughput target site-directed assays will allow the agrochemical industry to make use of these technologies to discover new insecticides with insect hormone action.

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