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Review

ECDYSONE AGONISTS: NEW INSECTICIDES WITH NOVEL MODE OF ACTIONS

AGONIS EKDISON: INSEKTISIDA BARU YANG MEMPUNYAI CARA KERJA BERBEDA DENGAN INSEKTISIDA SEBELUMNYA

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INTISARI

Perkembangan resistensi serangga terhadap insektisida merupakan salah satu faktor pendorong utama bagi industri untuk menemukan insektisida baru. Kesadaran dan permintaan masyarakat untuk tersedianya insektisida yang ramah terhadap lingkungan telah mengubah arah pengembangan dari insektisida yang berspektrum luas menjadi insektisida yang bersifat selektif. Perubahan ini telah membawa para peneliti untuk mencari sasaran baru selain sistem saraf pusat serangga. Senyawa pengatur pertumbuhan serangga (dikenal sebagai insect growth regulator,IGR*) adalah kelompok insektisida yang bersifat lebih selektif dibandingkan dengan insektisida konvensional, dan agonis ekdison merupakan anggota kelompok* IGR *terbaru yang saat ini sudah dipasarkan, contoh tebufenozide, methoxyfenozide, dan halofenozide. Agonis ekdison menempel pada reseptor ekdisteroid dan akan menimbulkan reaksi yang sama dengan hormon molting 20-hidroksiekdison. Hal ini selanjutnya akan memberikan tanda bagi larva atau nimfa untuk memasuki periode molting yang bersifat prematur dan akan mengakibatkan kematian pada serangga yang bersangkutan. Agonis ekdison dikembangkan untuk memenuhi kriteria selektifitas yang tinggi. Tebufenozide dan methoxyfenozide efektif untuk mengendalikan hama Lepidoptera, sedangkan halofenozide efektif untuk hama Coleoptera. Selektifitas agonis ekdison diantaranya disebabkan oleh perbedaan afinitas reseptor pada serangga dari bangsa yang berbeda. Hal ini menjadikan agonis ekdison mempunyai potensi yang lebih besar untuk dikombinasikan dengan metode pengendalian yang lain dalam program pengendalian hama terpadu di ekosistem pertanian.*

Kata kunci: insektisida baru, selektifitas, agonis ekdison

ABSTRACT

Development of insect resistance to insecticide has been the major driving force for the development of new insecticides. Awareness and demand from public for more environmentally friendly insecticides have contributed in shifting the trend from using broad spectrum to selective insecticides. As a result, scientists have looked for new target sites beyond the nervous system. Insect growth regulators (IGRs) are more selective insecticides than conventional insecticides, and ecdysone agonists are the newest IGRs being commercialized, e.g. tebufenozide, methoxyfenozide, and halofenozide. Ecdysone agonists bind to the ecdysteroid receptors, and they act similarly to the molting hormone 20-hydroxyecdysone. The binding provides larvae or nymphs with a signal to enter a premature and lethal molting cycle. In addition, the ecdysone agonists cause a reduction in the number of eggs laid by female insects. The ecdysone agonists are being developed as selective biorational insecticides. Tebufenozide and methoxyfenozide are used to control lepidopteran insect pests, whereas halofenozide is being used to control coleopteran insect pests. Their selectivity is due to differences in the binding affinity between these compounds to the receptors in insects from different orders. The selectivity of these compounds makes them candidates to be used in combinations with other control strategies to develop integrated pest management programs in agricultural ecosystems.

Key words: new insecticides, selectivity, ecdysone agonists

INTRODUCTION

Insecticides have played a key role in controlling many agricultural and medical insect pests. The types of insecticides used to control insect pests have followed the history of new insecticides being discovered (Brindley & Dicke, 1963; Brindley *et al.*, 1975; Ware, 1989). For example in corn, inorganic insecticides, such as arsenicals, were first used to control the European corn borer *Ostrinia nubilalis* (Hübner) in the early establishment of this species as a corn pest (Worthley & Caffrey, 1927). The discovery of synthetic organic insecticides led to growers switching to these insecticides to control *O. nubilalis* and the southwestern corn borer *Diatraea grandiosella* Dyar. A chlorinated hydrocarbon insecticide, DDT, was used to control these species (Bigger *et al.*, 1947; Cox *et al.*, 1956) before this insecticide was banned. A few organophosphate insecticides (EPN and diazinon) and carbamates (carbaryl and carbofuran) have been reported to be effective insecticides against these species (Harding *et al.*, 1968, Keaster & Fairchild, 1968). Conventional insecticides, including pyrethroids (permethrin, esfenvalerate), organophosphates (fonofos, chlorpyrifos, and methyl parathion), and carbamates (carbofuran and carbaryl) are currently used to control these species in several countries, such as USA (Munson & Bailey, 1996). Similar to corn, the use of insecticides in other crops has followed the same pattern. This trend is in part due to demand from the public for selective and environmentally friendly insecticides.

The effectiveness of insecticides for controlling insect pests depends on intrinsic factors, including the toxicity, type of formulation, systemic activity, and persistence, and extrinsic factors, including the application equipment and timing. Ineffectiveness of early arsenical insecticides was due to lack of satisfactory insecticide formulations and application equipment, and precise timing (Brindley & Dicke, 1963). Application of a granular insecticide with systemic activity applied over the row is often more favorable than that of a liquid insecticide with the same compound because a granular insecticide: 1) does not need mixing, 2) has less drift, 3) has less hazard to workers and non-target organisms, and 4) may control soil insect pests (Munson *et al.*, 1970; Straub, 1983; Mason *et al.*, 1996). Although improvement of the formulation and application techniques may minimize the impact on non-target organisms, the currently registered insecticides are mostly lethal to non-target organisms because of their broad spectrum of activity. For example, most conventional insecticides were lethal to the egg parasitoids, *Trichogramma* spp. (Stiner *et al.*, 1974; Bull & Coleman, 1985). Therefore, a need exists to develop selective biorational insecticides with minimal impact on non-target organisms. This shift has driven scientists to look for new target sites beyond the nervous system.

BIORATIONAL INSECTICIDES

Insect growth regulators (IGRs) are known as the third generation of insecticides that differ in their action from that of the first generation (naturally occurring rotenone and nicotine, kerosene, and the inorganic arsenicals) and the second generation (chlorinated hydrocarbons, organophosphates, and carbamates) of insecticides (Williams, 1967). Juvenile hormone (JH) mimics are the first commercially available IGR, followed by chitin synthesis inhibitors and ecdysone agonists. A few JH mimics have been registered and marketed for controlling insect pests; for examples: hydroprene for cockroaches, kinoprene for aphids and whiteflies, and fenoxycarb for fire ants. The first chitin synthesis inhibitor that was commercially available was diflubenzuron. This insecticide was registered in 1982 for gypsy moth, *Lymantria dispar* (L.), boll weevil,

Anthonomus grandis Boheman, and most forest caterpillars (Ware, 1989). Other benzoylphenyl ureas, such as teflubenzuron, triflumuron, and chlorfluazuron, have been registered in many countries, including Indonesia, for controlling several different insect pests. The ecdysone agonists are the newest IGRs being available in the market. Examples of this new insecticide group are tebufenozide, methoxyfenozide, and halofenozide. Tebufenozide and methoxyfenozide are commonly used for controlling lepidopteran insects, whereas halofenozide is for coleopteran insects.

ECDYSONE AGONISTS

Development. α-Ecdysone was first isolated and purified from pupae of the silkworm, *Bombyx mori* (L.), by Butenandt & Karlson (1954), and is a polyhydroxy sterol (Karlson *et al.*, 1965). α-Ecdysone is a prohormone that is converted into the active form β-ecdysone (20-hydroxyecdysone) by fat body and epidermal cells (Nijhout, 1994). 20-Hydroxyecdysone was first isolated from pupae of *B. mori* by Hoffmeister (1966). Shortly afterward, the first phytoecdysteroids, named ponasterones A, B, C, and D were isolated from a plant *Podocarpus nakaii* Hay and proved to have molting hormone activity (Nakanishi *et al.*, 1966). By 1974, 37 different phytoecdysteroids had been isolated (Hikino & Takemoto, 1974). The occurrence of phytoecdysteroids having similar activities with that of the molting hormone stimulated an intensive research to understand the role of these compounds in plants and their effect on insect host-relationship.

The inhibitive effects of 20-hydroxyecdysone, ponasterone A, and three synthetic ecdysone analogs on growth and reproduction of the house fly, *Musa domestica* L., using diet incorporation was first reported by Robbins *et al.* (1968). Then, Earle *et al.* (1970) reported that several ecdysone analogs inhibited larval development and egg production in *A. grandis*. These findings have showed the potential use of the ecdysteroids as insecticides. However, no insecticide has been developed based on the active steroids because: 1) they are expensive to synthesize due to their complex structures, 2) they are hydrophilic so they do not penetrate the insect cuticle, and 3) insects have the ability to eliminate ecdysteroids between molting (Koolman & Karlson, 1985; Hsu, 1991). Considering these problems, research in the last decade has focused on identifying non-steroidal compounds that mimic the action of 20-hydroxyecdysone. Up to now, three companies, Rohm and Haas (Spring House, PA, USA), Merck Research laboratories (Rahway, NJ, USA), and Sumitomo Chemical Co. (Takarazuka, Hyogo, Japan), had discovered non-steroidal ecdysone agonists from three different chemical classes.

In 1988, Rohm and Haas Company discovered that RH-5849 (1,2-diacyl-1-alkylhydrazine) (Fig. 1B) had ecdysone-like activity causing premature and lethal molting cycles in larvae of the tobacco hornworm, *Manduca sexta* (L.) (Wing *et al.*, 1988). RH-5849 is the prototype of non-steroidal ecdysone agonists. Although RH-5848 was 30 times less potent than was 20-hydroxyecdysone (Fig. 1A) to displace ponasterone A from its receptor extracted from the fruit fly, *Drosophila melanogaster* Meig. (Kc) cells, RH-5848 was 670 times more potent than was 20-hydroxyecdysone in inducing the molting of *M. sexta* larvae when these compounds were given orally (Wing, 1988; Wing *et al.*, 1988). Tebufenozide (RH-5992, N-tert-butyl-N-3,5-dimethylbenzoyl -N′-4-ethyl-benzoyl-hydrazine) (Fig. 1C) and methoxyfenozide (RH-2485, N-tert-butyl-N-3,5-dimethyl-benzoyl l-N′-3-methoxy-2-methyl benzoyl-hydrazine) (Fig. 1D) are other ecdysone agonists that have been reported to be more lethal against lepidopteran larvae than is

RH-5849. Tebufenozide was 60-75 times more lethal than was RH-5849 to the nutgrass armyworm, *Spodoptera exempta* (Walker), the beet armyworm, *S. exigua* (Hübner), and the cotton armyworm, *S. littoralis* (Boisduval) (Smagghe & Degheele, 1994a; b). Methoxyfenozide was 3-7 times more lethal than was tebufenozide to *S. littoralis* (Ishaaya *et al.*, 1995), the pandemis leafroller, *Pandemis pyrusana* Kearfott, and the obliquenbanded leafroller, *Choristoneura rosaceana* (Harris) (Brunner *et al.*, 1995), *O. nubilalis* (Trisyono & Chippendale 1997), and *D. grandiosella* (Trisyono & Chippendale 1998). Tebufenozide was the first ecdysone agonist to be available commercially (J. W. Long, personal communication).

In 1996, Elbrecht *et al.* (Merck Research Laboratories) isolated *Ajuga reptans* L. (Laminaceae) and identified a new class of non-steroidal ecdysone agonist, 8-*O*-acetylharpagide (Fig. 1E). The genus *Ajuga* has been known as producer of phytoecdysteroid, and isolation of a number of iridoid glycosides, including 8-*O*-acetylharpagide, has been reported early (Shimonura *et al.*, 1987; Takeda *et al.*, 1987; Assaad & Lahloub, 1988). A receptor binding assay using cMK9 cells, developed by transfection of *D. melanogaster* S2 cells with a plasmid containing the *Drosophila* EcR cDNA and a *Drosophila* metallothionein promoter (Koelle *et al.*, 1991), showed that 8-*O*-acetylharpagide and 20-hydroxyecdysone displaced ponasterone A from the receptor with 20-hydroxyecdysone being about 14,000 times more potent than was 8-*O*-acetylhargide.

In 1996, Mikitani (Sumitomo Chemical Co.) reported that 3,5-di-tert-butyl-4-hydroxy-N-isobutyl-benzamide (DTBHIB) (Fig. 1F) showed ecdysone-like activity in the Kc cells. Similar to RH 5849 and 8-*O*-acetylharpagide, DTBHIB was about 165 times less potent than was 20-hydroxyecdysone in displacing ponasterone A from the ecdysone receptor. These discoveries provide useful leads to find analogs with higher ecdysone agonist activity than that of their parent compounds.

Mode of action. Wing (1988) was the first to demonstrate, at the cellular level, that RH-5849 and 20-hydroxyecdysone bind and act through the same ecdysone receptor in Kc cells of *D. melanogaster*. The cells treated with these compounds showed similar effects, including formation of extended processes, clumping, and cessation of proliferation. Additional evidence that RH-5849 and 20-hydroxyecdysone cause similar effects was demonstrated when these compounds were given to the imaginal disc derived cell line from the Indian meal moth, *Plodia interpunctella* (Hübner) (Silhacek *et al.*, 1990), the midge, *Chironomus tentans* (Fabricius), cell line (Spindler-Barth *et al.*, 1991), and the embryonic cell line of *O. nubilalis* (Trisyono *et al.*, 2000). The other ecdysone agonists, 8-*O*-acetylharpagide (Elbrecht *et al.*, 1996) and DTBHIB (Mikitani, 1996), also caused similar effects on Kc cells as did by 20-hydroxyecdysone, including formation of the long processes, flattening of the cells, and inhibition of cell proliferation. These results showed that RH-5849, 8-*O*-acetylharpagide, and DTBHIB cause the typical effects of ecdysteroids at the cellular level (Courgeon, 1972; Cherbas *et al.*, 1980a).

The functional ecdysone receptors, e.g. in *D. melanogaster* and *B. mori*, are heterodimers containing the polypeptides EcR (ecdysone receptor) and USP (ultraspiracle) (Koelle *et al.*, 1991; Yao *et al.*, 1992; 1993; Swevers *et al.*, 1995). These subunits are incapable of binding to ligands. The formation of EcR-USP complex induces a conformation change in the EcR causing the complex becomes capable of binding to ecdysteroids and/or ecdysone response elements (EcREs), specific DNA sequences that regulate the expression of nearby genes. The binding of ecdysteroid and EcREs to EcR-USP

complex tranduces the ecdysone signal (Yao *et al.*, 1992).

At the genetic level, Retnakaran *et al.* (1995) showed that tebufenozide and 20-hydroxyecdysone acted similarly in regulating three genes in *M. sexta* epidermis during larval molting. These genes are the *Manduca* hormone receptor 3 (MHR3) gene, the larval endocuticle protein (LCP-14) gene, and dopa decarboxylase (DDC) gene. These compounds induced the MHR3 mRNA, suppressed the expression of LCP-14 gene, and induced the expression of DDC gene. The MHR3 gene is the steroid hormone receptor gene and expressed only during the molting (Pali *et al.*, 1992). The LCP-14 gene is repressed during the molt (Hiruma *et al.*, 1991). The DDC gene is expressed at the end of molting (Hiruma & Riddiford, 1985) and the expression of the DDC gene requires initial exposure to 20-hydroxyecdysone followed by a withdrawal of this molting hormone. However, treatment of tebufenozide caused a significant delay in the expression of this gene showing that tebufenozide has a longer effect than does 20-hydroxyecdysone.

The ecdysone agonists, RH-5849 and tebufenozide, bind to ecdysteroid receptors and provide larvae with a premature signal to enter a lethal molting cycle (Wing *et al.*, 1988; Retnakaran *et al.*, 1996; Smagghe *et al.*, 1996b, Trisyono & Chippendale 1998). Larvae of the spruce budworm, *Choristoneura fumiferana* (Clemens), received tebufenozide *per os* stopped feeding within 6 hr after treatment. Slippage of the old head capsule was observed within 24 hr after treatment. Even though the old cuticle split in the thoracic region, the larvae failed to completely ecdysed (Retnakaran *et al.*, 1996). Internally, tebufenozide caused a significant reduction in the formation of new endocuticular lamellae and the digestion of the old endocuticular lamellae in larvae of *C. fumiferana* and *S. exigua* (Retnakaran *et al.*, 1996; Smagghe *et al.*, 1996a). Because tebufenozide remains longer in the tissues than does 20-hydroxyecdysone, it causes a failure in the expression of genes that requires ecdysteroid withdrawal. This failure results in the inhibition of ecdysis, the formation of incomplete cuticle, or lack of tanning (Retnakaran *et al.*, 1996). Thus, death of treated larvae may be as a result of starvation due to malformation of the mouth parts or feeding cessation, or desiccation (Smagghe & Degheele, 1992; Retnakaran *et al.*, 1996).

The ecdysone agonists, RH 5849 and tebufenozide, caused a reduction in the number of eggs laid by females of several species of Coleoptera and Lepidoptera due to a decrease in the size of ovaries or the number of chorionated eggs, or inhibition of the formation of new ovarioles (Aller & Ramsay, 1988; Smagghe & Degheele, 1994a; Salem *et al.*, 1997; Sun & Barrett, 1999; Trisyono, 2000). These effects were similar to those caused by natural ecdysteroids (Robbins *et al.*, 1968).

In addition to its ecdysone-like activity, RH-5849 has neurotoxic effects by blocking the potassium channel causing prolong action potentials in insect nerves and muscles (Salgado, 1992a; b). Neurotoxic symptoms of RH-5849, including loss of balance, tremor, or paralysis, have been observed in several Coleoptera; for example, the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), the Mexican been beetle, *Epiláchna varivéstis* Mulsant (Aller & Ramsay, 1988), and the Japanese beetle, *Popilia japonica* Newman (Monthéan & Potter, 1992). Similar to RH-5849, tebufenozide caused tremor and paralysis in *L. decemlineata* (Samgghe & Degheele, 1994b). However, the neurotoxic activity of these ecdysone agonists was less prominent than was their ecdysone-like activity because the neurotoxic symptoms

existed only when these compounds were applied at a high concentration (Aller & Ramsay, 1988; Smagghe & Degheele, 1994b).

Selectivity. Although RH-5849 has a broader spectrum of activity than does tebufenozide, these compounds are more selective than are conventional insecticides. RH-5849 is lethal to several species of Coleoptera, Lepidoptera and Homoptera but it is significantly less lethal to several species of Blattaria, Orthoptera, Diptera, and Hemiptera (Wing *et al.*, 1988; Darvas *et al.*, 1992, Monthéan & Potter 1992; Smagghe & Degheele, 1994a). In contrast, its analog tebufenozide has been reported to be a selective for Lepidoptera (Heller & Mattioda, 1992; Smagghe & Degheele, 1994b; Dhadialla *et al.*, 1998; Pons *et al.*, 1999; Sun & Barret, 1999; Whiting *et al.*, 1999).

Thirteen different insect orders including beneficial, predatory, and parasitic have been reported to be unaffected by tebufenozide (Oakes, 1994). For example, Brown (1994; 1996) reported that tebufenozide had no effect on growth and development of the ectoparasitoid, *Hyssopus pallidus* Askew, and the endoparasitoid, *Ascogaster quadridentata* Wesmael, when they were fed with treated codling moth larvae, *Cydia pomonella* L. The endoparasitoid dies when the host's tissue deteriorates. Trisyono *et al.* (2000) reported that tebufenozide and methoxyfenozide was significantly less toxic to the lady beetle, *Coleomegilla maculata* DeGeer, than was carbaryl. In addition to its selectivity to beneficial arthropods, tebufenozide and methoxyfenozide are significantly less toxic to rats than other groups of insecticides such as carbaryl and chlorpyrifos. The oral LD_{50} values of tebufenozide and methoxyfenozide to rats are > 5000 mg/kg, whereas for carbaryl and chlorpyrifos are 264 mg/kg and 96-279 mg/kg, respectively (Anonymous, 1988; 1991; 1992a; 1992b). Furthermore, tebufenozide shows to be a soft insecticide to other non-target organisms (Table 1) (Anonymous, 1992a; Oakes, 1994).

Even though the selectivity of the ecdysone agonists are well documented, research to answer why they are selective is limited. Considering their mode of action, the selectivity of the ecdysone agonists may be due to differences in their binding affinity to the ecdysone receptors among insects from different orders. Using cultured imaginal wing discs, tebufenozide was found to have 60 times more affinity for the ecdysone receptors in *G. mellonella* than to those of *L. decemlineata* (Smagghe *et al.*, 1996b).

Table 1. Environmental toxicity of the ecdysone agonist tebufenozide to non-target organisms

Non-target organism	Toxicity
Bobwhite quail	Oral LD_{50}: >2150 mg/kg
Mallard duck	Dietary LC_{50}: >5000 mg/kg
Bluegill sunfish	96-h LC_{50}: 3.0 mg/L
Daphnia magna	48-h EC_{50}: 3.8 mg/L
Daphnia magna	21-d life cycle NOEL: 0.029 mg/L
Mysid shrimp	96-h LC_{50}: 1.4 mg/L
Tadpole	96-h LC_{50}: >100 ppm
Eastern oyster	96-h EC_{50}: 0.64 mg/L
Algae (Selanastrum)	120-h EC_{50}: >0.64 mg/L
Earthworm	NOEL: 1000 mg/kg

References: Anonymous 1992a; Oakes 1994

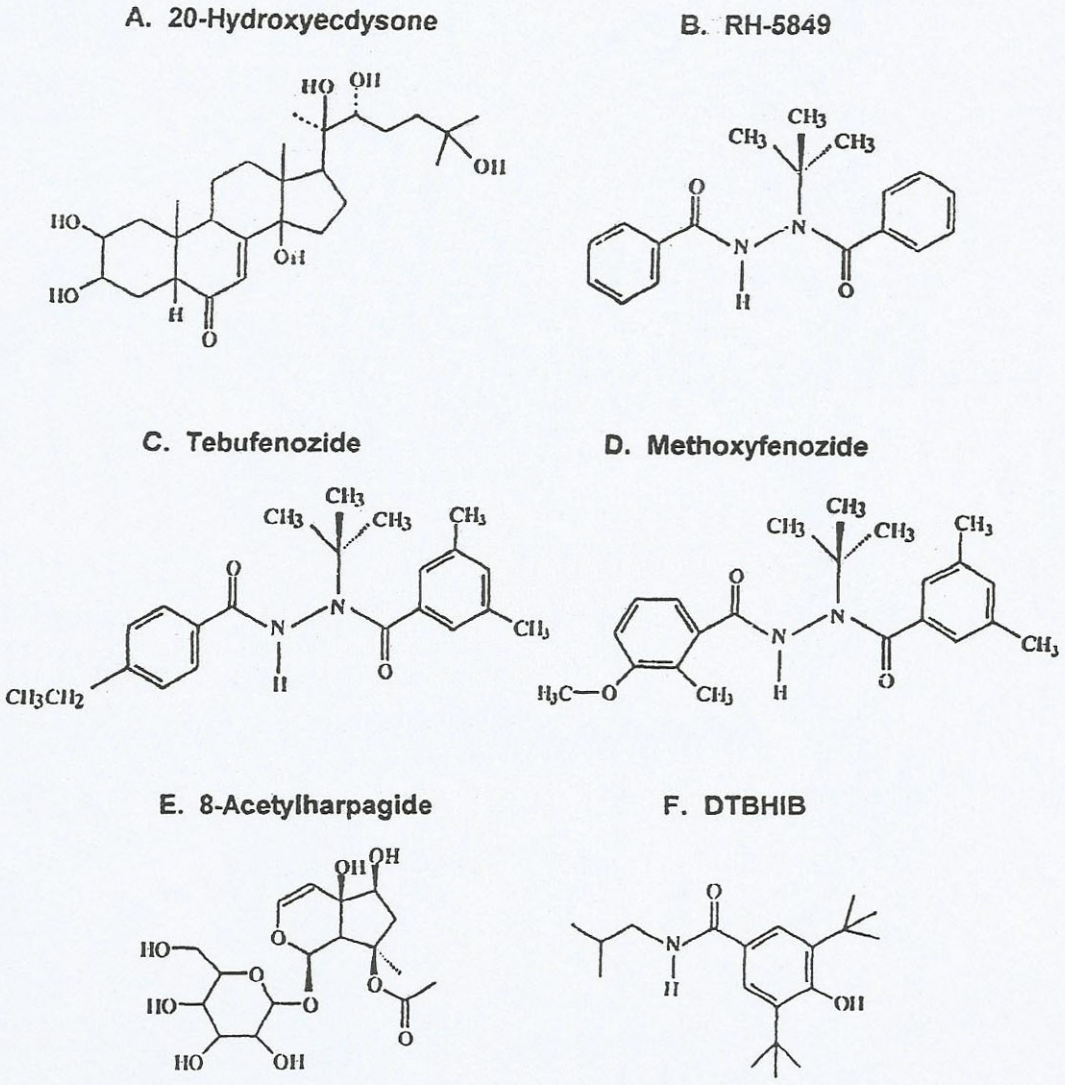

Fig. 1. The molting hormone 20-hydroxyecdysone (A) and the ecdysone agonists RH-5849 (B, Rohm dan Haas), tebufenozide (C, Rohm and Haas), methoxyfenozide (D, Rohm and Haas), 8-acetylharpagide (E. Merck), and DTBHIB (F, Sumitomo).

CONCLUSIONS

The ecdysone agonists have a novel mode of action by mimicking action of the molting hormone 20-hydroxyecdysone. With new mode action, ecdysone agonists may provide new tools for combating insects that have become resistant to other insecticides. In addition, the selectivity of ecdysone agonists, which is in part due to differences in the receptors among insects from different orders, may make them compatible with other control strategies in integrated pest management programs.

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