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Juvenoids: from basic research to practical use. A short review. (*)

INTRODUCTION

Crop losses caused by insect pests have reached, according to FAO data, incredible heights: 25% of wheat, 45% of rice, 30-35% of maize, fruit and cotton harvests throughout the world. Man has always attempted to control pests, and since World War II synthetic insecticides have been systemically used in plant protection. Yet three factors have restricted the use of pesticides: their high toxicity for non-target animals (including mammals), the increasing resistance of target insect pests to them and the increasing contamination of the environment by xenobiotics, including pesticides. It is thus necessary to develop an alternative method of pest control, and one of the promising ways to do this is the use of insect growth regulators, especially with analogues of juvenile hormones called juvenoids.

JUVENILE HORMONES AND JUVENOIDS

The source and structure of juvenile hormones are well known. There are at least four: JH 0, JH I, JH II, JH III (Mayer *et al.*, 1968; Judy *et al.*, 1973; Bergot *et al.*, 1980), the most common being JH III. Some other natural compounds with JH-activity have been isolated from various sources: farnesylmethylether from plants, yeast and insect excreta (Schmialek, 1961), juvabion and dehydrojuvabion from such conifers as *Abies pseudotsuga* (Sláma and Williams, 1965), sesamin from sesame oil (Bowers, 1968), echinolone from the American cone-flower *Echinacea angustifolia* (Jacobson *et al.*, 1975), and juvocimen from the sweet basil *Ocimum basilicum* (Bowers and Nishida, 1980). A listing of the chemical formulas of juvenile hormones and of the natural compounds with JH-activity is shown in figure IA.

Juvenile hormones are sesquiterpenoid compounds specific for Arthropods which regulate growth and metamorphosis by interaction with ecdysteroids (Williams 1956; Wigglesworth, 1936, 1963) and, in adults, the reproductive processes (Engelmann, 1970).

Juvenoids are true hormone mimetics possessing defined molecular size and

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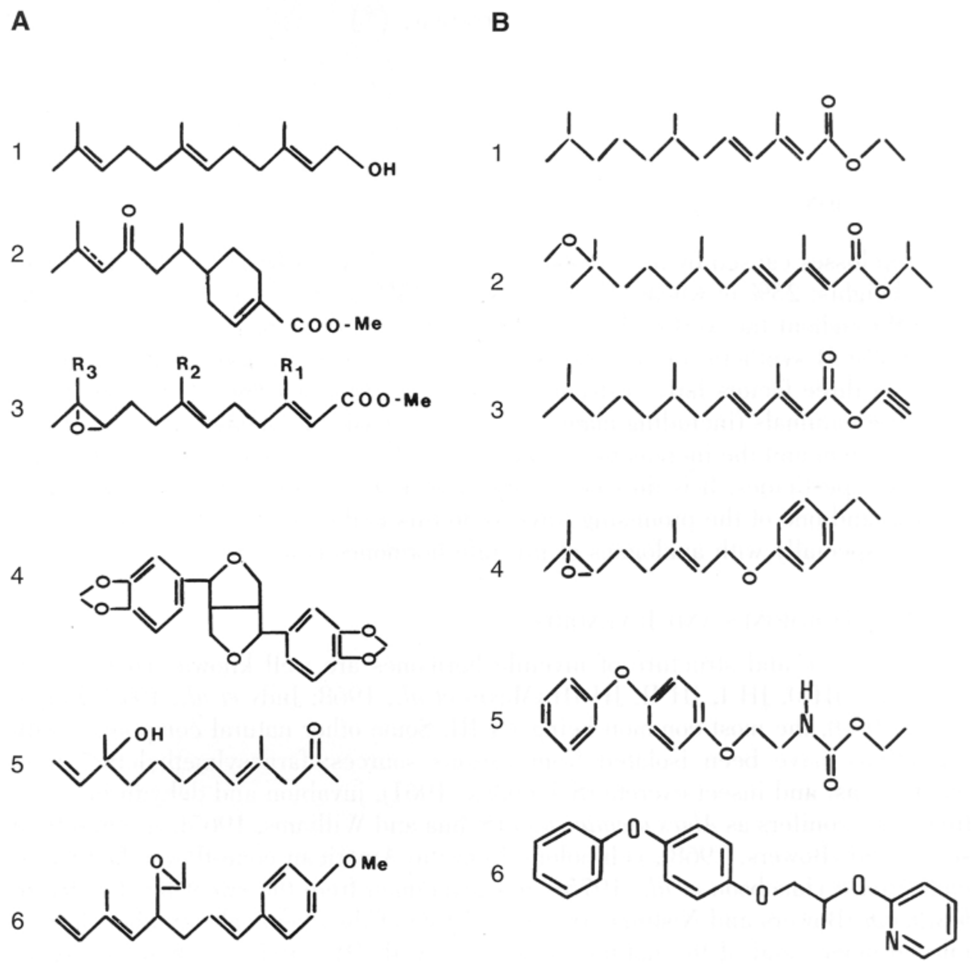

Fig. I - A. Natural compounds with juvenile hormone activity: 1. Farnesol; 2. Juvabion; 3. Juvenile hormone (JH 0: R1, R2, R3 all ethyls; JH I: R1 methyl, R2, R3 ethyls; JH II: R1, R2 methyls, R3 ethyl; JH III R1, R2, R3 all methyls); 4. Sesamin; 5. Echinolon; 6. Juvocimen. B. Chemical structures of practically used synthetic juvenoids: 1. Hydroprene; 2. Methoprene; 3. Kinoprene; 4. Epophenanone; 5. Fenoxycarb; 6. Pyriproxyfene specific structural features. They have characteristic physico-chemical properties like lipophility, relatively low polarity and more or less pronouced volatility. Besides the above mentioned natural compounds, about 4,000 synthetic JH analogues have been described to date (Sláma, 1985; Wimmer and Romaňuk, 1989).

The primary physiological action of juvenoids on insects is selective inhibition of metamorphosis and its replacement by isometric growth, thereby preventing pupal and adult emergence (Sláma *et al.*, 1974; Gilbert, 1976).

Juvenoids cause such malformation when applied to the late penultimate or last-instar larvae (Kikukawa *et al.*, 1989) that recipients are unable to mate (Kramer *et al.*, 1989). Exposure of larvae to juvenoids sometimes results in prolongation of the larval stage (Shaaya *et al.*, 1991) and increases mortality (Riddiford and Asburner, 1991) probably due to disturbances in the formation of internal organs (Sehnal, 1985). Juvenoids affect the differentiation of castes in termite colonies (Hrdý and Křeček, 1972), in ants (Glancey *et al.*, 1989) and other social insects (Žd'árek *et al.*, 1976; Rupeš *et al.*, 1978). The biologically active JH-mimics can also break diapause in the Colorado potato beetle (Koopmanschap *et al.*, 1989) and in pear psylla (Krysan, 1990). When applied to last instar larvae or early adults, juvenoids can severely disturb gametogenesis (Kikukawa *et al.*, 1989; King and Bennett, 1989), and even disrupt embryogenesis when applied to ovipositing females or freshly laid eggs (Myiake *et al.*, 1991; King and Bennett, 1990). A listing of juvenoid-sensitive periods is given in the following table 1.

Table 1 - Periods of sensitivity to juvenoids.

Treated stage	Effects of juvenoid
Larvae	Changes of phase, seasonal and caste polymorphism
Larvae (last instar in Endopterygota)	Diapause-like extension of last larval instar
Larvae (last or penultimate instar), pupae	Inhibition of metamorphosis
Pupae (pharate and diapausing)	Termination of diapause
Larvae, pupae, adults	Disturbance of gametogenesis and egg formation
Reproducing adults and eggs	Various derangements in embryogenesis

Methods of Juvenoid Application

Direct application

Given their lipophility, juvenoids can be (a) dissolved in acetone, oil or wax and directly applied to an insect's body surface, and (b) injection in solution into the body cavity. As most insects are relatively small, both these approaches require equipment for measuring the exact 0.1-1.0 µl juvenoid solutions with less than 5% standard error. These methods are commonly used in research laboratories for screening of juvenoid activity in insects.

Indirect application

These methods are designed for testing responses of developing insects to given concentrations of juvenoids in the environment. Special equipment is needed for treating various surfaces, which are sprayed or coated with a juvenoid solution of known volume so as to define the amount of juvenoid per square unit. The methods include spraying and dipping of the plant into the juvenoid solution, coating a surface with juvenoid, fumigation by juvenoid vapours (first tested on a coleoptera eggs by Walker and Bowers, 1970). The method of systemic application of juvenoids through the plant was first reported by Babu and Sláma (1972).

Field application techniques are a combination of the direct and indirect methods, the procedures used being similar to those for insecticides.

Juvenogens

In order to increase their stability (linked to their biological activity), juvenoids are usually bound to a suitable substrates (fatty acids, sugars etc.). The resulting complexes, called juvenogens, can be broken up by enzymes in the body of the target insect, thereby releasing the juvenoid and triggering its action (Sláma and Romaňuk, 1976; Sláma *et al.*, 1978). Juvenogens are still awaiting practical use.

Juvenoids: Practical Use

The advantage of juvenoids in comparison to other insecticides is their specific action on certain developmental stages, low toxicity and chemical lability in environment. The toxicity of methoprene, one of the most frequently used juvenoids, is 34,500 ppm for mice (Siddal and Slade, 1974). Their low chemical stability thus means that juvenoids are unlikely to accumulate in ecosystems (Siddal, 1976). The drawbacks associated with juvenoids include the late manifestation of their effects, which is often accompanied by the extension of feeding period, as mentioned above.

Well-timed juvenoid application disrupts development of target pests but preserves other insect species, including parasites and predators (Edwards and Menn, 1981; Sechser and Engelhardt, 1988; Nagai, 1990). Juvenoids are used also as a sterilant in the control of insects which are vectors of dangerous diseases, like mosquitoes (Mulligan and Schaeffer, 1990) or tse-tse flies (Langley, 1990; Langley *et al.*, 1988).

Because of its low toxicity to mammals, methoprene is even incorporated in the salt given to cattle for licking. Once it passes through the cattle's digestive tract, it remains in the faeces, where it can disrupt development and metamorphosis of various blood-sucking flies and Tabanids (Sehnal, 1985). Juvenoids stimulate growing of silk-glands in *Bombyx mori* (Kajiura and Yamashita, 1989) and can, in combination with a prolongation of feeding period, increase silk production by 25-30%.

Only several of the nearly 4,000 synthesised juvenoid compounds are registered for practical use (figure IB). The first to introduce juveniods as a practical tool was Zoecon Corporation, Palo Alto, California, when it marketed kinoprene, hydroprene and methoprene (Henrick *et al.*, 1973; 1976) and epophenonane (Pallos and Menn, 1972). The Dr. Maag Corporation (Switzerland) has contributed with fenoxycarb (Dorn *et al.*, 1980), which has broad spectrum of activity and good chemical stability.

The latest juvenoid likely to be registered for practical use is pyriproxyfen, a product of the Sumitomo Corporation, Osaka, Japan. It evinces about a 20-fold higher biological activity on the larvae of *Drosophila melanogaster* than methoprene (Riddiford and Ashburner, 1991), decreases populations of *Thrips palmi* at very low concentration yet preserves populations of predatory Anthocorid bugs (Nagai, 1990).

A comparison of the biological activity of commercial juvenoids as well as their target insects, formulations and dosages are given in table 2.

Table 2 - Commercially used juvenoids

Commercial name	Active substance	Use	Dosage and application
Enstar 5E	Kinoprene	Control of Homopterans in greenhouse	Spray (0.5%, 0.1 g/m^2)
Altosid	Methoprene	Control of mosquitoes	280 g/ha or more
Altosid	Methoprene	Control of hornfly	0.02% a.i. in salt block
Apex	Methoprene	Control of sciarid flies in mushroom cultures	1 g/m^2
Kabat	Methoprene	Control of *Ephestia elutella* and *Lasioderma serricorne* in stores	Spray (100 ppm a.i.)
Dianex	Methoprene	Control of various pests of stored products (pea nuts)	Aerosol (0.0207 g a.i./m^3)
Diacon	Methoprene	Control of various pests of stored products (pea nuts)	Spray (1.12 g a.i./100 Kg)
Pharoid (Lafarex)	Methoprene	Control of synatropic ants	0.5% a.i. in bait
Manta	Methoprene	Increase of silk production	Spray (2.5 ppm a.i.)
Precor	Methoprene	Control of fleas	?
Raid flea killer II Plus	Methoprene + Pyrethroid	Control of fleas	?
Gencor	Hydroprene	Control of cockroaches	Spray (0.03 g a.i./m^2)
Gencor Plus	Hydroprene 0.6% + Permetrin 0.25%	Control of cockroaches	As above
Ro 13-5223	Fenoxycarb	Control of Homoptera and Tortricids in orchards Control of mosquitoes Control of fire ants Control of stored products pests	250 g/ha per 2-3 applications 0.03-0.1 ppm 5-10 mg/colony 4-10 ppm
S-71639	Pyriproxyfen	Control of mosquitoes Sterilization of tsetse flies	100 g/ha Contaminated target pillows (approx. 2 ng/spec.)

SUMMARY

Several insect-hormones analogues can be used to control insect pests. Juvenoids, the true juvenile hormone mimics, have a molecular size and distribution of functional polar groups similar to juvenile hormones and are already being used in practical applications. A few dozen of the several thousand synthetic juvenoids currently available are more active than the best conventional insecticides and have no mutagenic or teratogenic effects and very low toxicity. They act primarily as inhibitors of morphogenesis. That is, because individual target cells may exhibit an "all or nothing" response and only some of them are sensitive to juvenoids at any given time, the insects treated with juvenoids become mosaics of morphologically advanced and retarded cells. This phenomenon leads to functional disturbances and then to death in the immature stages and to sterility in the adult insects. The advantages of juvenoids over conventional insecticides include low toxicity, very high specificity for certain taxonomic groups (Insecta) and low persistence in the environment. Their drawbacks are the limitation of their action on certain developmental stages of insects and the delay in manifestation of their effects.

Iuvenoidi: dalla ricerca di base all'applicazione pratica.

RIASSUNTO

Diversi analoghi degli ormoni prodotti dagli insetti possono essere usati per la lotta contro gli insetti dannosi. Gli iuvenoidi, i veri mimetici dell'ormone giovanile, hanno dimensione della molecola e distribuzione dei gruppi polari funzionali simili a quella dell'ormone giovanile e sono già stati utilizzati per applicazioni pratiche. Solo poche dozzine delle diverse migliaia di iuvenoidi disponibili possiedono un'efficacia maggiore rispetto agli insetticidi classici più attivi; a ciò si associa una tossicità molto bassa e la mancanza di effetti mutagenici o teratogenici nei confronti dei mammiferi. La loro attività principale consiste nell'inibizione della morfogenesi, dovuta alla risposta del tipo "tutto o niente" manifestata dalle cellule interessate dallo iuvenoide. Infatti ogni singola cellula è sensibile a tale composto solo in momenti ben precisi del proprio sviluppo; cosicché l'insetto trattato con lo iuvenoide risulta costituito da un mosaico composto da cellule che hanno proceduto nello sviluppo e altre nelle quali tale processo si è interrotto. Il complesso di tali fenomeni provoca disturbi funzionali che provocano la morte degli stadi immaturi, mentre negli adulti causano sterilità. I vantaggi che derivano dall'applicazione degli iuvenoidi, rispetto agli insetticidi classici, sono legati alla loro bassa tossicità, all'elevata specificità nei confronti del gruppo tassonomico degli Insetti e alla loro ridotta persistenza nell'ambiente. Tali composti, però, presentano alcuni svantaggi dovuti a un'efficacia limitata solo su ben precisi stadi di sviluppo dell'insetto e al ritardo con cui l'effetto si manifesta.

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