Enzyme Inhibitors in Biorational Approaches for Pest Control

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Abstract: Conventional insecticides of broad spectrum have been widely used as the main tools for controlling insect pests. However, as the consequence of their toxicity and deep environmental impact, new biorational, and more specific approaches have been developed. In this review we present an overview of those pest control approaches which have resulted from studies dealing with inhibition of the enzymes involved in the physiology, growth, molting, development and reproduction of insect pests. These approaches involve synthetic compounds from laboratory studies and natural chemicals present in the crop plants. Recent developments using inhibitors expressed in transgenic plants are also outlined.

INTRODUCTION

Insect pests affect virtually every major crop throughout the world causing notable losses to crops and human food. Until recently, the main tools for controlling the insect species responsible for these losses have been broad-spectrum neurotoxic insecticides. These insecticides, however, have a number of serious drawbacks, including their toxicity to humans and non-target organisms as well as their persistence in the environment. Another major problem associated with their utilization is the possible development by the insects of genetically based resistance to the compounds, which may lead to unexpected control failures. Therefore, the development of novel biorational, specific and non-toxic approaches to pest control is highly desirable.

Enzyme inhibitors play an important role in advancing our knowledge of many biochemical and physiological processes and, therefore, the developmnt of potent enzyme inhibitors is an area of pivotal importance in the pharmaceutical and agrochemical fields [1,2]. Illustrative of the significance of these compounds is, for example, the development of inhibitors of human neutrophil elastase for treatment of pulmonary emphysema [3], or inhibitors of neuropathy target esterase, the target site of certain neurotoxic organophosphorus compounds [4]. Particularly important are those inhibitors that function as substrate analogues and have been used to elucidate metabolic pathways and kinetic mechanisms of enzyme-driven reactions. In this review, we present an overview of the enzymatic inhibitors that have been found in the recent literature to affect the physiology, growth and development of insect larvae and/or modify the intraspecific communication of adults as well. The enzymes covered in this review mainly include esterases, oxidoreductases, aldehyde dehydrogenases, oxidases, and proteases, and the cited references mainly refer to papers published since 1990, although in some cases earlier papers have also been included because of their importance or impact on the subject. It should be noticed that although there have been a number of papers dealing with antagonism of insect pheromone responses by a variety of compounds, which might well be considered pest control agents, they are not cited here if no activity on any specific enzyme is reported.

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1. PHEROMONE-DEGRADING ENZYMES

Insects possess two distinct olfactory systems: a highly specific and sensitive pheromone olfactory system and a less specific general olfactory system, which participates in recognition of other type of odorants, like other semiochemicals, food volatiles, etc. The olfactory neurons corresponding to both types of systems are housed in distinct sensory hairs that are located on the antennae or the mouthparts. The pheromone-sensitive sensory hairs of many moths possess at least two soluble types of proteins that are engaged in pheromone processing: the pheromone binding proteins (PBPs) and the pheromone-degrading enzymes (PDEs). Whereas the first are involved in the transport of the pheromone through the aqueous lymph to the dendritic membrane, the PDEs are essential to prevent accumulation of pheromone molecules into the lymph/receptor. Once the pheromone molecules have interacted with the receptor, they must be inactivated to make the receptor sites accessible to new incoming pheromone molecules. The PDEs mostly studied in moths are esterases, aldehyde dehydrogenases, oxidases, and epoxide hydrolases.

1.1 Pheromone Esterases

General and pheromone-specific esterases are widely distributed on the body surfaces, including wing scales [5] and legs [6], to degrade conspecific and heterospecific pheromone components that can adsorb on the cuticle. Localization of specific esterases in sensory hair preparations has been demonstrated in the silk moth *Antheraea polyphemus* [7,8]. Odorant-degrading enzymes (ODEs), particularly pheromone esterases, have not been isolated or characterized yet possibly because of the very tiny amount present in the male antennae (four orders of magnitude lower than that of PBPs). Very recently, a bioinformatic approach has led to the cloning of cDNAs encoding a putative odorant degrading enzyme (ApolODE) and a putative integumental esterase from *A. polyphemus* [9].

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Incorporation of polyfluoroketone moieties into inhibitors exhibiting close structural analogy to the substrates has proven to be a useful strategy for generating strong inhibitors of diverse serine hydrolases, many of them being pharmacologically attractive targets [10-16]. The inhibition activity displayed by fluorinated compounds arises from unique physical features induced by fluorine, which closely mimics the steric requirement of hydrogen at the enzyme acceptor site. The strong electron-withdrawing character of fluorine induces that trifluoromethyl ketones (TFMKs) form stable hydrates of tetrahedral geometry in aqueous solutions resembling the transition state involved in the enzymatic hydrolysis of esters or peptides [17] (Fig. (**1**)). Therefore, TFMKs inhibit the action of a variety of serine esterases, like acetyl cholinesterase [10], juvenile hormone esterase [18] or mammalian carboxyl esterases [19].

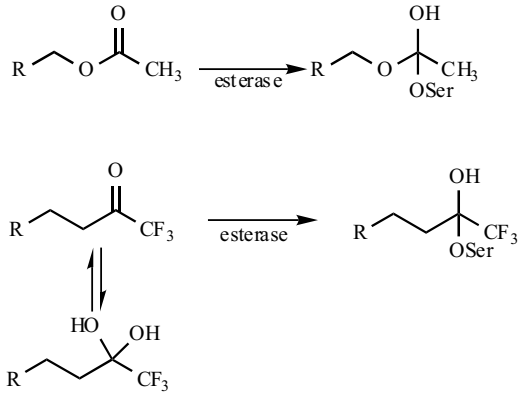

Fig. (1). Proposed tetrahedral intermediates in the mechanism of hydrolysis of a pheromone ester and the inhibition of esterases by TFMKs. The TFMKs are in equilibrium with their hydrated forms.

In insects, TFMKs also reversibly inhibit the antennal esterases responsible for the catabolism of pheromone molecules in the olfactory tissues of males [7,20-22]. We have proved by ^{19}F NMR studies that 3-octylthio-1,1,1-trifluoropropan-2-one (OTFP, **1**, Fig. (**2**) reversibly binds the active site of the enzyme by forming an adduct with a serine residue of the enzyme [23]. A more conclusive proof of the mechanism of action of these chemicals was provided by a single crystal X-ray analysis of the complex of porcine pancreatic elastase with a peptidyl TFMK that clearly shows a covalently bound hemiketal [24]. Long chain TFMKs behaved as tight slow-binding inhibitors, the β-thio derivatives being the most potent, particularly OTFP with an IC_{50} 0.08 μM [23]. Other linear TFMKs, especially those most structurally related to the pheromone structure, were also notably active (IC_{50} 0.14-0.23 μM) [23].

Inhibition of enzymatic catabolism of odorant molecules has been considered a potential approach for the disruption of pheromone reception as another strategy for pest control [25]. In this context, we have prepared [26] and tested [22] a variety of TFMKs to investigate the inhibition of the pheromone reception of the processionary moth *Thaumetopoea pityocampa*. In the field, the most closely related analogues to the pheromone displayed a potent inhibitory effect on male catches when mixed with the pheromone in different ratios [22]. However, in the Mediterranean corn borer *Sesamia nonagrioides*, (*Z*)-1,1,1-trifluoro-14-nonadecen-2-one, a pheromone analogue, increased male catches when added to the pheromone [27]. In the European corn borer (ECB) *Ostrinia nubilalis*, when both isomers of 1,1,1-trifluoro-14-heptadecen-2-one, an steric mimic of the pheromone, were applied to the antennae of both *Z*- and *E*-type insects the ketone was a weak inhibitor of the esterase in either strain [28]. In wind tunnel, the compound had no effect on male upwind flight response to the pheromone of both ECB types. Similarly, Prestwich and Streinz [20] found that a one carbon-elongated TFMK analogue of the pheromone of *Plutella xylostella* was also a weak esterase inhibitor of the pheromone.

In *Spodoptera littoralis* males treated with a variety of TFMKs, particularly OTFP (**1**) and (*Z,E*)-9,11-tetradecadienyl trifluoromethyl ketone (**3**), the most closely related analogue of the major component of the pheromone **2**, frequently exhibited erratic progress towards the plume, flying across the wind with high number of intersections with the plume [29]. Interestingly, the non-fluorinated analogue did not decrease the number of contacts with the source (synthetic pheromone or virgin females) and did not affect the regular flight track to the pheromone source, confirming the key role played by fluorine in the inhibitory action of these molecules. Compounds **1** and **3** also displayed good antiesterase activity (IC_{50}<10 μM) in "*in vitro*" biochemical assays [21,23].

A similar effect was found in *S. nonagrioides* males, in which the TFMK analogue **5** exhibited a remarkable inhibition of response in all steps of behavior [29]. The K_m and V_{max} values of the esterase were $1.61x10^{-7}$M and $1.25x10^{-7}$M.min^{-1} and the chemical exhibited an IC_{50} 123 μM. The TFMKs were more active than other difluorinated derivatives, such as difluoromethyl ketones and difluoroaldehydes [30].

Compound **1** behaves also as an oviposition deterrent and antifeedant and when added to the diet of the 2nd instar larvae of *S. littoralis* and *S. nonagrioides,* it reduced diet consumption and growth, pupation and adult emergence [31]. Also, in behavioral assays, adult males, when treated

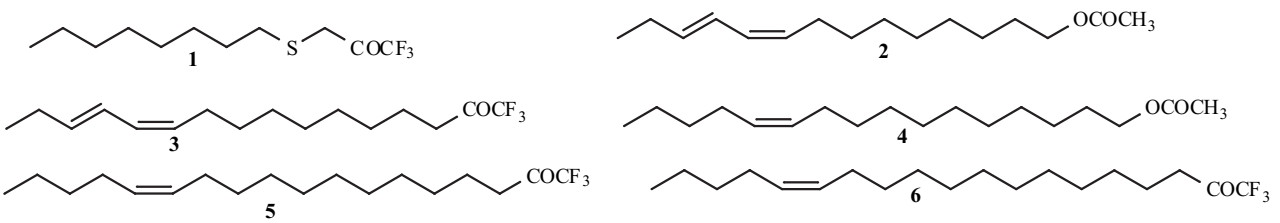

Fig. (2). Structures of OTFP (**1**) and the trifluoromethyl ketones **3**, **5** and **6,** analogues to the major components of the pheromones of *S. littoralis* (**2**) and *S. nonagrioides* (**4**).

with the chemical at the larval stage, were less attracted to the pheromone source or virgin females than regular untreated males. OTFP has also been shown as a good inhibitor of the JHE present in the haemolymph of 6th instar larvae of *S. littoralis* (IC_{50} 5.8x10^{-7}M) [32], so the chemical may be acting on other esterases or serine proteases of the gut as well.

Using radiolabeled analogues and in displacement reactions, some aliphatic TFMKs were bound and transported by the PBPs present in the sensory hairs of *T. pityocampa* and *A. polyphemus,* thus facilitating a productive interaction with the esterases responsible for pheromone catabolism [33,34]. In *Mamestra brassicae*, which also contains compound **4** as the major pheromone component, TFMK **5** also significantly diminished the behavioral responses of males to the pheromone in an actograph [35].

As putative pheromone carboxyl esterase inhibitors, Baker *et al*. [36] developed several dialkyl phosphorofluoridates and alkyl methyl phosphorofluoridates containing a (*Z*)-8-dodecenyl group, the alkyl substituent of the main pheromone component of the Oriental fruit moth *Grapholita molesta*. The compounds disrupted the pheromone-mediated behavior in a wind tunnel, possibly by inactivation of the pheromone carboxyl esterase, and resulted only weakly active against houseflies and mice [36].

As far as the toxicity of the TFMKs is concerned, compounds **1** and **5** showed little toxicity to mice, with an LD_{50} of 1 g/kg after the 6th day of administration to the animals, whereas the major component of the pheromone **1**, a naturally-occurring compound, presented an LD_{50} of 5 g/kg after the same period of treatment [37]. These data agree with the low acute toxicity to mice of several substituted TFMKs already reported [38,39]. All these data combined suggested the possible application of this type of chemicals in future biorational strategies for pest control.

1.2 Oxidases

Oxidases are enzymes that catalyze reactions directly involving molecular oxygen and that use FAD or NAD^+ as cofactors. The aldehyde oxidizing enzymes (AOEs), present at high levels in antennal tissues of adult moths, convert pheromone aldehydes to inactive carboxylic acids. Several types of analogues have been prepared for AOE inhibition in *H. virescens*, such as cyclopropanols, α-fluorinated aldehydes and α,β-unsaturated carbonyl compounds [40]. Cyclopropanols were suggested to act as AOE inhibitors possibly *via* oxidation to the unstable cyclopropanones through the action of an alcohol oxidase. The formed cyclopropanones would be acting as a transition state analogue by formation of a stable tetrahedral adduct with an active cysteine residue of the antennal AOE [40]. α-Fluor and α,α-difluoro substituted analogues of (*Z*)-9-tetradecenal, one of the major components of the pheromone of *H. virescens*, were shown to be modest inhibitors of AOE. The most potent inhibitors found were α,β-unsaturated carbonyl mimics of (*Z*)-11-hexadecenal (**7**), the other major component of the pheromone, and the inhibition appeared to be irreversible [40]. However, the activity of these chemicals on male behavior was not pursued.

Aldehyde dehydrogenases are enzymes that oxidize aldehydes to carboxylic acids by removal of hydrogen using NAD^+ or $NADP^+$ as cofactor. They are found, for instance, in leg and antennal tissues of male and female *H. virescens,* as determined in studies using tritium-labeled *Z*9-14:Ald or *Z*11-16:Ald (**7**) as substrates in the presence of NAD+ or NADP+ cofactors [41]. Compounds able to display antipheromone activity included *N*-methyl carbamate **8**, trifluoroacetate **9**, trichloroacetate **10** and methyl ketone **11** [42] (Fig. (**3**)).

These chemicals acted as competitive antipheromones displaying reversible inhibition of electrophysiological and behavioral responses of males, and methyl carbamate **8** resulted also a potent antagonist of oviposition on cotton [43]. The proposed mechanism of action involved tight binding of the chemicals to a nucleophilic receptor site forming a stable tetrahedral hemiketal adduct, similar to the mechanism proposed for the esterase inhibition by TFMKs. In the same context, Prestwich [25,44] developed analogues by replacement of the aldehyde hydrogen by fluorine in the structure of the pheromone **7** to produce, for instance, acyl fluoride **12**. This compound resulted to be a potent

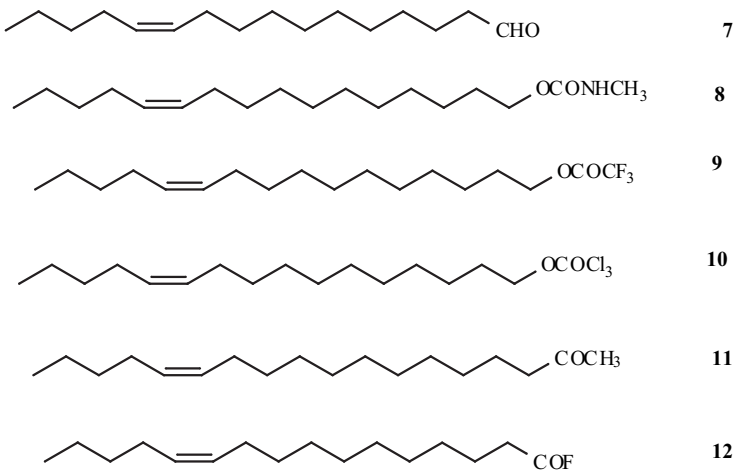

Fig. (3). Structures of the main pheromone component of *H. virescens* **7** and the N-methyl carbamate **8**, trifluoroacetate **9**, trichloroacetate **10** and methyl ketone **11** analogues.

hyperagonist in male *H. virescens* at high doses and a disorientation agent at lower doses [25]. The proposed mechanism of action involved irreversible N-acylation of the receptor protein by reaction of the acyl fluoride with a free amino group of the protein.

2. PHEROMONE BIOSYNTHETIC ENZYMES

It is known that biosynthesis of Lepidoptera sex pheromones takes place mainly through three main processes: fatty acid synthesis, desaturation and β-oxidation. Particularly appealing is the study of desaturases, which allow stereoselective introduction of specific double bond(s) in certain position(s) of the fatty acyl chain. The most common desaturases enzyme is Δ-11 desaturase although other desaturases with different regiospecificities have also been reported in different species [45]. Based on previously shown inhibition of desaturation of stearic to oleic acid by a cyclopropenic C-18 fatty acid, several inhibitors of *Z*-11 and *Z*-9 desaturation of palmitic acid in the biosynthetic pathway of *S. littoralis* were reported [46]. Cyclopropenic fatty acids with the cyclopropene ring at positions 10-11, 12-13 and 11-12 inhibited the biosynthesis of (*Z*)-9-tetradecenyl acetate and (*Z,E*)-9,11-tetradecadienyl acetate, two key components of the sex pheromone. 2-Halofatty acids have also been found as inhibitors of the sex pheromone production in *S. littoralis*, *T. pityocampa* and *Bombyx mori* [47]. Among them, 2-bromohexadecanoic acid was particularly active, being the *Z*-11 desaturase and acetyl transferase the target enzymes.

In spite of the promising features for "*in vivo*" application that these types of inhibitors can display, only one report has been found in the literature on the "*in vivo*" activity of these cyclopropenic fatty acids. Baird and coworkers [48] synthesized analogues of the pheromone of three different insects, *Musca domestica*, *Plutella xylostella* and *Ephestia eleutella*, in which the *Z* double bond was replaced by a cyclopropene group. The analogues interfered with the mating behavior of the insect and the inhibitory action was long-term [48]. However, details of the biological activity of the chemicals were not reported.

The β-oxidation chain-shortening step implies the successive loss of acetyl-CoA units and is also one of the primary steps in the biosynthetic pathways of insect sex pheromones. A variety of monofluorinated [49], acetylenic and cyclopropane fatty acids [50] were designed to block the enzymatic oxidation of palmitic into myristic acid through an acyl-CoA dehydrogenase, the first step of the biosynthesis of the major component of the pheromone of *S. littoralis*. Some compounds resulted good inhibitors of the process both in "*in vitro*" and "in vivo" assays, the most potent being the 2,3-dichlorocyclopropane analogue of palmitic acid in the experiments "*in vitro*" and 2-bromopalmitic acid in "*in vivo*" [51].

Insect neuropeptides can also be an important target in the study of new insect control agents since they regulate embryonic and post-embryonic development, homeostasis, migration, oviposition, mating, etc. Antagonists of these neuropeptides may disrupt and interfere the normal development of these processes by blocking the corresponding receptor, and therefore they can be considered receptor-selective, insect-specific insecticides. In this context, an important discovery is that the sex pheromone biosynthesis of *H. zea* is regulated by a neurohormone, called *pheromone biosynthesis activating neuropeptide* (PBAN) [52]. The hormone is a 33-aminoacid peptide produced by the suboesophageal ganglion, and has a molecular weight of 3900 Da. Since this pioneering study, other PBAN molecules have been isolated from *B. mori*, *Lymantria dispar*, *M. brassicae*, *Agrotis ipsilon* and *Helicoverpa assulta*, their primary structures have been determined and the c-DNA and genes have been cloned [53]. In structure-function relationship studies on *S. littoralis*, Altstein and coworkers found that the activity resided mainly on the presence of the sequence between amino acids 9 and 13 as well as on the C-terminal amide. Other neuropeptides, sharing the C-terminal pentapeptide of PBAN (Phe-Xxx-Pro-Arg-Leu-NH_2; Xxx=Ser, Gly, Thr, Val), have also been isolated from various insects, such as the pyrokinins, locusta myotropins, pheromonotropin and diapause hormone. In addition to stimulate pheromone biosynthesis, these peptides have been found to control several physiological and behavioral functions, such as melanization, egg diapause, acceleration of pupation, myotropic activity, etc. [53]. So far, however, there are no commercial insect control agents based on neuropeptides agonists or antagonists but more work is in progress in this direction.

3. GENERAL ESTERASES

As inhibitors of general esterases, TFMKs also inhibit esterase-mediated resistance to insecticides, and therefore they can be considered potential insecticide synergists. In fact, several TFMKs (f.i. OTFP) are insecticide synergists for azinphosmethyl-resistant tufted apple bud moth *Platynota idaeusalis* [54]. In this work, a 1-naphthyl acetate resistance-associated esterase was isolated from whole body homogenates of azinphosmethyl-resistant adult females, and the enzyme was inhibited by OTFP with an IC_{50} of $1x10^{-8.5}$ M. A second 1-naphthyl acetate esterase susceptible to the insecticide was also inhibited with similar IC_{50} value. In the Colorado potato beetle *Leptinotarsa decemlineata*, OTFP has also been found to be an effective inhibitor of a resistance-associated esterase suggesting the possible use of these chemicals as novel insecticide synergists [55].

4. ACETYL CHOLINESTERASE

Plant terpenes may be a good source of compounds for pest management, since they are environmentally friendly and offer strong resistance to insect attack. In this context, there is a wide spread effort focused on limonoids from plants of the Meliaceae family, flavonols from Asteraceae plants, sesquiterpenes from Celastraceae, etc. due to their strong resistance against insect attack. Thus, *ent*-clerodanes from aerial parts of *Gutierrezia microcephala* (Asteraceae) have exhibited insecticidal activity on larvae of *Spodoptera frugiperda*, as well as inhibition of growth, pupation and emergence [56]. The activity of these compounds was associated with a mechanism involving inhibition of AChE. In the same manner, β-dihydroagarofurans, isolated from *Maytenus sp*., caused significantly growth inhibitory effects

as well as larval mortality, delay of pupation time and adult emergence against *S. frugiperda*. These compounds turned out to be potent inhibitors of AChE (78-100% inhibition at 15 ppm) [57]. The same group reported similar results with insecticide triterpenes isolated from *Parthenium argentatum* [58].

5. JUVENILE HORMONES

In holometabolous insects, the presence of JH or a JH analogue (JHA) during the larval-pupal molt results in a supernumerary larva that is unable to give rise to normal adults. As consequence, many JHAs have been prepared and tested for insecticidal activity [59]. Some of them, like methoprene, resemble JH in their basic structure, but other several highly active compounds with less apparent similarity to JHs have been recently synthesized and tested. This is the case of fenoxycarb (registered by Roche/Maag), pyriproxyfen (registered by Sumitomo Chem. Co.) and diofenolan (registered by Ciba Geigy) (Fig. (**4**)). Application of these latter compounds induces morphological deformity and sterility in adults, suppress egg production, and inhibit oviposition. Therefore, fenoxycarb has been used for control of a number of coleopteran and lepidopteran pests of stored wheat and rice, tortricids, leafrollers, psyllids, diaspidid scales, fleas and mosquitoes [60], pyriproxyfen has been active against mosquitoes, housefly, scales whiteflies, aphids and pear psylla, and diofenolan has been used against lepidopteran pests in citrus, grapes and olives as well as against scale insects [60].

Biosynthesis of JH is also an attractive target for the biorational design of insect control agents [61-63]. Particularly appealing is the inhibition of the biosynthetic pathways controlled by the corpora allata (CA), such as farnesyl pyrophosphate hydrolysis, oxidation of farnesol to farnesal and farnesoic acid, esterification to methyl farnesoate and epoxidation of the 10,11-double bond [64]. Disruption of JH titer by surgical or chemical allatectomy is known to induce precocious metamorphosis or to inhibit reproduction in insects. The most active inhibitors of JH biosynthesis comprise compactin, isolated from the fungus *Penicillium brevicompactum* [65], piperonyl butoxide which inhibits the epoxidation step of the biosynthesis by acting on P-450 monooxygenases [66], fluoromevalonate (see below) and precocenes [67]. Fluvastatin (Sandoz Chem. Co.), an inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase, a key enzyme of JH biosynthesis, "*in vitro*" and "*in vivo*" inhibited JH biosynthesis by CA of *Locusta migratoria* [68]. Oxidation of farnesol to farnesal, another key step in insect JH biosynthesis, is mediated by a specific alcohol oxidase, and this enzyme can be weakly inhibited by 1,10-phenantroline (IC_{50} 11 mM) [64].

Another important group of inhibitors of JH synthesis comprises a variety of nitrogen heterocycles, which act as ligands of cytochrome P450 monooxygenase by coordination of their heteroatomic nitrogen lone electron pair with the iron atom. In this context, the activity of 1,5-disubstituted imidazoles as inhibitors of JH synthesis "*in vitro*" and "*in vivo*" in the cockroach *Diploptera punctata* has been described (IC_{50} 64-820 nM) [69]. Imidazoles with a JH-like terpene chain induced precocious metamorphosis in the silkworm *B. mori* after topical application to the larvae or when administered to the diet [70]. In a revision of the active extract of *Penicillium brevicompactum,* a heterocyclic oxime, called brevioxime, was also found to display "*in vivo*" anti-JH activity (precocious metamorphosis) on *Oncopeltus fasciatus,* possibly by inhibition of the final steps of JH III biosynthesis [71].

A variety of fluorinated mevalonates was prepared at Zoecon Co. (USA) as anti-JH (AJH) compounds. The 6,6-difluoromethyl compound was moderately active on *Manduca sexta* while 6-fluoromevalonate showed the highest activity (ED_{50} 0.7 mg/g body weight) [72]. This compound also prevented normal ecdysis to pupa when it was fed to *T. ni* larvae. The AJH activity was postulated to be due to inhibition of JH biosynthesis at the level of enzymatic phosphorylation of mevalonate and homomevalonate. In the line of substitution of fluorine for hydrogen as shown above, other analogues elicited anti-JH activity on *M. sexta* and *H. virescens* larvae [73].

Allatostatins are neuropeptides, which rapidly and reversibly inhibit JH biosynthesis by the CA of moths, cockroaches and crickets [74]. However, so far no attempts have been made to extend these studies to develop new approaches for pest control. Also, synthesis of JH III by isolated CA of the cockroach *D. punctata* can be inhibited by several phorbol derivatives and by 1-oleyl-2-acetylglycerol [75] but, again, no implications on practical issues have been disclosed.

5.1 Juvenile Hormone Esterase

Insect juvenile hormones (JH) regulate insect embryogenesis, larval growth, metamorphosis, reproduction and metabolism. The two primary metabolic degradation

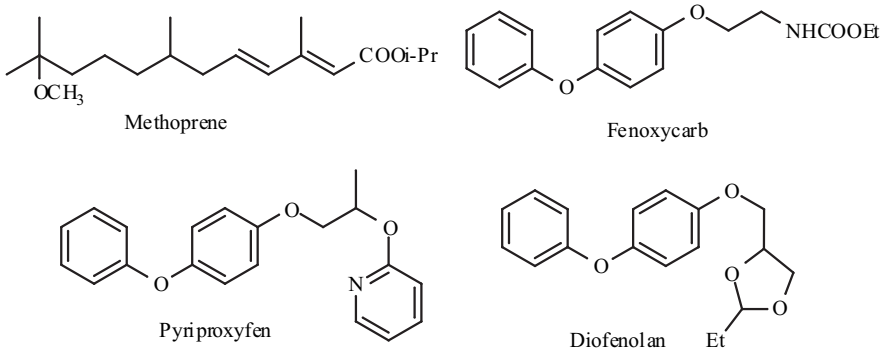

Fig. (4). Structures of the JHAs methoprene, fenoxycarb, pyriproxyfen and diofenolan.

pathways of JH in insects are ester hydrolysis by JH esterase and epoxide hydration by an epoxide hydrolase (EH). Degradation by specific JH esterases along with changes in the rate of JH biosynthesis is responsible for the regulation of insect development [76]. As cited above, TFMKs have been found to be potent inhibitors of JHE [38], particularly those containing a sulfur atom in β position to the carbonyl, like OTFP (**1**) [77]. It has been suggested that the extent of hydration of these compounds in aqueous solutions may be an important factor for activity [78,79]. Our own studies have indeed revealed that the most potent inhibitor was also the most hydrated but, in general, no clear correlation between the two parameters was apparent [30]. In this context, an intramolecular hydrogen bond between the hydroxyl group of the hydrate form and the sulfur in the most stable conformation of (*Z, E*)-9,11-tetradecadienyl trifluoromethyl ketone (**3**), the TFMK analogue of *S. littoralis* pheromone, has been reported [80]. In *T. ni* "*in vitro*" inhibition of JHE by β-thio-TFMKs is in the nM range (IC_{50} 3-8x10^{-9} M), that is 2-3 orders of magnitude higher than the S lacking derivatives [81]. Similarly, application of β-thio TFMKs on fifth-instar larvae delayed pupation and suppressed JHE activity in contrast to the compounds lacking the 4-thia substituent [82].

Oxidation of OTFP to the corresponding sulfone afforded the corresponding geminal diol, that was a potent "*in vitro*" selective inhibitor of JHE (IC_{50} 1.2 nM) and elicited "*in vivo*" juvenoid activity on the cabbage looper *T. ni* [83].

JH synthesis can be inhibited in *H. virescens* larvae by a recombinant baculovirus expressing antisense JHE mRNA [84]. A high proportion of larvae showed intermediate developmental forms, such as larval segmentation, pupal cuticle, size, and behavior, as result of attempted but not accomplished larval-pupal molt. These features are similar to those induced by application of JH or JHE inhibitors. Expression of genes coding for insect JHE has resulted in recombinant baculovirus with promise as biological insecticides [85]. These viruses are efficacious in the laboratory, greenhouse and in the field, and dramatically reduce damage caused by insect feeding. Moreover, the recombinant viruses may synergize and be synergized by classical pesticides, such as pyrethroids, and since they are highly selective for pest insects they can be used without disrupting biological control. A number of baculoviruses are currently used in pest control on several species of Lepidoptera [85].

5.2 Juvenile Hormone Epoxide Hydrolases

While in general JH esterase is more important than JH-EH in Lepidoptera, in the southern house mosquito *Culex quinquefasciatus* activity of the latter enzyme exceeded that of JH esterase throughout most of the 4th stadium by ca. 6-fold. This suggests that JH-EH and not JH esterase has a dynamic role in the initiation of metamorphosis [86]. Therefore, development of JH-EH inhibitors may be important for their potential application in pest control strategies. Several glycidyl ethers and epoxy alcohol JH analogues have been examined as inhibitors of JH-EH on *M. sexta* but only the analogues provided significant levels (μM range) of inhibition [87]. Roe and coworkers have found that the potential inhibitors designed to mimic a polarized or ionic transition state were moderately active against *T. ni* JH-EH, the most effective inhibitor being methyl 10,11-epoxy-11-methyldodecanoate (IC_{50} 80 μM) [88]. In structure-activity relationships studies with a series of glycidol- and epoxyesters, the authors established that the potency of the inhibitors was dependent on the absolute configuration of the epoxy group, the *R* configuration at C-10 being significantly more potent than the *S* configuration [89].

6. OXIDOREDUCTASES

Structurally diverse synthetic insecticides and acaricides have been shown to inhibit the proton-translocating NADH:ubiquinone oxidoreductase (complex I). This enzyme is the first electron transport complex of the mitochondrial respiratory chain, and oxidizes NADH transferring the electrons via a flavin mononucleotide cofactor to ubiquinone [90]. Rotenone, the active component of the extract of *Derris sp*. (Leguminosae) roots, and piericidin, isolated from cultures of *Streptomyces mobarensis*, have been known for long as high affinity inhibitors of complex I. Other dehydrorotenone and oxa-dehydrorotenone analogues have been recently isolated from extracts of the roots of *Lonchocarpus utilis* and *L. urucu,* and tested as inhibitors of complex I, and for toxicity to mosquito larvae, goldfish and mice, and cytotoxicity in three mammalian cell lines [91]. The most active analogue exhibited 50% inhibition of the oxidoreductase at 0.11 μM concentration and 50% mortality of goldfish at 1 ppm [91].

Other new potent inhibitors acting on mitochondrial respiratory chain have been recently discovered [90]. They comprise, among others, fenpyroximate (Nihon Nohyaku) and tebufenpyrad (Mitsubishi Kasei) as pyrazole derivatives; fenazaquin (DowElanco) and pyrimidifen (Ube Ind.) as pyrimidine-type compounds, and pyridaben (Nissan Chem.) as pyridazinone derivative. Fenpyroximate induced inhibition of the NADH:ubiquinone oxidoreductase in rat liver and in the spider mite *Tetranychus urticae* mitochondrial membrane enzymes (IC_{50} 0.4 and 0.08 μM, respectively), explaining its acaricidal activity [92]. Tebufenpyrad has been used against spider mites and some sucking insects, and pyrimidifen has also been utilized as an effective insecticide and acaricide against a broad spectrum of Lepidoptera, Coleoptera, aphids and bugs [93].

Annonaceae plants are known to contain potent bioactive secondary metabolites, called acetogenins, with pronounced insecticidal and antiparasitic activities [94]. Thus, acetogenins have been active against aphids, flies, the Mexican bean beetle and the diamondback moth. With regard to their mode of action, asimicin, an insecticidal acetogenin isolated from *Asimina triloba*, blocked oxygen consumption in mitochondria of *O. nubilalis* larvae, while thiangazole, isolated from a *Polyangium* strain and with insecticidal, acaricidal and antihelmintic activity, also inhibited complex I.

7. PROTEASES

The possible role of protease inhibitors (PIs) in plant protection was investigated as early as 1954 when trypsin inhibitors present in soybean were shown to be toxic to the

larvae of the flour beetle *Tribolium confusum*. Since then, there have been many reports of PIs in "*in vitro*" assays against gut proteases and in "*in vivo*" when applied on artificial diets [95].

Plant genetic transformation with exogenous genes encoding factors of resistance to phytophagous insects is a modern and attractive approach to control aggressive plant pests [96,97]. The first relevant results were obtained by engineering plants with crystal protein genes from *Bt*, due to the wide variety of toxic proteins it contains. A different but also attractive alternative to increase plant resistance to herbivores is the plant genetic transformation with genes coding for enzyme inhibitors [98-100]. Use of PIs in this context may be of interest due to their antinutritive effect and their activity on growth and development of phytophagous insects. Several major crop plants including rice, potato and rapeseed have been genetically transformed with PIs [101,102]. According to the active amino acid in the active center, proteases can be classified as serine, cysteine, aspartic and metallo-proteases.

7.1 Serine Plant Inhibitors

The serine class of proteases, such as trypsin, chymotrypsin and elastase, are responsible for the initial digestion of proteins in the gut of higher animals [103]. Serine plant inhibitors induce antinutritional effects on several lepidopteran insect species, reducing larval growth and producing mortality at certain doses. Thus, α_2-macroglobulin, Pefabloc, TLCK, SBTI, etc reduced larval growth and in some cases death of the Australian sheep blowfly, *Lucila cuprina* (Diptera: Muscidae), when incorporated into the artificial diet [104,105]. These results suggest that these proteases are key in protein digestion in this insect and that their inhibition leads to an almost complete blockade of digestion. Other serine-based inhibitors, such as saponins, a group of compounds that protect plants against insects attack, have been shown to reduce larval growth in the flower beetle *Tenebrio molitor* [106] and in the European corn borer *O. nubilalis* [107], among others. Saponins form complexes with proteins and by this mechanism they apparently inhibit proteases and curb digestion in insects gut [108]. Alfalfa saponins administered to the larval diet of *S. littoralis,* elicited prolongation of the larval and pupal stages, retarded growth, increased mortality and reduced fecundity and fertility [109]. The authors suggested that inhibition of the digestive enzymes and interference with the sterol metabolism could be involved in the effects of these compounds. Other insects, which have been treated with serine inhibitors, are *H. zea*, *Spodoptera exigua*, *Callosobruchus maculatus*, *M. sexta* [103] and the black field cricket *Teleogryllus commodus* [110].

Hymenopteran parasitic wasps have a good potential for use in integrated pest management (IPM) programs; for instance, the gregarious ectoparasitoid *Eulopholus pennicornis* was suggested as biological control agent for larvae of the tomato moth *Lacanobia oleracea* [111]. In this insect, the soybean Kunitz inhibitor (SKTI) inhibited serine protease activity (trypsin and chymotrypsin-like) in the gut by over 80% at $<10^{-5}$ M. When fed to larvae parasitized by *E. pennicornis*, the inhibitor was subsequently detected in the larval hemolymph of the parasitoid showing that protease inhibitors in the host diet can be delivered to a parasitoid *via* the host hemolymph [111]. In base to these results, the authors suggested that the use of protease inhibitors, genetically engineered into crop plants, could pose a potential threat to beneficial lepidopteran parasitoids, which could be used in biocontrol approaches of the pest species in question. However, this is in contrast to the report of Poppy and coworkers in which wasp parasitoids that had attacked *Bacillus thuringiensis* (*Bt*)-resistant larvae on transgenic plants, suffered no measurable adverse effects of *Bt* toxins either on their behavior as adults or on the survival of their larvae [112].

In *S. nonagrioides*, serine proteinases (trypsin, chymotrypsin and elastase) and exopeptidases (carboxypeptidase A, B and leucine aminopeptidase) are the major proteolytic enzymes [113]. "*In vitro*", the esterase inhibitor DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), present in maize and other cereals, inhibited the activity of esterase, carboxypeptidases, aminopeptidase and glutathione S-transferase, but exhibited no effect on trypsin, chymotrypsin and elastase. "*In vivo*", DIMBOA reduced the relative growth rate of *S. nonagrioides* larvae causing larval and pupal mortality when the larvae were fed on maize inbred plants [114]. The authors suggested that the activity of the compound could also be attributed to the reaction of the chemical with nucleophilic residues at the active center of the enzyme [115], a similar mechanism of action proposed for the action of TFMKs [23,116] (see above).

As cited above, several crop plants have been genetically transformed with PIs but the usefulness of the PIs approach for plant resistance development is still uncertain [117]. PIs do not induce immediate and massive effects on the physiology of herbivores and, moreover, their delayed effects can allow the insect to express adaptive biochemical, physiological or behavioral responses to maintain access to plant tissues. Narrow-spectrum PIs have also very limited impact on complex target enzyme systems [118] and are exposed to inactivation by non-sensitive proteases [119]. However, the mustard trypsin inhibitor MTI-2 has been demonstrated to be effective against *S. littoralis*, *M. brassicae* and *P. xylostella* when the MTI-2 encoding cDNA sequence was inserted in tobacco, arabidopsis and rapeseed [120]. The effect of the inhibitor expressed at different levels in transgenic tobacco lines has been also evaluated by feeding *S. littoralis* throughout its larval life [121]. Although feeding, growing, and development of larvae on the transgenic plants were not significantly different from controls, the females fertility was significantly decreased for plants expressing high levels of MTI-2 [121].

7.2 Cysteine Proteinase Inhibitors

A number of cysteine proteinases (CPs) (papain, calpin, asparagines and cystatins) have been isolated from midgets of several insect orders, particularly from Coleoptera and Hemiptera, and can be inhibited by several synthetic and naturally occurring cysteine proteinase inhibitors (CPIs) [122]. Out of eleven beetle species representing eleven different families, ten had gut proteinases that were inhibited by p-chloromercuribenzene sulfonic acid, a potent sulphydryl

reagent, indicating that the proteinases were of the cysteine-type. Leupeptin, a microbial inhibitor of cysteine and serine proteinases, induced significant reductions in the larval growth and development of the alfalfa weevil *Hypera postica* even after nine successive generations [123]. In addition, defoliation was significantly lower on alfalfa foliage treated with the inhibitor than on untreated foliage in all generations, suggesting that the weevil does not utilize or induce other proteinases (or digestive enzymes) to compensate for inhibition of one of its major proteinases [123].

The potato CPI was the major source of activity against a single major proteinase isolated from the corn rootworm *Diabrotica virgifera virgifera* (IC_{50} 31 nM), but other inhibitors were also notably active, such as E-64 (IC_{50} 35 nM) or chicken egg white cystatin (IC_{50} 121 nM) [124]. Incorporation of the potato CPI into the diet resulted in a significant increase in larval mortality and growth inhibition, suggesting that expression of these inhibitors by transgenic corn plants in the field is a potentially attractive method of plant resistance to the pest. As cited above, however, other reports doubt the usefulness of the proteinase inhibitors approach for plant resistance to pests. For instance, larvae of the Colorado potato beetle, *L. decemlineata*, feeding on transgenic potato foliage expressing oryzacystatin I (OCI), a specific cysteine proteinase inhibitor, were not affected as far as the relative growth rate or maximum weight was concerned [117]. The female reproduction ability or egg eclosion was also unaffected but the nutritional stress to females feeding on OCI foliage was evident, as reflected in their lower efficiency of conversion of infested foliage and increased foliage consumed per egg laid [117].

7.3 Aspartic and Metallo-proteinase Inhibitors

Aspartic proteinases (cathepsin D-like proteinases) have also been found in six families of Hemiptera. No aspartic proteinases have been found in Coleoptera but Wolfson and Murdock [125] reported that pepstatin, a potent and specific inhibitor of aspartyl proteinases, strongly inhibited proteolysis of the midgut enzymes of the Colorado potato beetle *L. decemlineata*, indicating that an aspartic proteinase was present in the midgut. Potato tubers contain an aspartyl proteinase inhibitor, cathepsin D, which inhibits not only cathepsin but also trypsin and chymotrypsin.

As metalloproteinase, potato and tomato plants have developed for their protection metallo-carboxypeptidases. The inhibitors of these enzymes are polypeptides that strongly and competitively inhibit a broad spectrum of carboxypeptidases from animals and microorganisms [103].

8. MISCELLANEOUS

α-Amylases are important enzymes in plant resistance to pests since they play a key role in carbohydrate metabolism of microorganisms, plants and animals [126]. Moreover, many insects, particularly seed weevils feeding on starchy seeds during larval and/or adult stages depend on their α-amylases for survival. Therefore, a variety of α-amylase inhibitors from different plant sources have been reported to be active against a number of mammalian and insect α-amylases [126,127], paving the way to control these pests through the use of transgenic technology [128]. However and in order to be of practical use for the production of transgenic plants, α-amylase inhibitors must not interfere with the action of endogenous α-amylases of recognized importance, for example in germination, and lack activity also against mammalian enzymes [126].

Inhibition of steroid metabolism is also, in principle, a potential target for insect-specific control agents, particularly the C-24 dealkylation pathway and the ecdysone biogenetic polyhydroxylation sequence [25]. In this regard, monofluorinated cholesterol and phytosterol derivatives were prepared to interfere with the side chain hydroxylations in ecdysone biogenesis. Among them, when 2-fluorophytosterols were fed a cholesterol-free diet they

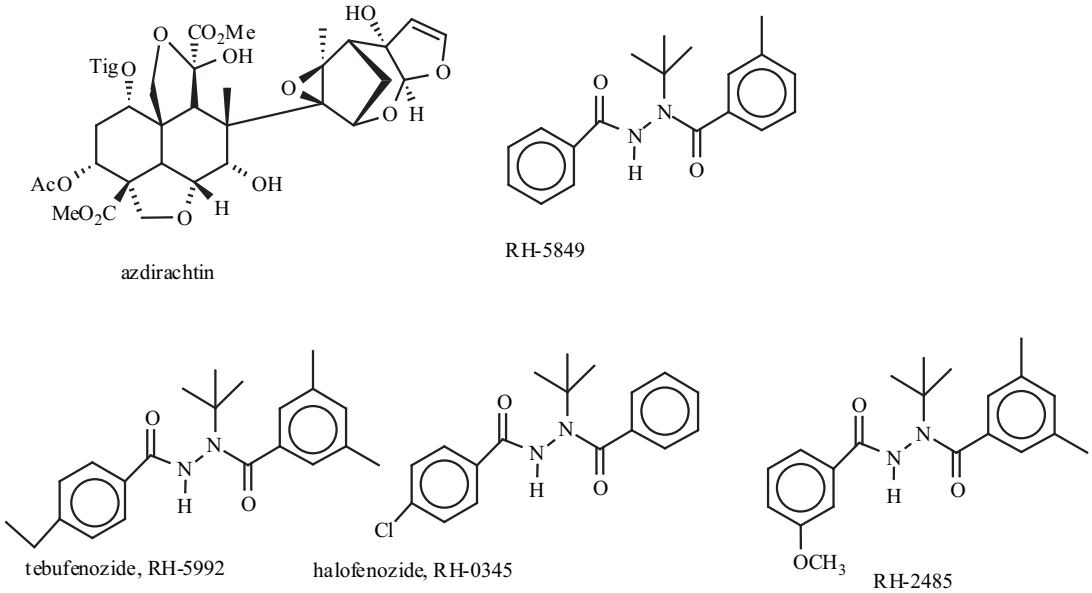

Fig. (5). Structures of azadirachtin and ecdysteroid agonists RH-5849, tebufenozide (RH-5992, halofenozide (RH-0345) and RH-2485.

induced reduction of larval growth, maximum weight, survivorship, and pupation. The authors suggested that inhibition of the C-24 dealkylation pathway occurred after release of a toxic 2-carbon fragment convertible "*in vivo*" to 2-fluorocitrate [25].

As inducers of abnormal molting and development, the molting hormones and related phytoecdysteroids have also been considered potential pest control agents [129], but their high cost, rapid degradation and low species specificity precludes attempts for practical application. However, compounds mimicking the action of natural ecdysteroid hormones have been used to control insect pests, such as azadirachtin. This compound has been isolated from leaves and berries of the neem tree *Azadirachta indica* and *Melia azedarach*, trees commonly occurring in India and East and West Africa. These parts of the trees are widely used for chewing sticks for cleaning teeth and as a remedy against malaria. Azadirachtin is also known for its very strong antifeedant activity against the desert locus *Schistocerca gregaria* and the graminaceous pest *Spodoptera exempta.* The chemical structure of azadirachtin (Fig. (**5**)) is too complex to be synthesized on a practical level. However, since the yield from nature is quite high (up to 800 mg from 300 g of seeds) and the tree is easy to cultivate, the compound has been traditionally used in India to control pests by reducing their feeding, survival and reproduction [130]. Azadirachtin elicits a delay or a permanent block of molting by inhibiting ecdysteroid secretion from the prothoracic gland or by inhibiting the conversion of ecdysone to the more active 20-hydroxyecdysone. At sublethal concentrations, azadirachtin prolonged larval instars and reduced food intake on *S. littoralis* when incorporated into artificial diet [131].

Other compounds with ecdysteroid activity mimic the action of natural ecdysteroid hormones by binding to the same hormone receptors, and therefore elicit abrupt cessation of feeding, interruption of morphogenesis, abnormal course of molt and disruption of reproduction [132].

Among them, synthetic diacylhydrazines with ecdysteroid agonist activity have been disclosed and marketed by Rohm and Haas Co.: RH-5849, tebufenozide (RH-5992), halofenozide (RH-0345) and RH-2485 [60] (Fig. (**5**)). Ingestion of these compounds with the diet leads, as result of a decrease in haemolymph ecdysteroid titers, to premature lethal molts in several lepidopteran, dipteran and coleopteran larvae, stop feeding within 4-16 h, reduction of egg production, ovicidal activity and disruption of normal spermatogenesis [60]. In addition, diacylhydrazines exert low acute toxicity to mammals, birds and fish. In the field, tebufenozide is highly active against *Cydia pomonella* and leafrollers in apples, *S. exigua* in cotton, *L. dispar* in forestry, *Diatraea saccharalis* in sugarcane and numerous other lepidopteran pests in vegetables and ornamentals. Halofenozide, in turn, is active against the scarab beetles, such as *Popillia japonica*, *Phyllophaga* spp., etc. and against caterpillar pests of apple, corn, cotton, grape, rice and vegetables. The effects of the bisacylhydrazines have been postulated to be due to induction of enzymes involved in the metabolic inactivation of ecdysteroids, as demonstrated with RH-5849 in *M. sexta* [133].

Phenol oxidase (PO), the enzyme responsible for the biosynthesis of melanin, is also an important component of insects immune system. The enzyme is also involved in other physiologically important processes, such as initiation of sclerotization of the cuticle, an essential step for the survival of most insects [134]. Moreover, in wound healing massive amounts of haemolymph loss is partly prevented by the action of PO by rapidly depositing melanin pigment at the wounding site. The first inhibitors of PO were identified by Tsukamoto [135] from pupal extracts of house flies as three low molecular weight proteins, which elicited competitive inhibition of endogenous PO activity. Sugumaran [134] has also isolated and characterized a glycoprotein from *M. sexta* larvae that appears to inhibit the PO responsible for the sclerotization of cockroach ootheca. In spite of their important role in regulation of PO oxidase, however, no further studies on the possible application of these inhibitors on pest control have been developed.

FUTURE PROSPECTS

Pest management continues to be an important challenge for the agricultural community. The continuously increasing concern over environmental health and public safety has led to the prohibition of some highly effective broad-spectrum chemicals from the market, and therefore new alternative approaches were developed to improve crop protection. As a result, new biorational agents, more specific and less toxic, have been developed and their mode of action disclosed. However, many of the new biorational products, which only constitute 2-3% of the insecticide market, have two important drawbacks: high production cost and limited applicability. Therefore, new studies at the target enzyme level will serve as the basis for the development of new highly effective and environmentally friendly insect control agents of relatively low cost. In this context, application of the latest advances in biotechnology and genetic engineering, the use of emergent recombinant DNA technology and expression of pesticidal proteins to induce plant natural defensive responses should play a key role in the development of such control agents.

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ABBREVIATIONS

PBP = Pheromone binding protein

PDE = Pheromone degrading enzyme

ODE = Odorant degrading enzyme

TFMK = Trifluoromethyl ketone

OTFP = Octylthiotrifluoropropanone

DTFP = Decylthiotrifluoropropanone

EAG = Electroantennogram

ECB = European corn borer

AChE = Acetylcholinesterase

PBAN = Pheromone biosynthesis activating neuropeptide

JH = Juvenile hormone

JHA = Juvenile hormone analogue

EH = Epoxide hydrolase

AJH = Antijuvenile hormone

PI = Protease inhibitor

CP = Cysteine proteinase

CPI = Cysteine proteinase inhibitor

PO = Phenol oxidase

AOE = Aldehyde oxidizing enzyme

CA = Corpora allata

Bt = *Bacillus thuringiensis*

REFERENCES

[1] Quinn, D. M. *Chem. Rev.* **1987**, *87*, 955.

[2] Powers, J. C.; Harper, J. W. Proteinase Inhibitors; Research Monographs in Cell and Tissue Physiology; Elsevier: Amsterdam, **1986**; Vol. *12*.

[3] Trainor, D. A. *Trends Pharm. Sci.* **1987**, *8*, 303.

[4] Johnson, M. K. *J. Neurochem.* **1974**, *23*, 785.

[5] Vogt, R. G.; Riddiford, L. M. *J. Chem. Ecol.* **1986**, *12*, 469.

[6] Ferkovich, S. M.; Oliver, J. E.; Dillard, C. *J. Chem. Ecol.* **1982**, *8*, 859.

[7] Vogt, R. G.; Riddiford, L. M.; Prestwich, G. D. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 8827.

[8] Klein, U. *Insect Biochem.* **1987**, *17*, 1193.

[9] Ishida, Y.; Leal, W. S. *Insect Biochem. Mol. Biol.* **2002**, *32*, 1775.

[10] Gelb, M. H.; Svaren, J. P.; Abeles, R. H. *Biochemistry* **1985**, *24*, 1813.

[11] Parisi, M. F.; Abeles, R. H. *Biochemistry* **1992**, *31*, 9429.

[12] Angliker, H.; Wikstrom, P.; Rauber, P.; Stone, S.; Shaw, E. *Biochem. J.* **1988**, *256*, 481.

[13] Abdel-Aal, Y. A. I.; Hammock, B. D. *Insect Biochem.* **1985**, *15*, 111.

[14] Ashour, M.-B. A.; Hammock, B. D. *Biochem. Pharm.* **1987**, *36*, 1869.

[15] Street, I. P.; Lin, H. K.; Laliberté, F.; Ghomashchi, F.; Wang, Z.; Perrier, H.; Tremblay, N. M.; Huang, Z.; Weech, P. K.; Gelb, M. H. *Biochemistry* **1993**, *32*, 5935.

[16] Patel, D. V.; Rielly-Gauvin, K.; Ryono, D. E. *Tetrahedron Lett.* **1988**, *29*, 4665.

[17] Liang, T. C.; Abeles, R. H. *Biochemistry* **1987**, *26*, 7603.

[18] Székács, A.; Abdel-Aal, Y. A. I.; Philpott, M.; Matolcsy, G.; Hammock, B. D. In *Biotechnology for Crop Protection*; Hedin, P. A., Menn, J. J., Hollingworth, R. M., Eds.; American Chemical Society: Washington DC, **1988**; Vol. ACS Symposium Series No. 379, p 215-227.

[19] Abdel-Aal, Y. A. I.; Hammock, B. D. *Science* **1986**, *233*, 1073.

[20] Prestwich, G. D.; Streinz, L. *J. Chem. Ecol.* **1988**, *14*, 1003.

[21] Durán, I.; Parrilla, A.; Feixas, J.; Guerrero, A. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2593.

[22] Parrilla, A.; Guerrero, A. *Chem. Senses* **1994**, *19*, 1.

[23] Rosell, G.; Herrero, S.; Guerrero, A. *Biochem. Biophys. Res. Comm.* **1996**, *226*, 2887.

[24] Takahashi, L. H.; Radhakrishnan, R.; Rosenfried, R. E.; Meyer, E. F. Jr., Trainor, D. A. *J. Am. Chem. Soc.* **1989**, *111*, 3368.

[25] Prestwich, G. D. *Pest. Sci.* **1986**, *37*, 430.

[26] Parrilla, A.; Villuendas, I.; Guerrero, A. *Bioorg. Med. Chem.* **1994**, *2*, 243.

[27] Riba, M.; Eizaguirre, M.; Sans, A.; Quero, C.; Guerrero, A. *Pest. Sci.* **1994**, *41*, 97.

[28] Klun, J. A.; Schwarz, M.; Uebel, E. C. *J. Chem. Ecol.* **1991**, *17*, 317.

[29] Bau, J.; Martínez, D.; Renou, M.; Guerrero, A. *Chem. Senses* **1999**, *24*, 473.

[30] Quero, C.; Rosell, G.; Jiménez, O.; Rodriguez, S.; Bosch, M. P.; Guerrero, A. *Bioorg. Med. Chem.* **2003**, *11*, 1047.

[31] Reddy, G. V. P.; Quero, C.; Guerrero, A. *J. Agric. Food Chem.* **2002**, *50*, 7062.

[32] Galcerán, M. Ph.D., University of Barcelona, **1996**.

[33] Feixas, J.; Prestwich, G. D.; Guerrero, A. *Eur. J. Biochem.* **1995**, *234*, 521.

[34] Pophof, B.; Gebauer, T.; Ziegelberger, G. *J. Comp. Physiol. A* **2000**, *186*, 315.

[35] Renou, M.; Berthier, A.; Guerrero, A. *Pest Manag. Sci.* **2002**, *58*, 839.

[36] Malik, M. S.; Vetter, R. S.; Baker, T. C.; Fukuto, T. R. *Pestic. Sci.* **1991**, *32*, 35.

[37] Riba, M.; Sans, A.; Bau, P.; Grolleau, G.; Renou, M.; Guerrero, A. *J. Chem. Ecol.* **2001**, *27*, 1879.

[38] Hammock, B. D.; Wing, K. D.; McLaughlin, J.; Lovell, V. M.; Sparks, T. C. *Pest. Biochem. Physiol.* **1982**, *17*, 76.

[39] Abdel-Aal, Y. A. I.; Roe, R. M.; Hammock, B. D. *Pest. Biochem. Physiol.* **1984**, *21*, 232.

[40] Ding, Y.-S.; Prestwich, G. D. *J. Chem. Ecol.* **1988**, *14*, 2033.

[41] Prestwich, G. D. In *Pheromone Biochemistry*; Prestwich, G. D., Blomquist, G. J., Eds.; Academic Press: New York, **1987**, p 473-527.

[42] Albans, K. R.; Baker, R.; Jones, O. T.; Jutsum, A. R.; Turnbull, M. D. *Crop Prot.* **1984**, *3*, 501.

[43] Baker, R.; Green, A.; Justum, A. R.; Turnbull, M. D., Eur. Patent Appl. EP 42,448, 1981

[44] Prestwich, G. D., US Patent 4.544.504, 1985

[45] Roelofs, W.; Bjostad, L. *Bioorg. Chem.* **1984**, *12*, 279.

[46] Gosalbo, L.; Fabriàs, G.; Camps, F. *Arch. Insect Biochem. Physiol.* **1994**, *26*, 279.

[47] Hernanz, D.; Fabriàs, G.; Camps, F. *J. Lipid Res.* **1997**, *38*, 1988.

[48] Al Dulayymi, J. R.; Baird, M. S.; Simpson, M. J.; Nyman, S. *Tetrahedron* **1996**, *52*, 12509.

[49] Delgado, A.; Ruiz, M.; Camps, F.; Hospital, S.; Guerrero, A. *Chem. Phys. Lipids* **1991**, *59*, 127.

[50] Camps, F.; Hospital, S.; Rosell, G.; Delgado, A.; Guerrero, A. *Chem. Phys. Lipids* **1992**, *61*, 157.

[51] Rosell, G.; Hospital, F.; Camps, F.; Guerrero, A. *Insect Biochem. Molec. Biol.* **1992**, *22*, 679.

[52] Raina, A. K.; Jaffe, H.; Kempe, T. G.; Keim, P.; Blacher, R. W.; Fales, H. M.; Riley, C. T.; Klun, J. A.; Ridgway, R. L.; Hayes, D. K. *Science* **1989**, *244*, 796.

[53] Altstein, M.; Gilon, C. In *Biochemical sites of insecticide action and resistance*; Ishaaya, I., Ed.; Springer-Verlag: Berlin, **2001**, p 151-166.

[54] Devorshak, C.; Roe, M. *Pest. Biochem. Physiol.* **2001**, *69*, 48.

[55] Anspaugh, D. D.; Kennedy, G. G.; Roe, R. M. *Pest. Biochem. Physiol.* **1995**, *53*, 84.

[56] Calderón, J. S.; Céspedes, C. L.; Rosas, R.; Gómez-Garibay, F.; Salazar, J. R.; Lina, L.; Aranda, E.; Kubo, I. *Z. Naturforsch. C* **2001**, *56*, 382.

[57] Céspedes, C. L.; Alarcón, J.; Aranda, E.; Becerra, J.; Silva, M. *Z. Naturforsch. C* **2001**, *56*, 603.

[58] Céspedes, C. L.; Martinez-Vázquez, M.; Calderón, J. S.; Salazar, J. R.; Aranda, E. *Z. Naturforsch. C* **2001**, *56*, 95.

[59] Retnakaran, A.; Granett, G.; Ennis, T. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*; Kerkut, G. A., Gilbert, L. I., Eds.; Pergamon: Oxford, **1985**; Vol. *12*, p 529-601.

[60] Dhadialla, T. S.; Carlson, G. R.; Le, D. P. *Annu. Rev. Entomol.* **1998**, *43*, 545.

[61] Feyereisen, R. In *Pesticide Science and Biotechnology*; Greenhalgh, R., Roberts, T. R., Eds.; Blackwell Scientific: Oxford, **1987**, p 113-116.

[62] Sparks, T. C. In *Safer Insecticides*; Hodgson, E., Kuhr, R. J., Eds.; Marcel Dekker Inc.: New York, **1990**, p 103-154.

[63] *Insecticidas Biorracionales*; Bellés, X., Ed.; Consejo Superior de Investigaciones Científicas: Madrid, **1988**.

[64] Sperry, A. E.; Sen, S. E. *Insect Biochem. Molec. Biol.* **2001**, *31*, 171.

[65] Monger, D. J.; Lim, W. A.; Kezdy, F. J.; Law, J. H. *Biochem. Biophys. Res. Commun.* **1982**, *105*, 1374.

[66] Staal, G. B.; Henrick, C. A.; Bergot, B. J.; Cerf, D. C.; Edwards, J. P.; Kramer, S. J. In *Regulation of Insect Development and*

Behaviour; Sehnal, F., Zabza, A., Menn, J. J., Cymborowski, B., Eds.; Wroclaw Tech. Univ. Press: Wroclaw, **1981**, p 323-340.
[67] Bowers, W. S.; Ohta, T.; Clerre, J. S.; Marsella, P. A. *Science* **1976**, *193*, 542.
[68] Debernard, S.; Rossignol, F.; Couillaud, F. *Gen. Comp. Endocrinol.* **1994**, *95*, 92.
[69] Andersen, J. F.; Ceruso, M.; Unnithan, G. C.; Kuwano, E.; Prestwich, G. D.; Feyereisen, R. *Insect Biochem. Molec. Biol.* **1995**, *25*, 713.
[70] Asano, S.; Kuwano, E.; Eto, M. *Appl. Ent. Zool.* **1986**, *21*, 305.
[71] Castillo, M.; Moya, P.; Couillaud, F.; Garcerá, M. D.; Martínez-Pardo, R. *Arch. Insect Biochem. Physiol.* **1998**, *37*, 287.
[72] Quistad, G. B.; Staiger, L. E.; Cerf, D. C. *J. Agric. Food Chem.* **1982**, *30*, 1151.
[73] Quistad, G. B.; Cerf, D. C.; Kramer, S. J.; Bergot, J.; Schooley, D.A. *J. Agric. Food Chem.* **1985**, *33*, 47.
[74] Veelaert, D.; Devreese, B.; Schoofs, L.; Van Beeumen, J.; Broeck, J. V.; Tobe, S. S.; De Loof, A. *Mol. Cell. Endocrinol.* **1996**, *122*, 183.
[75] Feyereisen, R.; Farnsworth, D. E. *FEBS Lett.* **1987**, *222*, 345.
[76] Roe, R. M.; Venkatesh, K. In *Morphogenetic Hormones of Arthropods*; Gupta, A. D., Ed.; Rutgers University Press: New Brunswick, NJ, **1990**, p 125-179.
[77] Hammock, B. D.; Abdel-Aal, Y. A. I.; Mullin, C. A.; Hanzlik, T. N.; Roe, R. M. *Pest. Biochem. Physiol.* **1984**, *22*, 209.
[78] Allen, K. N.; Abeles, R. H. *Biochemistry* **1989**, *28*, 8466.
[79] Linderman, R. J.; Jamois, E. A.; Roe, M. In *Reviews in Pesticide Toxicology*; Hodgson, E., Roe, R. M., Motoyama, N., Eds.; North Carolina State Univ.: Raleigh, NC, **1991**; Vol. *1*, p 261-269.
[80] Filizola, M.; Rosell, G.; Guerrero, A.; Pérez, J. J. *Quant. Struct.-Act. Relat.* **1998**, *17*, 205.
[81] Linderman, R. J.; Graves, D. M.; Garg, S.; Venkatesh, K.; Anspaugh, D. D.; Roe, M. *Tetrahedron Lett.* **1993**, *34*, 3227.
[82] Prestwich, G. D.; Eng, W. S.; Roe, M.; Hammock, B. *Arch. Biochem. Biophys.* **1984**, *228*, 639.
[83] Roe, M.; Anspaugh, D. D.; Venkatesh, K.; Linderman, R. J.; Graves, D. M. *Arch. Insect Biochem. Physiol.* **1997**, *36*, 165.
[84] Hajós, J. P.; Vermunt, A. M. W.; Zuidema, D.; Kulcsar, P.; Varjas, L.; De Kort, C. A. D.; Závodszky, P.; Vlak, J. M. *Insect Mol. Biol.* **1999**, *8*, 539.
[85] Inceoglu, A. B.; Kamita, S. G.; Hinton, A. C.; Huang, Q.; Severson, T. F.; Kang, K.; Hammock, B. D. *Pest Manag. Sci.* **2001**, *57*, 981.
[86] Lassiter, M. T.; Apperson, C. S.; Roe, M. *J. Insect Physiol.* **1995**, *41*, 869.
[87] Casas, J.; Harshmann, L. G.; Hammock, B. D. *Insect Biochem. Mol. Biol.* **1991**, *21*, 17.
[88] Roe, R. M.; Kallapur, V.; Linderman, R. J.; Viviani, F.; Harris, S. V.; Walker, E. A.; Thompson, D. M. *Arch. Insect Biochem. Physiol.* **1996**, *32*, 527.
[89] Linderman, R. J.; Roe, M.; Harris, S. V.; Thompson, D. M. *Insect Biochem. Molec. Biol.* **2000**, *30*, 767.
[90] Lümmen, P. *Biochim. Biophys. Acta* **1998**, *1364*, 287.
[91] Fang, N.; Rowlands, C.; Casida, J. E. *Chem. Res. Toxicol.* **1997**, *10*, 853.
[92] Obata, T.; Fujii, K.; Yoshiya, H.; Tsutsumiuchi, K.; Yoshioka, H. *Pest. Sci.* **1992**, *34*, 133.
[93] Hollingworth, R. M.; Ahammadsahib, K. I. *Rev. Pest. Toxicol.* **1995**, *3*, 277.
[94] Lewis, M. A.; Arnason, J. T.; Philogene, B. J. R.; Rupprecht, J. K.; McLaughlin, J. L. *Pest. Biochem. Physiol.* **1993**, *45*, 15.
[95] Koiwa, H.; Bressan, R. A.; Hasegawa, P. M. *Trends Plant Sci.* **1997**, *2*, 379.
[96] Meeusen, R. L.; Warren, G. *Annu. Rev. Entomol.* **1989**, *34*, 373.
[97] Estruch, J. J.; Carozzi, N. B.; Desai, N.; Duck, N. B.; Warren, G. W.; Koziel, M. G. *Nature Biotech.* **1997**, *15*, 137.
[98] Ryan, C. A. *Annu. Rev. Phytopathol.* **1990**, *28*, 425.
[99] Schuler, T. H.; Poppy, G. M.; Kerry, B. R.; Denholm, I. *Trends Biotech.* **1998**, *16*, 168.
[100] Jouanin, L.; Bonadé-Bottino, M.; Girard, C.; Morrot, G.; Giband, M. *Plant Sci.* **1998**, *131*, 1.
[101] Michaud, D.; Vrain, T. C. In *Recombinant proteins from plants: production and isolation of clinically useful compounds*; Cunningham, C., Porter, A. J. R., Eds.; Humana Press: Totowa, N.J., **1998**, p 49-64.
[102] Gatehouse, A. M. R.; Gatehouse, J. A. *Pestic. Sci.* **1998**, *52*, 165.
[103] Lawrence, P. K.; Koundal, K. R. *Electronic J. Biotech.* **2002**, *5*, http://www-ejb.org/content/vol5/issue1/full/3.
[104] Bowles, V. M.; Feehan, J. P.; Sandeman, R. M. *Int. J. Parasitol.* **1990**, *20*, 169.
[105] Reed, B. J.; Chandler, D. S.; Sandeman, R. M. *Int. J. Parasitol.* **1999**, *29*, 839.
[106] Pracros, P. *C.R. Acad. Agric. Fr.* **1982**, 1279.
[107] Nozzolillo, C.; Arnason, J. T.; Campos, F.; Donskov, N.; Jurzysta, M. *J. Chem. Ecol.* **1997**, *23*, 995.
[108] Potter, S. M.; Jiménez-Flores, R.; Pollack, S. M. *J. Agric. Food Chem.* **1993**, *41*, 1287.
[109] Adel, M. M.; Sehnal, F.; Jurzysta, M. *J. Chem. Ecol.* **2000**, *26*, 1065.
[110] Sutherland, P. W.; Burgess, E. P. J.; Philip, B. A.; McManus, M. T.; Watson, L.; Christeller, J. T. *J. Insect Physiol.* **2002**, *48*, 327.
[111] Down, R. E.; Ford, L.; Mosson, H. J.; Fitches, E.; Gatehouse, J. A.; Gatehouse, A. M. R. *Parasitology* **1999**, *119*, 157.
[112] Schuler, T. H.; Potting, R. P. J.; Denholm, I.; Poppy, G. M. *Nature* **1999**, *400*, 825.
[113] Ortego, F.; Novillo, C.; Castañera, P. *Arch. Insect Biochem. Physiol.* **1996**, *33*, 163.
[114] Gutiérrez, C.; Castañera, P. *Invest. Agrar.: Prod. Prot. Veg.* **1986**, *1*, 109.
[115] Ortego, F.; Ruiz, M.; Castañera, P. *J. Insect Physiol.* **1998**, *44*, 95.
[116] Linderman, R. J.; Leazer, J.; Roe, R. M.; Venkatesh, K.; Selinsky, B. S.; London, R. E. *Pest. Biochem. Physiol.* **1988**, *31*, 187.
[117] Cloutier, C.; Jean, C.; Fournier, M.; Yelle, S.; Michaud, D. *Arch. Insect Biochem. Physiol.* **2000**, *44*, 69.
[118] Michaud, D. *Trends Biotech.* **1997**, *15*, 4.
[119] Giri, A. P.; Harsulkar, A. M.; Deshpande, V. V.; Sainani, M. N.; Gupta, V. S.; Ranjekar, P. K. *Plant Physiol.* **1998**, *116*, 393.
[120] De Leo, F.; Bonadé-Bottino, M.; Ceci, L. R.; Gallerani, R.; Jouanin, L. *Insect Biochem. Mol. Biol.* **2001**, *31*, 593.
[121] De Leo, F.; Gallerani, R. *Insect Biochem. Mol. Biol.* **2002**, *32*, 489.
[122] Wolfson, J. L.; Murdock, L. L. *J. Chem. Ecol.* **1990**, *16*, 1089.
[123] Elden, T. C. *J. Entomol. Sci.* **2000**, *35*, 70.
[124] Fabrick, J.; Behnke, C.; Czapla, T.; Bala, K.; Rao, A. G.; Kramer, K. J.; Reeck, G. R. *Insect Biochem. Molec. Biol.* **2002**, *32*, 405.
[125] Wolfson, J. L.; Murdock, L. L. *Ent. Exp. Appl.* **1987**, *44*, 235.
[126] Franco, O. L.; Rigden, D. J.; Melo, F. R.; Grossi-de-Sá, M. F. *Eur. J. Biochem.* **2002**, *269*, 397.
[127] Alfonso, J.; Ortego, F.; Sanchez-Monge, R.; García-Casado, G.; Pujol, M.; Castañera, P.; Salcedo, G. *J. Chem. Ecol.* **1997**, *23*, 1729.
[128] Morton, R. L.; Schroeder, H. E.; Bateman, K. S.; Chrispeels, M. J.; Armstrong, E.; Higgins, T. J. V. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 3820.
[129] Kubo, I.; Klocke, J. A.; Asano, S. *J. Insect Physiol.* **1983**, *29*, 307.
[130] Schmutterer, H. *J. Insect Physiol.* **1988**, *34*, 713.
[131] Martinez, S. S.; Emden, H. F. *Bull. Entomol. Res.* **1999**, *89*, 65.
[132] Adel, M. M.; Sehnal, F. *J. Insect Physiol.* **2000**, *46*, 267.
[133] Williams, D. R.; Chen, J. H.; Fisher, M. J.; Rees, H. H. *J. Biol. Chem.* **1997**, *272*, 8427.
[134] Sugumaran, M. *Adv. Exp. Med. Biol.* **2001**, *484*, 289.
[135] Tsukamoto, T.; Ichimaru, Y.; Kanegae, N.; Watanabe, K.; Yamaura, I.; Katasura, Y.; Funatsu, M. *Biochem. Biophys. Res. Commun.* **1992**, *184*, 86.