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PRACTICAL DEVELOPMENT OF NON-TOXIC ANTI-INSECT AGENTS

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REPUBLIC OF HUNGARY

Terminal report*

Prepared for the Government of the Republic of Hungary
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

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Vienna

* This document has not been edited.

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CONTENTS

I. GENERAL CONSIDERATIONS

Our aim was to contribute, by means of basic research strategies, to the international efforts aimed at developing anti-insect agents which are harmless to humans or beneficial organisms and present no environmental damage. Development of new anti-insect agents fulfilling these requirements and offering possibilities for immediate practical utilization served as short term objectives of our work. The long term aim was to reveal new, hitherto unknown insect-selective activities and compounds providing these properties in order to establish a novel theoretical basis for new research trends on the field of chemicals to be applied against insect pests in agriculture.

II. THE PRESENT STATE OF THE FIELD, POSSIBILITIES AND LIMITATIONS

Without crop protection insects, fungi, weeds and other damaging organisms would decrease the world's agricultural production with about 35 per cent, the damage caused by insect participating with 10 per cent. The value of pesticides used for crop protection amounts to 19 billion US$ annually, 37 per cent of which being accounted for anti-insect agents.

The well known harmful side effects of pesticides, first of all their toxicity on humans and beneficial organisms and the increasing chemical contamination of the environment is highest in case of insecticides. The explanation of this roots in the

fact that the point of attack of most of the presently used traditional insecticides is the nervous system, therefore their extremely broad spectrum of activity encompasses the whole human and major part of animal population, permitting a narrow margin for the development of chemicals acting specifically against insects.

A fundamental solution of this problem can be brought about only by the development of chemicals which are directed against biochemical or physiological functions characteristic specifically to insects. This concept manifests itself in the research on synthetic chemicals which either imitate or inhibit the activity of the natural insect hormones, such as the juvenile hormones and the molting hormone (ecdysterone) regulating the cycle of insect metamorphosis; in research on substances inhibiting the formation of insect cuticle; and in search for behavior-controlling compounds, such as sex-pheromones, feeding and oviposition deterrents.

1. The metamorphosis cycle of insects is controlled by two type of insect hormones, the juvenile hormones and the molting hormone (ecdysterone). Shortly after the discovery of the juvenile hormones in the late sixties the idea was raised that synthetic juvenile hormone analogs (juvenoids) imitating the activity of the natural hormones and also compounds blocking the biosynthesis of juvenile hormones - both of them disrupting the hormonal equilibrium - could be employed as highly specific anti-insect agents in crop protection and other fields of insect control. Out of the two alternatives the application of juvenile hormone analogs (juvenile hormone mimics) obtained greatest practical importance and 4-5 of about 3,000 known juvenile hormone analogs described in the literature have reached

practical application and regulatory status. Yet their field of application is limited to specific areas. Insufficient chemical stability, delayed action and narrow range of insecticidal spectrum manifested the main factors limiting the overall use of these products. The insufficient chemical stability could be overcame lately by the development of more stable juvenile hormone analogs, but the other disadvantageous properties necessitate further research on this field.

2. Another approach to the disruption of the hormonal equilibrium is the development of specific inhibitors of enzymes involved in the biochemical processes of hormone biosynthesis and metabolism.

3. High costs, excessive chemical persistence and undesirable physical properties are those main factors limiting the extended application of the benzoyl-urea type chemicals acting as inhibitors of formation of chitin, the main component of the insect cuticle.

4. The other large group of insect-selective, environmentally friendly anti-insect agents consists of chemicals modifying behavioral pattern of insects. The most extensively studied field within this overall approach is focused on the application of synthetic chemicals identical with the long distance volatile signaling compounds of insects (pheromones). The main areas of their application rests on using pheromone treated traps and disturbance of orientation to prevent mating.

5. A further subgroup of insect behavior controlling compounds is represented by feeding and egg-laying deterrents.

Research on this field, taking back to four decades, has not reached the level of practical applicability but results of the latest years have stimulated interest in this direction.

6. The above mentioned modes of action, each of which represents a separate research trend, offer significant possibilities as regard to replace the presently used harmful insecticides by environment-friendly anti-insect agents harmless to humans and beneficial organisms. Yet their utilization created a number of new problems to be solved. Therefore research can not remain within the framework of already known modes of action and further research is needed to discover new, hitherto unknown insect-specific activities.

III. WORK SCHEDULE SPECIFICATION

Based on joint consideration of

- the overall objective of the project as outlined under Chapter I,
- the perspectives of the research field as outlined in Chapter II and
- the research possibilities of the project team,

we outlined the main trend of the project as follows:

1. Design, synthesis and biological evaluation of new juvenile hormone analogs (compare Chapter II, Point 1) in order to obtain derivatives possessing better biological properties as compared to those known earlier.

2. Development of inhibitors of insect-specific enzymes, such as cytochrome P-450 dependent enzymes, in order to inhibit the enzyme methyl-farnesoate-epoxidase participating in the biosynthesis of natural juvenile hormone and to inhibit ecdysone-20-monooxygenase participating in the formation of the molting hormone (ecdysterone); search for inhibitors of juvenile hormone-esterase, an enzyme playing crucial role in the biochemical breakdown of the vital juvenile hormone (see Chapter II, Point 2).

3. Search for compounds selectively inhibiting feeding and egg-laying of insects (see Chapter II, Point 5).

4. Research on compounds possessing new, hitherto unknown selective anti-insect activity

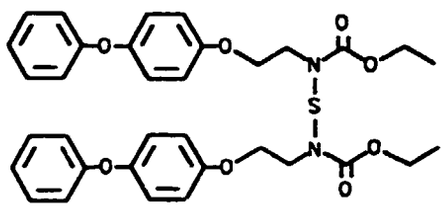
IV. EXPERIMENTAL METHODS, RESULTS AND THEIR DISCUSSION

1. Research on novel juvenile hormone analogs

a) Compounds synthesized

In the design and synthesis of new juvenile hormone analogs possessing higher efficacy or different spectrum of insecticidal activity than the presently known other juvenoids, the "pro-drug" principle commonly used in pharmaceutical research as well as the "pro-insecticide" principle introduced into the research of conventional insecticides by Fukuto and co-workers and by Drabek and co-workers, were applied. In essence a derivative of a known medicament or pesticide molecule is synthesized which may exhibit

more favorable features in its uptake, translocation, action or other characteristics than the parent compound, and which, when entering the organism and before reacting with the site of action, splits off the "pro-drug" moiety and gives birth of the original molecule. In our work, we designed and synthesized several derivatives of a juvenile hormone analog known as fenoxycarb where nitrogen atoms of two fenoxycarb molecules were coupled through a sulfur atom or a sulfoxy group as well as other derivatives where a sulfur atom or a sulfoxy moiety links the fenoxycarb molecule to a biologically indifferent carbamate ester bearing significance, however, on the uptake and translocation.



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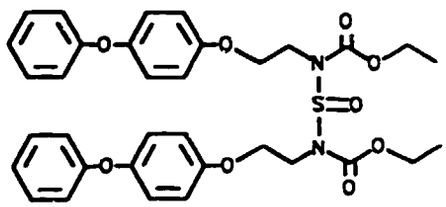

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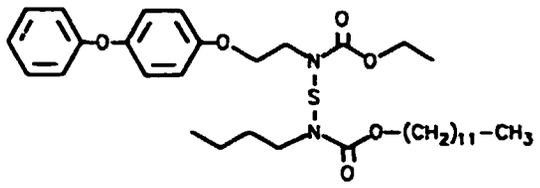
In the synthesis of compounds 1 and 2, fenoxycarb was brought into reaction with sulfenyl chloride or thionyl chloride, respectively, in pyridine as a medium. In the case of compounds 3, 4 and 5, as the first step, N-sulfenyl or N-sulfinyl derivatives of the corresponding carbamate ester were synthesized followed by the reaction with fenoxycarb in pyridine as a medium.



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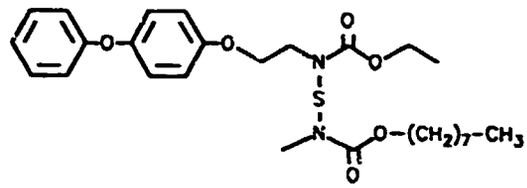

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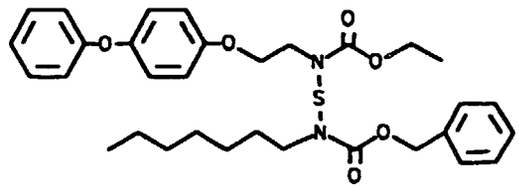

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b) Laboratory bioassays

In laboratory tests carried out on larvae of the large white butterfly (Pieris brassicae), a typical representative of the insect order Lepidoptera, the activity of the compounds was expressed in ID_{50} or LD_{50} (μg/specimen) values, respectively. These data represent dosages evoking a medium average score of larval-pupal intermediates or introducing morphogenetic malformations in 50% of the treated larvae. As a reference compound, the similarly acting and commercially available juvenoid, fenoxycarb was used. Results of these assays show satisfactorily good activity of three experimental chemicals which were in this respect superior to fenoxycarb:

		ID_{50}	LD_{50}
2	NKI-35120	0.0026	< 0.0001
3	NKI-43049	0.0028	< 0.0001
4	NKI-43050	0.0026	< 0.0001
fenoxycarb (standard)		0.021	< 0.0001

In the laboratory assay on the San José scale (Quadraspidiotus perniciosus), a representative of Homoptera, scale insect populations reared on the surface of pumpkins and sprayed with 5 mM emulsions of NKI-35120 and NKI-43049, exhibited 100 % mortality 3 months after treatment.

When the compounds were tested on the grey fleshfly (Neobelliata bullata, Diptera), NKI-35117 and NKI-46015 proved to be the most effective surpassing the activity of fenoxycarb by one order of magnitude:

		LD_{50}
1	NKI-35117	0.03
5	NKI-46015	0.06
fenoxycarb (standard)		0.25

A series of investigations conducted on the insect species, cotton stainer (Dysdercus cingulatus, Heteroptera) gave the

conclusion that NKI-43049 and NKI-35120 exhibited the highest activity surpassing that of fenoxycarb by one order of magnitude.

On our proposal UNIDO commissioned Slough Laboratory (functioning under the supervision of the British MAFF) to perform assays with our compounds for their potential as control agents against some insect pests of stored grain (Sitophilus granarius, Tribolium castaneum). Results were positive also in this case but our juvenoids could surpass fenoxycarb in activity only in a topical assay on Tribolium pupae.

Investigations of Prof. G. Prestwich (New York State University, Stony Brooks, N.Y., USA) revealed that NKI-43050 influenced the social behavior of termites in a unique way and, therefore, it may have good chance in the elaboration of new, selective control measures against termite species.

Duphar B.V. in the Netherlands carried out extensive laboratory assays (using large series of dosages) with our experimental compounds with the aim to establish their efficacy and activity spectrum in insects.

Without mentioning numerical data in detail, the main conclusions can be summarized as follows:

On larvae of the yellow fever mosquito (Aedes aegypti), NKI-35120 proved to be 3-10-fold more active than the reference compound, the commercially available fenoxycarb.

Against the woolly aphid (Eriosoma lanigerum) neither NKI-35120, nor fenoxycarb was effective. On the basis of these negative results it can be stated with a great probability that no effect of juvenoids can be expected on this species.

In the case of the tobacco budworm (Heliothis virescens) ovicidal action was tested in a quantitative assay. The efficacy of NKI-35120 and fenoxycarb was essentially the same but 100% mortality of the eggs was never observed.

On a population of the apple mussel scale (Lepidosaphes ulmi) NKI-35120 did not show any effect, in contrast to fenoxycarb which proved to be rather efficient.

Against larvae of the Colorado potato beetle (Leptinotarsa decemlineata) NKI-35120 produced much the same efficacy as fenoxycarb but both of them was surpassed significantly by another experimental compound, NKI-46015.

When our compounds were applied to the larvae of the housefly (Musca domestica), NKI-43049 was prominently the best juvenoid being superior in activity both to NKI-35120 and diflubenzuron (Dimilin), a known product of Duphar but even S-31183, a preparate of Sumitomo (Japan) already on the market as a potent, selective control agent against housefly under the trade name Sumilarv.

On the peach aphid (Myzus persicae) NKI-35120 exhibited slightly better efficacy than fenoxycarb but none of them could reach the potency of pyrimicarb, a carbamate aphid presently used against species resistant to the environmentally hazardous phosphoric acid esters. Against this insect pest juvenoids are generally not suitable at present to replace directly toxic insecticides.

Against the brown rice leafhopper (Nilaparvata lugens) all experimental juvenoids and fenoxycarb showed rather weak effectiveness.

In the case of the diamond back moth (Plutella xylostella) the rate of ovicidal action was studied. The experimental compounds and fenoxycarb exhibited merely medium or low activity.

On larvae of the desert locust (Schistocerca gregaria) the best efficacy was found in the case of NKI-43049 which was superior in this respect to fenoxycarb. While NKI-43049 resulted in 100% mortality when sprayed in a concentration of 10 mg/liter,

fenoxycarb could produce the same efficacy at a concentration of 100 mg/liter.

Against the larvae of the Egyptian cotton leafworm (Spodoptera littoralis) all experimental juvenoids and fenoxycarb gave excellent efficacy. The chemicals produced 100% effectiveness when tested in concentrations of 100, 10 and 1 mg/liter but they gave 84 to 100% larvicidal efficiency even in 0.1 mg/liter.

On the red spider mite (Tetranychus cinnabarinus) all experimental compounds as well as fenoxycarb proved to be ineffective.

When the chemicals were tested on the onion thrips (Thrips tabaci) and the effectiveness was expressed as the percentage of leaf-damage, fenoxycarb proved to be the best compound (8% damage after using a concentration of 100 mg/liter) followed by NKI-35120 (13% damage). The effectiveness of propoxur used as a standard was significantly lower (23% of leaf damage) which can be an indication that NKI-35120 can be considered as a perspectivic compound also against this pest species.

Against the greenhouse white fly (Trialeurodes vaporariorum) NKI-43050 exhibited very good efficacy surpassing also that of fenoxycarb.

In our laboratory tests carried out on larvae of the cereal bug (Eurygaster austriaca), a representative of a dangerous wheat pest bug family, NKI-35120 gave the best effectiveness being more active than the reference compound, fenoxycarb.

From the viewpoint of the practice, the results of these laboratory assays reflect promising effectiveness of our new juvenoid preparates. Depending on the test species, however, various chemicals exhibited the highest bioactivity. It was considered to use all of these compounds in the field trial

planned for the next period. Due to our restricted possibilities, however, in our further investigations we focused on NKI-35120 because

- its synthesis is the most economic; and
- on the majority of pest species this compound provided the maximum effectiveness.

We took into consideration (but unfortunately could not realize) the large-scale evaluation and practical development of NKI-43049 as a specific agent against the housefly. The fact which substantiated this intention was the high effectiveness found by Duphar B.V. This compound is the best in comparison to other well-known and commercially used juvenoid preparates and its activity surpasses that of the Japanese preparate, S-31183 by one order of magnitude.

c) Field experiments

During the spring of 1990, a field trial with penultimate and last instar (L_4 and L_5) larvae of the summer fruit tortrix moth (Adoxophyes orana, Lepidoptera: Tortricidae) was set up in apple tree branches covered with isolating bags. The foliage was sprayed with 0.005% and 0.0005% aqueous emulsion of NKI-35120. The commercial WP formulation of fenoxycarb served as a standard preparate. The insect material was recovered after 15 days and the numbers of the dead individuals perished due to the specific juvenoid action, were determined. The efficiency calculated after Abbot proved to be in average 16% higher in groups treated with NKI-35120 than that found after applying fenoxycarb.

In another experiment, caterpillars of the gypsy moth (Lymantria dispar, Lepidoptera: Lymantriidae) were reared on an artificial diet in the laboratory. L_5 instar male and L_6 instar female larvae were put into the isolating bags placed, after treatment, on oak tree branches. NKI-35120 was used in 0.02% and 0.002% concentrations and, in comparison, fenoxycarb was tested in the same concentrations. In this case our preparate, NKI-35120 could surpass the efficiency of the reference compound only by 3-4%.

The Dutch firm, Duphar B.V. conducted a field trial in Egypt where the effectiveness of NKI-35120 was investigated on a cotton plantation against the tobacco whitefly (Bemisia tabaci). Treatments were carried out twice on experimental plots of 87 m^2 size. Fenoxycarb was used as a reference compound. The effects were evaluated 7 times after treatment in regular intervals. Data on the last check made on the 21st day after treatment, are summarized in the table below. Calculated according the Henderson-Tilton formula, these results reflect the decrease of nymphal and adult populations:

Preparate	Dosage (a.i.) g/ha	Decrease of population (as percent of untreated control) nymphs	adults
NKI-35120	50	51.53	4.51
	100	54.52	6.58
	200	62.43	7.57
fenoxycarb	100	42.63	0
	200	46.26	0
	400	48.15	0

Results show that, though NKI-35120 surpassed in effectiveness fenoxycarb tested as a standard, the reduction of the adult population did not prove to be sufficient for an effective control of the tobacco whitefly.

With the financial support of UNIDO, the Pakistan Agricultural Research Council (PARC), Islamabad, undertook a field trial to assess the control potential of NKI-35120 against the tobacco whitefly (Bemisia tabaci) and certain lepidopteran cotton pests (Earias spp., Heliothis armigera, Pectinophora gossypiella) parallel with fenoxycarb and a presently used toxic insecticide, a phosphoric acid ester. Results obtained in this field test demonstrated that NKI-35120 surpassed fenoxycarb by 10-15% but the mortality values remained below those found after the use of the phosphoric acid ester. At the same time, both NKI-35120 and fenoxycarb reduced significantly the appearance of normal adults. This finding draws our attention to the possibility that on long-term scale NKI-35120 could provide us with an appropriate effectiveness necessary in the practice.

In Egypt (near Mansoura), in an orange plantation NKI-35120 was tested against the mussel purple scale (Lepidosaphes beckii) and it was found that this compound surpassed the effectiveness of fenoxycarb by 40%. At a concentration of 0.1% it caused 88% mortality and reduced the egg production of surviving females.

d) Prospects of product development, patent status

The Hungarian national Patent Office has granted a patent for this family of molecules (Hungarian Patent No. 196,055). Innofinance General Innovation Bank Corp., Budapest acquired

subsequently the exploitation right of this patent from the Plant Protection Institute of the Hungarian Academy of Sciences and made patent pendings for these compounds in several countries. The judgement of the pendings are still under process in some countries but in a few others the patent has been granted (among others: Belgian Patent No. 1,000,120 and US Patent No. 4,745,128).

Duphar B.V. in the Netherlands took the exploitation of this invention into its own pesticide development program and performed extensive pharmacological investigations with these compounds. The studies gave evidence on the beneficial practical characteristics of the compounds studied. However, Duphar merged the Belgian firm, Solvay and the latter subsequently abolished the whole pesticide profile. At present, two firms, FMC in the US and Toyo-Menka in Japan assess the practical potential of these juvenoid compounds for the sake of an optimal decision concerning a future product development.

Results obtained in the research of juvenile hormone analogs can be summarized as follows:

1) A novel group of insect juvenile hormone analogs was invented. These new compounds exert their action through the interference with the hormonal balance regulating insect development and, therefore, they are harmless to man, other living organisms and the environment, but at the same time, they seem to be perspectivic for replacing the present-day used anti-insect agents in agriculture and hygiene.

2) The scientific value of these results are emphasized by the facts that, on one hand, the authors could elaborate this new

family of molecules on the basis of fundamental research using a biorational, theoretical approach of molecular design, in contrast to the random selection method widely used in the industrial research necessitating also a large screening apparatus, and, on the other hand, only 4-5 anti-insect agents of JH analog-type could reach the pesticide market with no respect to the intensive research conducted in this field.

3) The majority of compounds exhibited very good effects on a series of pest insects having significance in the agriculture and hygiene. Depending on the insect species used in the assays, various compounds gave the best efficacy, a fact that makes the selection of a single compound for practical development more difficult. Nevertheless, in many cases the use against a single species may represent such a large application area that, in itself, may render the practical development of a juvenoid agent economic.

For example, it was NKI-35120 which exhibited the best effectiveness against the large white butterfly (Pieris brassicae), the San José scale (Quadraspidiotus perniciosus), the Egyptian cotton leafworm (Spodoptera littoralis), as well as the mussel purple scale (Lepidosaphes beckii), the cereal bug (Eurygaster austriaca), the rust-red flour beetle (Tribolium castaneum), the cotton stainer (Dysdercus cingulatus), the yellow fever mosquito (Aedes aegypti), while NKI-46015 proved to be the most active on the Colorado potato beetle (Leptinotarsa decemlineata) and NKI-43049 on the housefly (Musca domestica). This latter compound can compete with other, selective chemicals available in the market against the housefly.

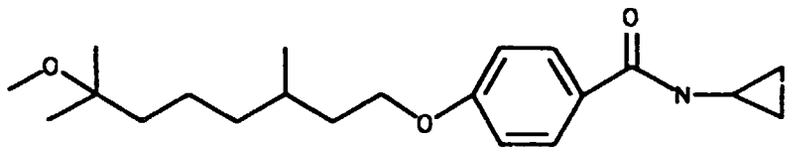
4) The novel, selective, environmentally sound anti-insect agents elaborated in our institute received patent protection in Hungary and in certain other countries, including the US and Belgium, and patent pendings are in progress in other countries. The chemicals are investigated at FMC in the US and Toyo-Menka in japan for assessing the possibility of practical development.

2. Research of selective inhibitors acting on cytochrome P-450 systems

The sesquiterpenoid juvenile hormones (methyl esters of epoxidized farnesoic acid or its homologs: JH) appear to be unique to insects. The "poly-hydroxylated" steroid molting hormones ecdysteroids (E) are also especially typical to non-vertebrates. We have concentrated our efforts on an enzyme class which is crucial to the biosynthesis of both these hormones. Specifically, the research project aimed at developing novel anti-insect agents acting on cytochrome P-450-linked monooxygenase systems, i.e. anti juvenile hormone agents (AJH) acting and anti ecdysterone agents (AE) acting on on methyl farnesoate epoxidase (MFE) and ecdysone 20-monooxygenase (E-20-M), respectively. The synthesis of anti hormone agents inhibiting or inactivating cytochrome P-450 and thus preventing the formation of these important hormones may offer promising approaches to disrupting insect development and reproduction (see Chapter II, Point 2).

N-Cyclopropylbenzylamines are highly potent inactivators of microsomal cytochrome P-450. N-Acylcyclopropylamines (N-cyclopropylamides) too, have inhibitory activity. Therefore, the molecule bearing a N-Cyclopropylamine moiety can be expected to

inhibit MFE selectively. Compound 6, a N-cyclopropylamide derivative of a known juvenoid, is a good in vitro inhibitor (EC_{50} = 42 μM) of the biosynthesis of JH in cockroach (Diploptera punctata), but it has significantly lower activities in the assays for inhibition of microsomal cytochrome P-450. No in vivo JH-antagonist activity of this compound was detected. This might be caused by an intrinsic JH-agonist activity, the agonist activity may be masked by the activity of compound 6 as a JH analog.



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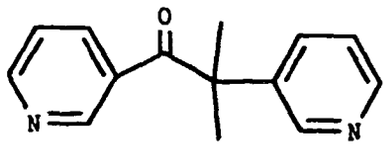
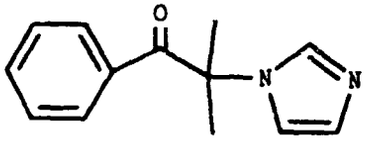
Metyrapone (compound 7) is one of the best competitive inhibitors of steroid hydroxylases. In addition to its potent inhibitory activity on 11-ß-hydroxylase cytochrome P-450 (P-$450_{11ß}$) of the adrenal cortex, it binds to phenobarbital-inducible forms of cytochrome P-450 from various tissues as well as has good in vitro inhibitory action on ecdysone 20-monooxigenase from different insects. The possible agricultural utilization of metyrapone derivatives of anti ecdysterone action has been first recognized by the scientists having taken part in this project. At the beginning of the design of the inhibitors of E-20-M metyrapone was considered as the lead compound because this was the only molecule of simplest structure that had been known as potent E-20-M inhibitor. We were aware of the fact that metyrapone was not selective for mammals, therefore, one of our

first aims was to alter its structure toward selectivity. Structure-activity studies have shown that only the B-pyridine ring takes part in the ligand formation with the heme of cytochrome P-450$_{11\beta}$, the A-pyridine ring therefore could be replaced by a phenyl group without loosing activity. A-phenyl-metyrapone, moreover is a better inhibitor than metyrapone itself. Another structural requirement found to maintain activity was the presence at least one methyl group in position 2. A good correlation has also been observed between the molecular size of metyrapone analogs and their 11β-hydroxylase inhibition potency. Taking these structural requirements into consideration, along with the observation that many imidazole derivatives are more potent inhibitors of cytochrome P-450 than pyridines, we replaced the A-pyridine ring to a phenyl group and the B-pyridine ring with an imidazole moiety or a 1,2,4-triazole ring to obtain A-phenyl-B-imidazolyl-metyrapone (PIM, 8) and A-phenyl-B-triazolyl-metyrapone (PTM, 9) respectively.



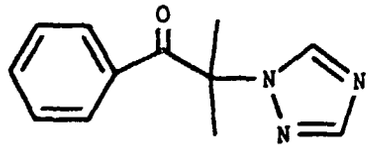
METYRAPONE

7



PIM

8

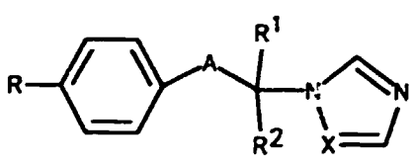


PTM

9

PIM shows good inhibitory activity of E-20-M (IC_{50} = 7.89 x 10^{-7} M) from the fleshfly (Neobelliata bullata) and delayed significantly the pupariation of the same insect, possibly as consequence of E-20-M inhibition. PIM also showed acute toxicity (LC_{50} = 148 ppm) and caused molting disturbances as well as spiracle and mandible malformations. PTM was less active than PIM. PIM and PTM proved to be potent inhibitors of microsomal cytochrome P-450 monooxygenase in the housefly and cockroach midgut aldrin epoxidation tests.

In our further work we considered PIM as lead compound. The general structure of the compounds have been prepared for studying the structure-activity relations is shown by the figure.

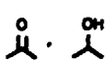


10

R = H, Cl, MeO, $PhCH_2O$

R^1 = H, Me, Et, *i*-Bu

R^2 = H, Me

A = 

X = CH, N

From the results of the examination of the compound on the larval development of the fleshfly (Neobelliata bullata) the following conclusions could be drown:

a) The imidazole derivatives are more active than the ones containing triazole;

b) PIM is 3.7 times more active than metyrapone;

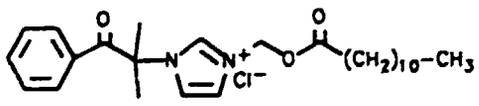
c) At least one methyl group should be present at the carbon atom adjacent to the carbonyl function to maintain activity;

d) The reduction of the carbonyl group to hydroxy decreased the activity in the case of PIM, while enhanced it in the case of PTM;

e) The imidazole derivatives lengthened the time of the larval development, the survivors reached the white pre-pupal stage later than the untreated control;

f) More compounds caused malformations of spiracles and mandibles.

We anticipated the possibility that compounds having good activity in cell-free systems may be less effective <u>in vivo</u> due to physico-chemical problems of penetration or adverse metabolism. We endeavored to improve the bioavailability, the performance of PIM and PTM molecules by derivatizing them to bioactivatable soft alkylated pro-inhibitors (sPIM and sPTM, compound 11 and 12). A parallel goal with the soft-alkylation was also to improve persistence, the easy (optimal) natural degradation.



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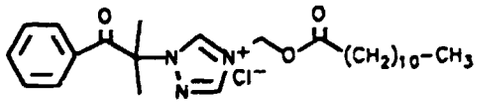

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sPIM delayed the pupariation of the larvae of <u>Neobelliata bullata</u>. This action could be reversed by the simultaneous

application of 20-hydroxy-ecdysone. sPTM had poor activity in this test. In vitro, however, both compounds were moderate inhibitors of E-20-M. If these compounds were let stand for one day in aqueous buffer before application the action was increased in the case of PIM, while decreased in the case of PTM. The facts show unambiguously that these soft-alkylated molecules can not be considered pro-derivatives because

i) They inhibit the enzyme even in systems where there are no enzyme(s) (hydrolases) present that could release the active compound;

ii) In aqueous medium, the molecules slowly decompose to form the more potent PIM or the less active PTM, respectively. The action of sPIM and sPTM is possibly due to their own quaternary structure.

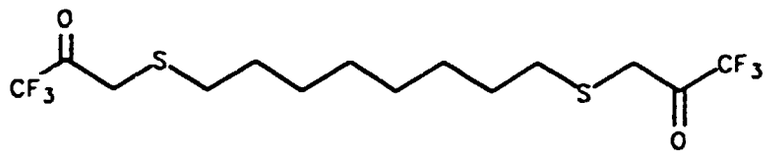
Our results are promising for developing anti-insect agents acting on physiologically essential P_{450} systems similarly to the success of the recent development of agrochemical azole-type fungicides based upon the inhibition of the P_{450} catalyzed lanosterol metabolism.

3. Research on transition state analog inhibitors of juvenile hormone esterase

The insect enzyme, juvenile hormone esterase (JHE) is of key importance in the proper timing of insect metamorphosis. A possible avenue for the development of selective anti-insect agents is, therefore, the creation of specific inhibitors of JHE (see Chapter II, Point 2). Our group participated in such a program conducted according to this research strategy at the

laboratory of Prof. Bruce D. Hammock at the Departments of Entomology and Environmental Toxicology, at the Davis campus of the University of California.

As potential transition state analog inhibitors of JHE, series of 3-alkyl-, 3-aryl-, or 3-heteroaryl-thio-1,1,1-trilfuoro-2-propanones, as well as α,α'-bis(2-oxo-3,3,3-trifluoro-propil-thio)alkanes were prepared in the reaction of the appropriate thiols or dithiols and 3-bromo-1,1,1-trifluoro-2-propanone. JHE obtained from the larvae of the cabbage looper (Trichoplusia ni) was inhibited by some of these compounds even at the low nanomolar range. 1,1,1,16,16,16-Hexafluoro-4,13-dithia-2,15-hexadecadione (compound 13) with a molar I_{50} value against JHE of 8.2×10^{-10} was found as the most potent inhibitor within the series.



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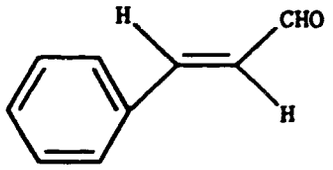
Applied at doses of 0.2 μmol/larva the compound has strongly repressed pupation of the larvae. With mathematical methods involved, we studies a quantitative relationship between the chemical structure of the JHE inhibitors and their biological activity, and concluded that mainly hydrophobic interactions control the molecular level of the mode of action.

Based on these results the application of the transition state analog theory of enzyme inhibition seems to be an effective approach in the design of selective inhibitors of JHE and thus, the development of selective anti-insect agents.

4. Research of insect antifeedants and ovipositional deterrents

The research of insect antifeedants and ovipositional deterrents is a novel tendency within the efforts to seek selective methods of plant protection. The resistance developing against the traditional insecticides in various insect species as well as the increasingly stricter registration procedures concerning the toxicological and environmental risk assessment have turned the attention to representatives of these novel modes of action.

Hollingworth, Cowles and Miller (Michigan State University, East Lasting, Mich, USA) have established that cinnamaldehyde is a stronger ovipositional deterrent to onion fly (Delia antiqua) than other compounds with the same action, reported earlier. Based on this observation we have synthesized about 50 cinnamyl-, cinnamoyl- and phenethyl-derivatives with the modification of cinnamaldehyde (**14**) as base compound.



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The syntheses of a part of the compounds are described in the literature, but in many cases we have elaborated new or improved synthesis methods. (E)-α,β-unsaturated fatty acids (**15**) were reduced to allyl-alcohols followed by oxidation to give the required end-product [(E)-**17**]:

$$\underset{\textbf{15}}{RCH{=}CHCOOH} \longrightarrow \underset{\textbf{16}}{RCH{=}CHCH_2OH} \longrightarrow \underset{\textbf{17}}{RCH{=}CHCHO}$$

(E)-$PhCH{=}CHCH_2CH_2CHO$ aldehyde was obtained by the bromination of (E)-cinnamalcohol (**16**) followed by a multistep "malonester synthesis" on the obtained bromine derivative.

The oxidation of the alcohol, obtained with a Wittig-reaction on 5-hydroxypentanal, gave the compounds $PhCH{=}CHCH_2CH_2CH_2CHO$ (E/Z = 8:2).

The preparation of the corresponding carbamates (**20**) were carried out from substituted cinnamalcohols (**16**, R = aromatic) as starting materials. An improved procedure has been developed for the preparation of acid azides, using phase transfer catalysis method. The corresponding carbamates (**20**) were obtained from the reaction of isocyanates (**19**), formed <u>in situ</u> from azides (**18**) and alcohols (**16**).

$$R'COCl + NaN_3 \longrightarrow \underset{\textbf{18}}{R'CON_3} \longrightarrow \underset{\textbf{19}}{R'NCO} \longrightarrow \underset{\textbf{20}}{R'NHCOOCH_2CH{=}CHR}$$

The ovipositional deterrent tests of the compounds prepared, were conducted by the research group headed by Prof. Hollingworth. The outstanding activities of certain compounds tested, surpassing the action of cinnamaldehyde itself to a great extent, are hopeful from the point of view of future practical applications. From the results the general conclusion can be drown that moderately polar substituents R and R', respectively, are advantageous for the good activity, although they should be of a certain volatility.

The compounds synthesized were tested for antifeeding action by Dr. Árpád Szentesi at our institute on larvae of the potato beetle (Leptinotarsa decemlineata). The compounds showing antifeedant action were almost the same ones which revealed positive action in the ovipositional deterrent tests as well. This fact indicates that a parallelism might exist between the structural requirements of these two activities. From this, a similarity in their mode(s) of action may also be anticipated. The only difference could be observed that some more polar and less volatile compounds, bearing more substituent on the aromatic ring showed antifeedant action, but at the same time they were inactive as ovipositional deterrents.

As the results obtained from these preliminary tests have been encouraging we are preparing our laboratories for a longer work for the synthesis and biological evaluation of antifeedant and ovipositional deterrent compounds.

The significance of our work carried out so far and giving positive results is given by the fact that the research on the development of synthetic antifeedants and ovipositional deterrents is going on only with moderate intensity over the world, so our compounds have contributed to the relations between the structural requirements and biological actions to a large extent, enlarging the circle of the synthetic antifeedants and ovipositional deterrents.

5. Research on compounds possessing new, hitherto unknown selective anti-insect activity

The type of compounds representing modern, alternative trends of selective insect control (compare to Chapter II, Points

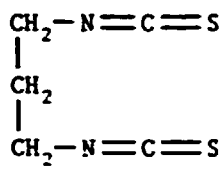
1-5) could fulfill the initial expectations on partial areas only. While they seemed to abolish greatest drawbacks of the traditional insecticides, namely their toxicity to humans and beneficial organisms as well as damaging effects on environment, they raised a number of new problems as regard to method of application, unfavorable timing, narrow range of biological activity and other aspects, therefore they proved to be unsuitable to replace the traditional insecticides on the whole field of their application.

We considered therefore that research must exceed the boundaries of the presently studied alternative insect-selective activities and must envisage also the search for new, hitherto unknown anti-insect effects and of chemicals possessing such biological properties. Our choice fell on seeking for compounds selectively interfering with processes of cuticle formation, a highly insect-specific physiological function, and starting from analogy considerations, we focused on bivalent sulphur compounds, including thio-, dithio-carbamates and isothiocyanates.

We entered cooperation with the Department of Zoology of the Washington State University, as the research team working under the direction of Prof. Lynn M. Riddiford represents highest level investigation stduies on the field of insect cuticle-biochemistry and -physiology. This department performed the highly specific biological investigation of the compounds synthesized by the project team.

A significant outcome of our work was the finding that some aliphatic and cycloaliphatic 1,3- and 1,4-bis-isothiocyanates can selectively inhibit the formation of spiracles and of crochets in larvae emerging from previous instar stages, treated with these chemicals in extremely low dosages. While the compounds inhibiting the formation of spiracles (the openings on the larval

integument needed for respiration) result in a lethal upset of respiration, those inhibiting formation of crochets (needed for the larvae to grasp the plant), might cause falling to the ground and starving to death.



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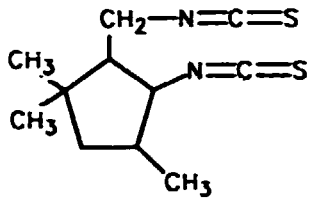

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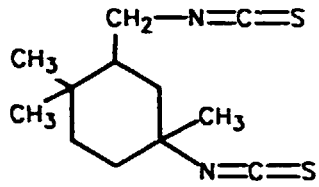

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Investigating the chemical structural requirements for this activity led to the conclusion that only bis-isothiocyanates are able to exert this effect, while mono-isothiocyanates are inactive. This indicates that the bifunctional character of the bis-isothiocyanates is the decisive factor. Our finding on inhibited formation of spiracles and crochets is a new insect-selective activity which might serve as a starting point in the research on new strategies on selective insect control.

This outcome of the project necessitates the continuation of research on this line with regard both to theoretical aspects (investigating chemical structural requirements and mechanism of action) and to the aim of practical application. Work on this field will continuously progress even after termination of the present project, in close cooperation with the Department of Zoology, Washington State University.

6. Exemption of the dithiocarbamate type fungicides from the carcinogenic metabolite ethylenethiourea (unplanned spin-off of the project)

The theoretical background of our work which led us to the development of a new juvenile hormone analog type anti-insect agent (see Chapter IV, Point 1), offered itself to be employed also in research on another field of crop protection: the control of fungal diseases of agricultural crops by the application of fungicides.

The aforesaid juvenile hormone analog type was developed by us on the basis of the pro-drug principle, a research strategy successfully employed in drug research. This strategy rests on preparing rationally modified derivatives of biologically active compounds in order to improve activity pattern by modification of its stability, physicochemical and chemical properties as well as other characteristics, playing role in the biological effectivity. After fulfilling its function, the modifying moiety is split off to restore the active substance.

Our research on the fungicide field was motivated by the fact that dithiocarbamates, a class of the most frequently used types of agricultural fungicides, participating with about 12 per cent in the world's total pesticide consumption, are partially converted into a carcinogenic metabolite, ethylenethiourea. Due to the formation of this product, the application of the dithiocarbamate type fungicides was become severely limited. In our efforts to exclude the possibility of formation of this harmful substance, we made use of the pro-drug approach by preparing a metal-complex type slow-release pro-fungicide containing a dithiocarbamate fraction different from that permitting the formation of the hazardous metabolite. A cleavage

of the complex forming bonds results in releasing the active principle.

The product showed outstanding fungicidal properties, exceeding those of the former products forming the hazardous metabolite. Patent application procedure is under way in Hungary and in a number of foreign countries and investigations aimed at practical utilization are in progress within chemical industry framework abroad.

V. SUMMARIZED ESTIMATION

The part of our work intended to immediate practical utilization resulted in the development of a new group of juvenile hormone analog type, selective, environment-friendly anti-insect products. Some members show the practice-required activity against a number of economically important insect pests damaging agriculture, while a further member of the new group reveals activity against the housefly, exceeding the potency of the most active products being in commercial usage. The new group obtained patent protection in Hungary and in a number of foreign countries in Europe and in the USA. Detailed examinations aimed at practical application are in progress in the US and in Japan.

The significance of this result can be estimated only in the light of the fact that only 4-5 similar product has reached regulatory status and practical use, in spite of the fact that about 3,000 have been prepared and studied extensively in different research centers (see Chapter IV, Point 1).

The theoretical part of our work, leading towards practical application only indirectly and on the long term, was divided into two sections. One of them joined already accepted research

trends in environmentally friendly, selective insect control. Thus we prepared several compounds which block the biosynthesis of enzymes regulating insect-specific developmental processes (inhibitors of cytochrome P-450 enzymes), contributing to the knowledge on insect anti-hormones and revealed correlations which can be utilized in practice-oriented research directed to insect control on the basis of the anti-hormone concept (see Chapter IV, Point 2). A further notable outcome was the development of inhibitors of the enzyme juvenile hormone esterase, opening new possibilities in insect specific activities based on disrupting the hormonal balance and at the same time enriching its fundamental principle the transition state theory, gaining increasing importance in rational research on the field of medicines and pesticides (see Chapter IV, Point 3). We developed a new family of compounds acting as inhibitors of egg-laying and feeding of insects, extending towards economically producible products the international efforts focusing on indirect control of the damaging activity of insects, instead of killing them (see Chapter IV, Point 4).

The second section of the theoretical part in our project has not joined any of the known research trends and resulted in the discovery of a sixth anti-insect activity, besides the five ones known already. This new activity, inhibition of formation of spiracles needed for respiration and of crochets needed to grasp the plant, may serve as starting point in a new approach to environmentally friendly, selective and safe insect control methods. This fact has motivated our decision to continue our research also after termination of our project, in cooperation with our partner, the Department of Zoology, Washington State University (see Chapter V, Point 5).

VI. OUTCOMES OF THE PROJECT BEYOND RESEARCH RESULTS

Apart from research results, a substantial achievement of the project was the enrichment of knowledge and experience of the project team members, representing a benefit equivalent with the research outcomes. The short and long term study tours enabled establishing contacts and acquiring training at various scientific centers representing the highest level on this field. At the same time the high number of publications and lectures of the project team members helped in disseminating the project results, facilitating their application by other research centers. All these factors have contributed to the international recognition of the project team.

One of the objectives of the project, the buildup of a complex institutional-industrial research system capable of functioning as a reference unit in the development of selective anti-insect agents, has been attained only partially, at the institutional level. This is mainly caused by the fact that the chemical companies acting as potential partners in realization of project results declined to establish development activity on the basis of permanent bilateral contact and performed development procedure according to the company's standard schedule, without the interference of the project team. Nevertheless in the course of development activities within the institutional framework the project team turned into a unit capable of performing the institutional (non-industrial) developmental processes aiming at practical realization of the research achievements. This will serve as a decisive positive factor in the future efforts of the

project team, as realization of further practice-oriented research outcomes will gain importance in future plans.

LIST OF PARTICIPANTS

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PUBLICATIONS

Books, book chapters

Matolcsy, G., Nádasy, M., Andriska, V.: **Pesticide Chemistry**, Akadémiai Kiadó, Budapest, 1988.

Matolcsy, G., Nádasy, M., Andriska, V.: **Pesticide Chemistry**, Elsevier Publ., Amsterdam - New York, 1989.

Székács, A., Bordás, B., Hammock, B.D.: Transition state analog enzyme inhibitors: Structure-activity relationships of trifluoromethylketones. In: "Rational Approaches to Structure, Activity and Ecotoxicology of Agrochemicals", (Draber, W., Fujita, T., Eds.), CRC Press, Boca Raton, FL, pp. 219-250, 1992.

Papers, articles in journals and periodicals

Abd El-Kareim, A.I., Davas, B., Kozár, F.: Effects of fenoxycarb, hydroprene, kinoprene and methoprene on first instar larvae of Epidiaspis leperii (Hom., Diaspidiae) and on its ectoparasitoid, Aphytis mytilaspidis (Hym., Aphelinidae). **J. Appl. Entomol.**, 106, 270-275, (1988).

Bélai, I., Darvas, B.: New azole derivatives of metyrapone and their anti-ecdysterone action on Neobellieria bullata (Dipt., Sarcophagidae). **Abstract 7th Int. Congr. Pesticide Chemistry** (Freshe, H., Kesseler-Schmitz, E., Conway, S., Eds.), Hamburg, 1990, 1:37 (01A-25)

Bélai, I., Darvas, B., Matolcsy, G.: Synthesis of alkoxy-benzenes and alkoxyvinylbenzenes and their chemosterilizing and toxic activity on Planococcus citri (Hom., Pseudococcidae) **Agric. Biol. Chem. (Japan)**, 51, 17 (1987).

Bélai, I., Darvas, B., Rees, H.H.: Inhibition of ecdysone 20-monooxygenase from the fleshfly Neobellieria bullata by some metyrapone analogues. **Abstr. 20th FEBS Meeting**, Budapest, 1990, p. 51, (P-MO-158)

Bélai, I., Darvas, B., Tag El-Din M.H., Rees, H.H.: Action of azole deriatives of metyrapone on Neobellieria bullata (Dipt., Sarcophagidae) in vivo and on inhibition in vitro of ecdyson-20-monooxigenase, **Proc. Xth Ecdysone Workshop**, Liverpool, p 78, 1992.

Bélai, I., Matolcsy, G., Farnsworth, D.E., Feyereisen, R.: Inhibition of insect cytochrome P-450 by some metyrapone analogues and compounds containing a cyclopropylamine moiety and their evaluation as inhibitors of juvenile hormone biosynthesis. **Pestic. Sci.** 24, 205-219 (1988).

Bordás, B., Székács, A.: Construction of 2D maps for the design of compounds with required potency and spectral properties. In: "QSAR: Rational Approaches to the Design of Bioactive Compounds", **Proc. 8th European Symp. on QSAR**, (Silipo, C., Vittoria, A., Eds.), Elsevier, Amsterdam, the Netherlands, pp. 205-208, 1991.

Cowles, R.S., Miller, J.R., Hollingworth, R.M., Abdel-Aal, M.T., Szurdoki, F., Bauer, K., Matolcsy, G.: Cinnamyl derivatives and monoterpenoids as non-specific ovipositional deterrents of the onion fly. **J. Chem. Ecol.**, 16, 2401-2428 (1990).

Darvas, B.: Precoccious pupariation caused by cytochome P-450 inhibitors (phenarymol, KK-110) and inducers (barbital, phenobabital). **Abstr. 2nd Intl. Congr. Dipterology**, Bratislava, p. 45, 1990.

Darvas, B., Bélai, I., Fónagy, A., Kulcsár, P., Tag El-Din, M.H.: Lethal disturbances in larval development of Neobellieria bullata caused by A-phenyl-B-imidazolyl- and A-pheny-B-triazolyl-metyrapone treated diet. **Pestic. Sci.**, 32, 133-140 (1991).

Darvas, B., Kulcsár, P., Bélai, I., Matolcsy, G.: Inhibitory effect of an in vivo activated soft analogue of A-phenyl-B-triazolyl-metyrapone during pupariation in Sarcophaga bullata. **Entomol. Exp. Appl.** 44, 295 (1987).

Darvas, B., Rees, H.H., Kuwano, E., Bélai, I., Matolcsy, Gy., Tímár, T., Hoggard, N., Tag El-Din, M.H: Some inhibitors of microsomal ecdysone 20-monooxygenase. **Abstr. IXth Ecdysone Workshop**, Paris, **Invert. Repr. Develop.**, 18, 110 (1989).

Darvas, B., Timár, Varjas, L., Kulcsár, P., Hosztafi, S., Bordás, B.: Synthesis of novel 2,2-dimethyl chromene derivatives and their toxic activity on larvae of Pieris brassicae (Lep., Pieridae) and Leptinitarsa decemlineata (Col., Crysomelidae). **Acta Phytopathol. Entomol. Hung.**, 24, 455-472 (1989).

Darvas, B., L. Varjas, Farag, A.I., van Mellaert, H.: Effects of benzyl-1,3-dioxoles on development of sensilla trichodea of Hyphantria cunea larvae (Lep., Arctidae). In: "Endocrinological Frontiers of Physiological Insect Ecology" (Sehnal, F., A. Zabza, A., Denlinger, D.L., Eds.), Wroclaw Technical University Press, Wroclaw, pp. 581-586, 1988.

Darvas, B., Zsellér, H.I.: Effectiveness of some juvenoids and anti-ecdysones against the mulberry scale, Pseudaulacaspis pentagona (Homoptera: Diapididae). **Acta Phytopathol. Acad. Sci. Hung.**, 20, 341-346 (1985).

Fónagy, A., Tímár, T., Darvas, B., Kulcsár, P., Varjas, L., Bordás, B.: Comparative morpohegenetic and toxicological evaluation of 25 novel precocene derivatives on larvae of Oncopeltus fascinatus (Heteroptera) and Pieris brassicae (Lepidoptera). **Gen. Comp. Endocrinol.**, 74, 312-313 (1989).

Fónagy, A., Tímár, T., Sebők, P., Darvas, B., Kulcsár, P., Varjas, L.: Morpohegenetic and toxic activity of seven novel 2,2-dimethylcromene derivatives on larvae of Oncopeltus fascinatus (Heteroptera) and Pieris brassicae (Lepidoptera). **J. Pestic. Sci.**, 16, 267-269 (1991).

Gadenne, C., Varjas, L., Mauchamp, B.: Effects of the non-steroidal ecdysone mimic, RH 5849 on diapause and non-diapause larvae of the European corn borer, Ostrrinia nubialis. **J. Insect Physiol.**, 36, 555-559 (1990).

Hammock, B.D., Harshman, L.G., Philpott, M.L., Székács, A., Ottea, J.A., Newitt, R.A., Wroblewski, V.J., Halarnkar, P.P., Hanzlik, T.N.: Strategies for the discoveries of insect control agents: exploitation of biomechanisms regulating insect development. In: "Beltsville Symposia in Agriculture Research", Vol 12, "Biomechanisms Regulating Growth and Development", (Steffens, G.L., Rumsey, T.S., Eds.) Kluwer Acad. Publ., USDA, Beltsville, pp. 137-173, 1988.

Hammock, B.D., Székács, A., Hanzlik, T., Maeda, S., Philpott, M., Bonning, B., Posse, R.: Use of transition state theory in the design of chemical and molecular agents for insect control. In: "Advances in the Chemistry of Insect Control", (Crombie, L., Ed.), Royal Society of Chemistry, Cambridge, UK. pp. 256-277, 1989.

Hanzlik, T.N., Harshman, L.G., Philpott, M.L., Wroblewski, V., Székács, A., Hammock, B.D.: Biology and molecular biology of juvenile hormone esterase. **Serologica**, 28, 70 (1988).

Kulcsár, P., Darvas, B., Brandtner, H., Koolman, J., Rees, H.: Effects of the pyrimidine containing cytochrome P-450 inhibitor, fenarimol on the formation of 20-HO-ecdysone in flies. **Experientia**, 47, 261-263 (1991).

Matolcsy, G., Bélai, I., Fónagy, A., Varjas, L., Tombor, Á., Laczay, I.: Use of novel approaches for designing safer drugs in insecticide research: soft alkylating agents. **Pestic. Sci.** 24, 9-19 (1988).

Matolcsy, G., Feyereisen, R., Van Mellaert, H., Pál, Á., Varjas, L., Bélai, I., Kulcsár, P.: Molecular modifications of benzylphenol and benzyl-1,3-benzodioxole types of insect chemosterilants. **Pestic. Sci.** 17, 13 (1986).

Matolcsy, G., Ujváry, I., Riddiford, L.M., Hiruma, K.: Alkylene-bis-isothiocyanates: Novel insect growth regulators. **Z. Naturforsch.** 41c, 1069 (1986).

Okot-Kotber, B. M., Ujváry, I., Mollaaghababa, R., Szurdoki, F., Matolcsy, G., Prestwich, G. D.: Physiological influence of fenoxycarb-pro-insecticides and soldier head extracts of various termite species on soldier differentiation in Reticulotermes flavipes (Isoptera). **Sociobiology**, 19, 77-90 (1991).

Pap, L., Hegedüs, E.R., Bauer, K., Ujváry, I., Matolcsy, G.: A rapid method for the evaluation of nerve conduction blocking compounds. **Comp. Biochem. Physiol.** 85C, 347 (1986).

Polgár, L., Darvas, B.: Effects of a nonsteroidal ecdystroid agonist, RH 5849 on a host/parasite system, Myzus persicae/Aphidius matricariae. In: "Behaviour and Impact of Aphidiophage (Polgár, L., Ed.), SPD Academic Publ., the Hague, pp. 255-259, 1990.

Polgár, L., Darvas, B., Wing, K.: Effects of a nonsteroidal ecdystroid agonist, RH 5849 on Aphidius matricariae (Hymanoptera: Aphidiidae). **Abstr. Vol. Aphidiophaga 4th Conf.** (Kulcsár, P., Eastop, V., Eds.) Gödöllő, p. 50, 1990.

Székács, A., Bordás, B., Matolcsy, G., Hammock, B.D.: Quantitative structure-activity relationship study of aromatic trifluoromethyl ketones. **ACS Symposium Series** 413, 169-182 (1989).

Székács, A., Halarnkar, P.P., Olmstead, M.M., Prag, K., Hammock, B.D.: Heterocyclic derivatives of 3-substituted-1,1,1-trifluoro-2-propanones as inhibitors of esterolytic enzymes. **Chem. Res. Tox.**, 3, 325-332 (1990).

Székács, A., Hammock, B.D., Abdel-Aal, Y.A.I., Halankar, P.P., Philpott, M., Matolcsy, G.: New trifluoropropanone sulfides as highly active selective inhibitors of insect juvenile hormone esterase. **Pestic. Biochem. Physiol.** 33, 112-114 (1989).

Székács, A., Hammock, B.D., Abdel-Aal, Y.A.I., Philpott, M., Matolcsy, G.: Inhibition of juvenile hormone esterase by transition-state analogs. A tool for enzyme molecular biology. **ACS Symposium Series** 379, 215-227 (1988).

Szurdoki, F., Szentesi, A., Abdel-Aal, M. T., Székács, A.: Horváth, J., Bauer, K., Matolcsy, G., Jermy, T.: Novel feeding deterrents against the Colorado beetle. In: "Insect Chemical Ecology", **Proc. Conf. on Insect Chemical Ecology** (Hrdý, I. Ed.), Academia Praha, Czechoslovakia, pp. 251-254, 1991.

Thomas, T.C., Székács, A., Rojas, S., Hammock, B.D., Wilson, B., McNamee, M.G.: Characterization of neuropaty target esterase using trifluoromethyl ketone transition state analogs. **Biochem. Pharm.**, 40, 2587-2596 (1990).

Ujváry, I., Matolcsy, G., Riddiford, L.M., Hiruma, K., Horwath, K.: Inhibition of spiracle and crochet formation and juvenile hormone activity of isothiocyanate derivatives in the tobacco hornworm, Manduca sexta. **Pestic. Biochem. Physiol.** 35, 259-274 (1989).

Ujváry, I., Hiruma, K., Riddiford, L.M., Matolcsy, G., Roseland, C.R., Kramer, K.J.: Role of ß-alanine in pupal cuticle coloration in tobacco hornworm, Manduca sexta. Effects of ß-alanine analogues on melanization and catecholamine levels. **Insect Biochem.** 17, 389 (1987).

Varjas, L., Mauchamp, B.L.: Summer and winter diapause in pupae of Mamestra brassicae: The possible role of juvenile hormone. In: "Endoc. Front. Physiol. Ins. Ecol.", (Eds.: Sehnal, F., Zabza, A., Denlinger, D.L.) Wroclaw Techn. Univ. Press, Wroclaw, 325-330 (1988).

Invited plenary lectures

Matolcsy, Gy.: Perspectices of environmentally safe pesticides. "Seminar on pesticide Production and use" Jakarta, Indonesia, Nov. 1988.

Matolcsy, Gy.: Environment-Friendly Pesticides. International Seminar "Recent Developments in the Field of Pesticides and Their Application to Pest Control in China and Other Developing Countries of the Region", Shenyang, China, Oct. 1990.

Ujváry, I., Matolcsy, Gy.: Novel sulfur-containing compounds with juvenile hormone activity, Intl. Conf. "Insecticides - Mechanism of Action and Resistance", Rheinhardsbrunn, Germany, Apr. 1991.

Lectures

Darvas, B., Rees, H.H., Kuwano, E., Bélai, I., Matolcsy, Gy., Tímár, T., Hoggard, N., Tag El-Din, M.H, Some inhibitors of microsomal ecdysone 20-monooxygenase. Abstracts IXth Ecdysone Workshop, Paris, **Invert. Repr. Develop.**, 18, 110 (1989).

Matolcsy, Gy.: Application of Pro-drug Principles in Search for Selective Insecticides. University of California, Riverside, 1988.

Székács, A.: Trifluoromethyl ketones as transition state analog inhibitors of juvenile hormone esterase. University of California, Davis, 1989.

Posters

Bordás, B., Székács, A.: Multivariate analysis for the design of compounds with required potency and spectral properties. **8th European Symp. on QSAR**, Sorrento, Italy, 1990.

Szurdoki, F., Abdel-Aal, M.T., Bauer, K., Matolcsy, G., Cowles, R.S., Miller, J.R., Hollingworth, R.M.: Synthesis of Cinnamyl and Cinnamoyl Derivatives, Ovipositional Deterrents to onion fly. **7th Intl. Cong. of Pesticide Chem. (IUPAC)**, Hamburg, 1990

Patents

Ujváry, I.,Matolcsy, G., Bélai, I., Varjas, L., Darvas, B.: Preparations with insecticidal and acaricidal activity containing carbamic acid derivatives and proceeding for preparation of the active ingredients., Hung.Pat. 196 055 (1985), Belg.Pat. 1 000 120 (1988), US Pat. 4,745,128 (1988), Spain Pat. 8,603,526 (1988), French Pat. 8,700,427 (1989), Brit Pat. 2,199,578 (1989), Dutch Pat. 8,700,074 (1989).

Patent procedure is in progress in further countries: Japan, Canada, Germany, Switzerland and the Union of Independent States.

UNIDO Comments

The terminal report gives the complete summary of the research work carried out by the counterparts to develop interesting compounds that could act as potential juvenile hormones. The research carried out is of international calibre and is reflected in the number of publication generated in the whole process. It also indicates the number of areas in which one could look for environment friendly pesticides.

The whole report, giving different approaches, is likely to surface in the near future as possible approaches for developing bio-pesticides or bio-rationals where the compounds are mainly applied so as to have specific biological activity without any interference to man and his environment.

The work carried out has acclaimed international appreciation and would be very useful for future work in this area.