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Correlations in the spectrum of activity of juvenile hormone analogues

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Modern pest control sets high standards for new active substances which cannot always be easily met :

1. The substances are expected to be of a low order of toxicity to mammals.

2. The substances should leave no residues following practical application, or, in other words, should break down biologically.

3. The active substances used must not disturb the natural balance, that is to say, they should only be active on the pests to be controlled and have the lowest possible influence on other living organisms.

The toxicity for humans is very low with most of the substances tested so far, that is to say, more than 5000 mg/kg body weight (acute oral toxicity for rats). Many of the tested substances have a relatively low vapour pressure and for this reason a limited residual activity on dead material. They are, however, broken down by plant enzymes so that, according to human judgment, no serious residue problems are to be expected. In this lecture we shall be dealing with the question of specificity of juvenile hormone derivatives.

Starting point

Roeller extracted his substance from the Lepidoptera species *Hyalophora cecropia* and carried out his tests on the Coleoptera species *Tenebrio molitor*. With tests arranged in this way a very specific juvenile hormone could not be determined at all. It was to be expected, therefore, that beside the two species mentioned, others might also respond to the isolated substance. In the meantime this has been confirmed by different authors. Like the original isolated substance most of the derivatives have a notable action on various insects of widely differing families. In some cases, however, it was found that, under certain circumstances, very large differences in the action on various species may occur, pointing to a certain specificity of the substances. As extensive results are now available, the important question is whether there is a real

specificity, or if the differences must be attributed to the particular arrangement of the tests.

We are now trying to show by some examples that there are very characteristic differences in the activity of the substances so that, in our opinion it is doubtful if conclusions on the general activity of the substance can be drawn by analogy with its activity in a certain test.

The selection of our test methods

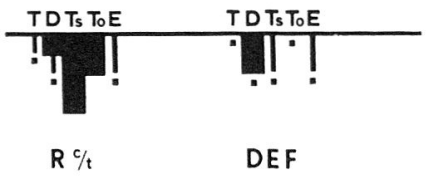


Fig. 1. — Test results obtained with standard compounds
Left: Mixture of the isomers of a synthetic preparation of the hormone isolated by Roeller.
Right: Ethyl ester of dichlorofarnesenic acid (Ethyl trans-7, 11-dichloro-3, 7, 11-trimethyl-2-dodecenoate (Romanuk)

a) *Tenebrio molitor*

The classic test on *Tenebrio molitor*, as described in the literature used for the selection of active substances in a screening programme, has certain merits because in most cases it gives a very flat dose activity curve, and for this reason the application of different dosages is not necessary in each series of tests. If a larger test series is carried out with a uniform dosage of 10^{-5} g/animal, the classification of different substances according to their activity is quite possible, although the final assessment is only possible with the usual dilution series. It is, however, questionable if a generally valid assessment of a group of substances can be made on the basis of a single classification of such substances by the *Tenebrio* test.

The results with *Tenebrio* were made available to us by Roche Nutley. We wish to thank our collegue Dr. Mitrovic most sincerely for them.

b) *Pyrrhocoris*

The linden bug *Pyrrhocoris apterus* has been used in extensive test series, particularly by Czech scientists, after it was shown that this bug is very sensitive to juvenile hormone active substances. Since the breeding of this bug may prove difficult under certain circumstances and this species is unimportant in crop protection we have not used it so far in large test series but another, related bug instead.

c) *Dysdercus sp.*

Bugs of the *Dysdercus germes* can be bred easily and used for tests with juvenile hormone. We ourselves have used the species *Dysdercus cingulatus* which is nearly as sensitive to many juvenile hormone derivatives as *Pyrrhocoris apterus*. Tests by topical application as well as residual contact produce very useful results, using larvae of the last larval stage as well as young adults. In the first case the effect is metamorphic disturbances, in the second case there is a sterilizing effect, as will be demonstrated by my colleague Homberger in one of the following papers.

d) *Tineola*

The cloth moth has proved itself as quite a suitable test object in our experiments, although this species is relatively resistant and responds only to high dosages. In residual feeding tests the effect is repeated moulting of the larvae, mostly without significant metamorphic disturbances. During recent years we have limited our tests to the sterilizing and ovicidal effects.

e) *Ephestia*

We have used eggs of *Ephestia* in our tests to determine the ovicidal effect, as we found that the eggs of *Ephestia* are highly sensitive to juvenile hormone derivatives and other substances. We treated filter paper discs with an acetonic solution and placed newly laid flour moth eggs on them. The samples were kept at a constant temperature and humidity, and counts were made after hatching. The assessment of all the results shows clearly that the effects observed must under no circumstances be interpreted in the sense of a clear juvenile hormone effect. On the contrary, we are of the opinion that the test is suitable for the general assessment of an active substance in respect of its suitability as a biocide. It is therefore possible that a substance will show high activity in the *Ephestia* test but, on the other hand, fails in morphogenetic tests on *Tenebrio* or *Dysdercus*.

Beside the experiments mentioned we have carried out a number of other test series whose results we cannot present in detail for lack of time.

The graphic presentation of the test results

In order to present a large number of test results clearly, a simple graphic diagram is needed to show the interaction between dosage and activity, as well as the specificity of the substance. In the following we show the diagram used by us. As is usual with this kind of investigations we used logarithmic dilution series with the factor 10 and tried to make, at the first working stage, a rough estimate of that dosage which will give an effect of 50% (ED_{50}). The different dilution grades

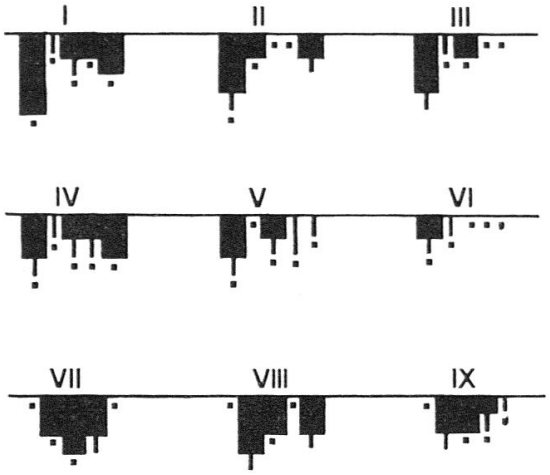


Fig. 2. — Test results of some selected compounds

I–III : Three compounds exhibiting good activity in the Tenebrio test show various results in other tests ;

IV–VI : Three compounds exhibiting medium activity in the Tenebrio test show various results in other species ;

VII–IX : Three compounds with no activity in the Tenebrio test show remarkable activity in other tests.

This summary indicates that the considerable differences in the activity spectrum offer the possibility of finding compounds that exhibit the desired specificity.

are given by beginning with the highest concentration on top. Where the effect is 100% the respective square is filled out, a vertical line shows that there is some effect ; a dot indicates that the result is negative. The diagram used gives a characteristic and clearly defined spectrum of activity. In our tests we indicate the dosages as follows :

10^{-x} g/cm^2 e.g. with Ephestia

or

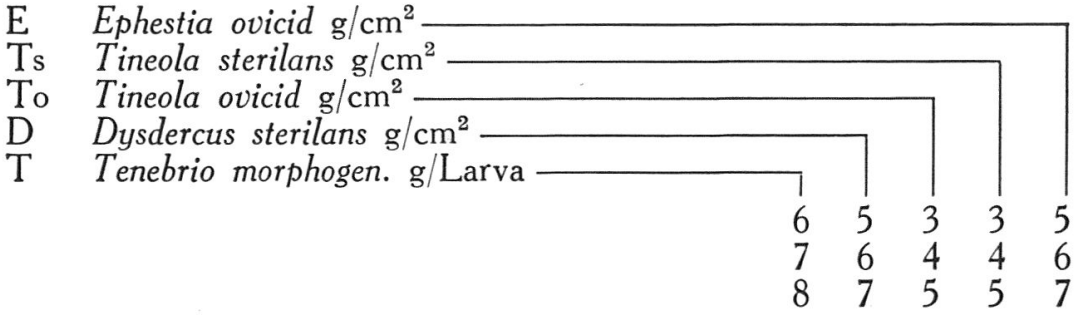
10^{-7} g/Tenebrio larva

or

10^{-5} g/cm^2 filter paper (Dysdercus)

This manner of expressing the dosage is very simple and clear. Sometimes, however, it may give rise to misunderstandings, as the results expressed in powers of ten are not always comparable, depending on the test method and units referred to (cm^2 filter paper, grams of food, individual animals, etc.). In preparing diagrams comprising different test results it is therefore necessary to make sure that they are comparable. We know from experience that in our *Dysdercus sterilans* test many substances are active at a dosage rate of $10^{-5}{}_g$ per cm^2 filter paper but only in a few cases they are active against grain beetle at $10^{-5}{}_g$ active substance per gram of wheat grains. This difference is

not due to a variable sensitivity that is mathematically determinable, but to the different test arrangements. Also, it is practically impossible with the Tenebrio test to use higher dosages than 10^{-5} g/pupa.



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