CHAPTER–III

BIOLOGICAL ACTIVITY

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ABSTRACT

This Chapter briefly surveys the current methods which can be performed under more or less standardized conditions for evaluating biological activity, and also summarises the results of biological evaluation of the compounds synthesized in the present study.

CURRENT METHODS FOR EVALUATING JH-ACTIVITY

Here some special assays are given, which are for some reason significant in JH-mimic research, but the main emphasis has been given to those assays which can be performed under more or less standardized conditions.

The Tenebrio Unit assay

Pupae 1 to 25 hours-old are injected intersegmentally between the 4th and 5th abdominal sternite with 1 µl of olive oil containing the active compound. Adults which emmerse after 6-8 days at 30° and 40% R.H are inspected, the positive effect is revealed by a patch of pupal cuticle over the area of sample deposition. The Tenebrio Unit concentration indicates the amount of JH-mimic producing pupal patches in 40% out of the total 50 pupae injected.[1,2] The assay is very sensitive and can be used for assaying compounds with low activity. The Tenebrio Unit doses are 100 to 1000 times < ID-50 Morphs units.

Tenebrio Topical assay

The ventral side of the thorax or abdomen of 1 to 20 hours-old pupae is treated topically with 1 µl acetone-juvenoid solution. After 6-8 days at 27° the percentage of retention

of the pupal epidermal patterns is evaluated and used for calculation of the ID-50 Morphs units of activity. This assay is less sensitive than the former one, but more important for practical use of juvenoids.

Galleria Wax Wound test

The wound of approximately 1 mm^2 is made on 1 to 20 hrs. pupae, and sealed by a mixture of paraffin wax with olive oil 1:1 using warmed needle or electrically heated platinum wire. The olive oil contains graded amount of the active compound. After 6-8 days positive responses are indicated by patches of cuticle with pupal coloration and architecture in the excised area, surrounded by adult cuticle. One Galleria Unit is indicated by an amount of extract which causes positive responses in 50% pupae tested.[4] This is one of the most sensitive test which is convenient for determination of JH-activity in crude extracts. However, it is less specific than the tests based on morphological influence.

Galleria Metamorphosis assay on Larvae and Pupae

The last instar larvae are either injected with 1 ul of olive oil solution, or treated topically with 1 µl of acetone-juvenoid solution. After 3-4 days ID-50 Morph values

are calculated according to the degree of retention of the old larval epidermal patterns.[5] The same procedure can be used with 0 to 20 hrs. old pupae, and the ID-50 Morph values are determined according to retention of the pupal structures. This assay is less sensitive than wax wound test, but it's results are more important from practical point of view.

Metamorphosis assay on Hemiptera

The last instar larvae 0 to 20 hrs.-old are treated topically with 1 µl of acetone-compound solution, or injected with 0.5 ul of olive oil solution, or they are exposed to contact with substrate previously treated with active compound. After 5 to 10 days at 27° the larvae moult into normal adults, larval adult intermediates, or extra-larvae depending on the dose of the juvenoid used.[6] Then ID-50 Morph concentrations are calculated, according to the degree of retention of the larval patterns. The assay is convenient for all kinds of indirect application methods.

Metamorphosis assay on Rhodnius

The last instar larvae 2 to 3 weeks after ecdysis are fed and treated with the sample solution, one day after

blood meal. For topical application 1 µl of acetane solution, and for injection 1 µl of vegetable oils are used. The ID-50 Morph values are determined some 20 days later, after ecdysis. The test is an example of atypical position of the sensitive period to juvenoids. Initiation of the sensitive period is affected by the ~~bled~~ blood meal which allows to obtain experimental animal with synchronized development.

Ovicidal assay

Adult females of Pyrrhocoris 2-days old are treated topically with 1 µl of acetone-juvenoid solution. The females are incubated together with active males in glass jars or plastic cylinders with a netting on the bottom. The eggs are either collected daily or 2-3-days intervals. After 8 to 9 days of incubation at 27° the proportion of free egg chorions (indicating hatched larvae) to undeveloped egg is determined. The ID-50 ovic. values are calculated from the number of hatched larvae related to the egg hatchability in controls. This method has wide applicability on many Exopterygote and and other insects.

Tribolium population assay

Male and female adults 1 to 2 weeks old are separated into groups, each containing 50 specimens. Each group is then transferred into breeding containers with 100 gm of

wheat (previously treated with known amount of juvenoid in organic solvent). These colonies are incubated at 27° until the adults of the new generation emerge. Their number is counted and the ID-50 popul. values are calculated with respect to the control experiment. This method can be applied to many insect stored product pests.

Drosophila Population assay

Five pairs of freshly emerged adults are placed in rearing tubes containing the rearing medium (10 ml) contaminated superficially with juvenoids. The colonies are incubated under standard conditions until new adults emerge. The ID-50 Popul. is calculated from percentage of the adults which emerge the next generation related to that ethanol treated controls. The assay is very instructive but little specific, being quite sensitive to various side effects. Characteristic action of juvenoids in the assay is understood by inhibition of pupariation, formation of atypical puparia, and formation of pupal-adult intermediates.

Special Population assay

The last instar larvae or pupae 0 to 20-hrs old are individually treated by juvenoids using doses $\leqslant$ID-50 Morphs. Adults which emerge after 5 to 10 days from the treated larvae or pupae are incubated under conditions favourable for

reproduction. The eggs are collected and allowed to hatch. The amount of hatching larvae is counted and used to determine ID-50 Popul. units. The unit values are related to the amount of hatching larvae produced by control groups of larvae or pupae of the same number and sexual ratio. The dose for ID-50 popul. is given in ug of juvenoid per specimen which was applied on larvae or pupae in the previous generation.

Field Assay on Hemipteran

Young larvae of Eurygaster intergriceps are collected on the wheat fields. At the time of appearance of the last larval instars, the localized field areas are sprayed with JH-mimics formulated as H_2O emulsions (1% light mineral oil soln of JH-mimc with equal amount of commercial emulsifiers used for insecticides, and water), or aerosol sprays. Evaluation of the results is initiated 5 days after the first treatment by collecting samples (30 to 100 larvae) at 3 or 5 days intervals of fully grown, and last instar larvae which have spent the sensitive period to JH-mimics in the treated fields. Each samples are incubated in small containers provided with food and water, either under field or laboratory conditions. It can be scored in a usual way. All the samples showing higher percentage of morphological influence than 30% are unable to reproduce and can be considered as a positive field control, as well as specimens with ecdysial failures

which soon die. Differences in activity or eventually disappearance of the activity in samples collected after successive intervals are used retrospectively for calculations of the actual persistence of the JH-activity, in the field. Description of this assay illustrates a real necessity to know factors such as sensitive periods, developmental rate and dispersion of developmental stages in a given insect, and persistence of the JH-mimic to be used before any field assay with JH-mimics is initiated.

JH-ACTIVITY OF THE SYNTHESIZED COMPOUNDS

JH-activity of the compounds synthesized in the present study has been tested on 4 hrs old last instar nymphs of red cotton bug, Dysdercus koenigi, using 10, and 1 µg of each compound in acetone as a topical application and evaluating the results in terms of inhibition of metamorphosis. Farnesyl methyl ether was used as a reference compound. Summary* of the results is given in Table 1.

From an analysis of the JH-activity data (Table 1), it is clear that (4) and (5) are more active than (7). Incorporation of epoxide function at C-6,7 position slightly supresses the

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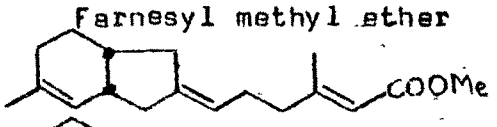
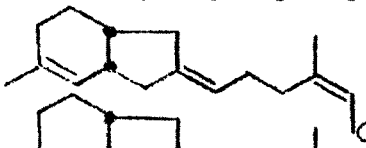
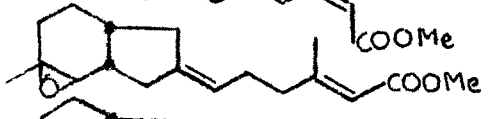
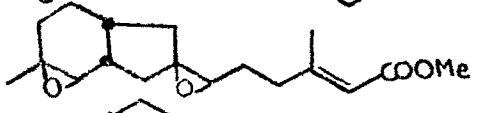
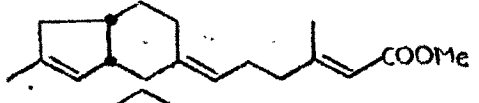
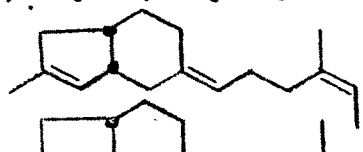
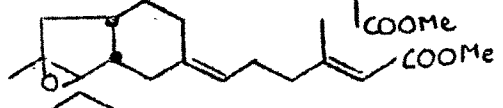
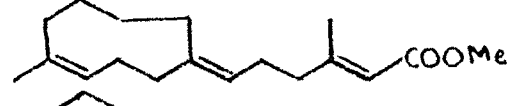
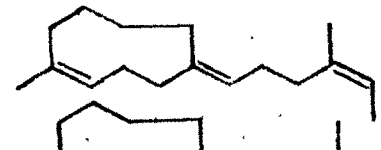
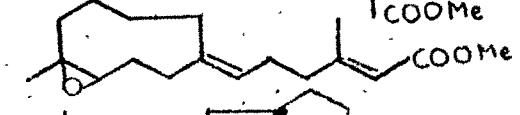
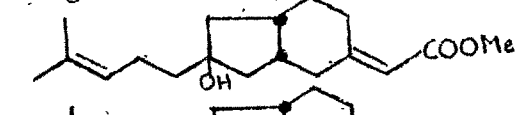
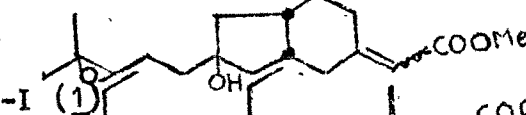
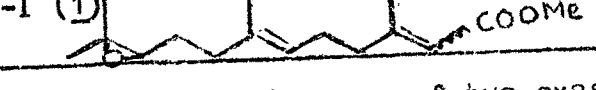
*Bioassay were carried out by Drs. N.K. Joshi and H.B. Mansukhani, through the courtesy of Dr. M.S. Chadha, at Bhabha Atomic Research Centre (Bio-Organic Division), Bombay. Author would like to place on record his grateful thanks for this help from this group.

TABLE 1

JUVENILE HORMONE ACTIVITY OF THE COMPOUNDS SYNTHESIZED IN THE PRESENT STUDY

No.	Compound	Dose/(µg) nymph 10	1	0.1
		Score[a]		
	Farnesyl methyl ether	1.5	0.0	
22		3.6	0.4	
23		3.6	1.1	
4		4.0	3.0	
25		3.3	2.6	
33		3.0	0.0	
34		4.0	1.5	
5		3.5	0.8	
55		2.4	0.0	
56		1.3	0.0	
7		2.9	0.0	
39		1.9	0.0	
40		2.8	0.0	
JH-I (1)				2.0[b]

[a] Score represents the mean of two experiments, each of which was carried out using three replicates of 15 nymphs each. Nymphs which successfully moulted were characterised and scored as: normal adults (0), adult-nymphs (1), intermediates (2), nymph-adults (3), and sixth instar nymphs (4).

[b] This is a converted[8] score of ID-50 (0.1) in Oysdercus.

activity (25) vs. (4). The presence of OH group at C-7 position has no adverse effect on activity, e.g. (40). Incorporation of cis-perhydroindane ring between C-3 and C-7 positions is less suitable as compared to between C-7 and C-11 positions in JH-I for good JH-activity (4) and (5) vs. (40) .

The data in Table 1 also permit us to evaluate the relationship between stereochemical features and JH-activity. The "freezing of two free-ethyl groups of JH-I either in the form of cis-perhydroindane or cyclononane ring decreases the activity. Of course, freezing of two free ethyl groups in the conformations of cis-perhydroindane ring is somewhat better than that of the cyclononane ring (4) and (5) vs. (7); and (40) vs. (7) .

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