BIOLOGICAL ACTIVITIES OF N-(1-METHYL-2-OXO-2-PIPERIDINO-ETHYL) BENZENESULPHONAMIDE

Biological activities of N-(1-methyl-2-oxo-2-piperidino-ethyl-)benzene sulphonamide (2.36) :

Juvenile hormone activity of a large number of junenoids including the type (2.6 - 2.6b) against various insect species have been extensively reviewed and reported in the book" Insect Hormones and Bioanalogues" (167). Since we were contemplating to synthesize various JH like compounds of the above type by introduction of sulphonamide function in the side chain (Chapter - II), we thought it desirable to undertake biological testing of one representative N-(1-methyl-2-oxo-2-piperidino-ethyl)benzene sulphonamide (2.36). This compound was chosen because biological activities of sulphonamides and hetrocyclic juvenoids were already known separately, but this compound contained both, the sulphonamide function and the heterocyclic ring. Compound (2.36) was tested for juvenile hormonal and chemosterilizing activity against potato tuber moth Phthorimea operculella. The investigations were undertaken with the help of colleagues in the Department of Bio-Sciences, Himachal Pradesh University, Summer Hill, Shimla, using their laboratory facilities.

Review of Literature

Numerous studies on the effect of various alkylating compounds on the hatchability and viability of eggs were undertaken by different workers more than four decades ago. The names of Goldsmith (207-208), Goldsmith and Frank (209) and Mitlin et al. (210-213) figure amongst the earliest workers in the field. A large number of compounds like

apholate, aphomide and aphoxide were studied for their biological activity as chemosterilant in a large number of insects. Mustafa and Naidu (214), Kern and Nair (215), Iba et al. (216), Nifantev et al. (217), Borkovec (218), Hatez et al. (219) who studied the effects of Tepa, Metepa and Hempa on the Anopheles pharoensis, the main vector of malaria in Egypt, concluded that Tepa was the most effective chemosterilant amongst the compounds studied.

After the introduction of alkylating agents as insect chemosterilants, a large number of chemicals including organophosphorus compounds, sulphonamides, melamines ,dithiobiurets, dithiazolium salts, anthamycin derivatives and various non-alkylating agents were introduced. The biological activities of various synthetic compounds were tested by numerous workers including Borkovec (220-222), Pillai (223-224) amongst others. With the advancement of the knowledge on the metabolic pathways in the biological systems, a large number of metabolic antagonists were synthesized and there action studied. Levinson et al. (225) studied the action of lipid antagonist, ethyl-p-chloro-phenoxy isobutyrate on insect growth and reproduction. Lofgren et al. (226) and Chang et al. (227-228) reported the sterilizing action of aziridinyl phosphine oxides and sulphides. Chinnarajan et al. (229) investigated the effect of tetramycin whereas Pare et al. (230) investigated the influence of reserpine on ovarian function in insects.

It is quite evident from the literature that the recent advances in the evolution of chemosterilization of insects were made by using compounds which mimic the neuroendocrine secretion, particularly the juvenile hormones. Hentschel (231) studied the effect of 6-hydroxydopamine on the process of ootheca and cocoon formation in the females of Periplanata americana. Malhotra and Kaur (232) investigated the effects of a

new pyridine derivative on the ovarian development in red cotton bug Dysdercus cingulatus.

The effect of juvenile hormones on female insect sterility and the physiological conditions required for such effects have been briefly mentioned by Slama et al. in their book "Insect Hormones and Biolanalogues" (166). The juvenoids generally produce female insect sterility by depositing defective eggs of very low or zero hatchability. The sterilizing effects are dependent on the dose of the juvenoid applied. Excessive doses can sometimes induce permanent sterility in a single application. In hemipterans, the sterilization of females can be achieved by the application of juvenoid at any moment of the reproduction cycle. It also includes the stages when the eggs were already chlorinated (233). In certain Endopterygote insects where oocytes develop before adult emergence, juvenoids may cause more pronounced sterility effects when applied at a certain determined stage of oocyte development (234). The other biological effects of juvenoids have already been described in Chapter I.

It is pertinent to point out that the above given review of literature is limited and fragmentary and is in no way complete in any respect. It may easily be appreciated that a more detailed study of literature on the subject cannot fall in the purview of the present dissertation.

Aims of Study

The present investigation on the biological activities of N-(1-methyl-2-oxo-2-piperidino-ethyl) benzenesulphonamide (2.36) has been undertaken to see if this compound has any JH and /or chemosterilizing properties. Also, it was aimed to assess if the

compound had any action in enhancing or delaying the ovarian development in the insects. Keeping the above in view, a preliminary attempt has been made to study the effect of compound (2.36) on the eggs as also on the adult female specimens of the potato tuber moth.

Materials and Methods:

Specimens of the potato tuber moth (male and female) were obtained from the Central Potato Research Institute, Shimla and were maintained in suitable transparent polypropylene containers tightly covered with black georgette. The insects were provided with fresh potato slices which were replenished every day. The eggs laid in the black georgette covering were collected daily and the old potato slices were thoroughly teased and shredded manually to collect larval stages if any.

The eggs were divided into four groups of 10 each and were maintained in covered petri-dishes. Filter paper (Whatmann No.1) was placed at the bottom of the petri-dishes. The compound was dissolved in acetone in quantities of 10 μg, 25 μg, and 50 μg per ml of acetone and three groups of eggs were treated with these respectively. The acetone solution of the compound was spread on the filter paper in the petri-dishes and once it was evenly distributed the acetone was allowed to evaporate and the eggs were placed on the filter paper. The petri-dishes were kept covered. The fourth group of eggs was maintained as control group. Six sets were run for each experiment.

The effect of the compound on the hatchability of eggs was studied by counting the larvae formed and viability by counting the number of adults obtained in each set. The chemosterilizing activity of the compound was studied by the histological preparations of the ovaries dissolved out of the females, 2 and 5 days after the formation of the adults.

Similar study was conducted on the adult female specimens treated with 10 µg, 25 µg, and 50 µg of the compound 2 and 5 days post-treatment. The method employed for histological slides of the ovaries is as follows:

The ovaries of the control as well as treated insects dissected after 2nd and 5th days were fixed in Bouin's fixative for 20 hrs. These were washed in 70% alcohol till the material was free from excess of picric acid. After complete dehydration and clearing, these were embedded in parafin wax of 60-62°C. Microtome sections were cut at 7 µ. Ribbons were stretched on albuminized slides and were processed for Haematoxyli-Eosin staining technique. Dewaxed and hydrated sections were stained in Delafied's Haematosylic for 2 minutes. These were differentiated in acid alcohol and made alkaline in 0.1% ammonia solution. After dehydrating upto 70% alcohol, these were counterstained in Eosin for 5 minutes. After complete dehydration the sections were cleared in xylene and finally mounted in DPX for study.

Bouin's Fluid

Formalin 40%-25 cc

Glacial acetic acid - 5 cc

Picric acid (saturated) - 75 cc

Delafield's Haematoxylin

Haematoxylin - 3.2 g

Aluminium ammonium sulphate - 30g

Absolute alcohol - 100 ml

Glycerol - 80 ml

Distilled water - 320 ml

The above mixture was shaken vigorously and was allowed to mature and ripen for 3 months. It was used after filtration.

Alcoholic Eosin

Eosin dye - 1 g

70% alcohol - 100 ml

Observations:

The development of the ovary in the control specimens reared from egg to the adult was normal. Each oocyte was associated with some trophocytes or nurse cells. The epithelial covering of oocytes was well demarcated and encloses distinctly identifiable cells of the follicular epithelium. The immature oogonia were present in a bunch and the nurse cells were accumulated towards the anterior cells. Intercalated interfollicular tissue was developed between some of the neighboring oocytes. Tube-like connection between the oocytes and trophocytes were visible in many sections in some of them. In some of the sections the germinal vesicle could be distinctly identified in many oocytes.

Egg 48 hrs and 5 days after 10 µg treatment

The adults developed from eggs treated with 10 µg had almost normal appearance. There was not much difference between the control and the treated eggs.

Egg 48 hrs after 25 µg treatment

With 25 µg of treatment the ovarioles exhibited strings of oocytes. The mature oocytes depicted ooplasmic disintegrations, and in many of them the yolk exhibited strong basophilia. The immature ones were still intact but had developed slightly thick outer coating. Also , the intercalated follicular plugs were slightly developed and were disproportionately thick. The trophocytes were few and far between and had a distorted

structure. Under the influence of this compound, oocytes hypertrophy was observed in some specimens. The large sized oocytes became almost hollow. Few trophocytes migrated around the usually separated hypertrophied ones and formed tubular extensions for the supply of nutrients to this aberrant egg cells. In many cases the tuber failed to join the oocytes.

Egg 5 days after 25 μg treatment:

After 5 days of treatment with this compound the ovarioles were in complete disarray. Most of the ova were damaged and those retaining structural identity, acquired odd shapes because of the formation of large number of finger like projections. The immature component of the ovariole was completely shriveled and so were the trophocytes.

Egg 48 hrs after 50 μg treatment:

After treatment with 50 μg for 48 hrs, the ovary depicated disruption of oocytes and trophocytes many of which lost their structural integrity. In some alternations in their shapes and breakages in their epithelial lining were clearly visible. Many of the oocytes depicted a clear ooplasitc background devoid of any yolk platelets or granulations. Such cells aggregated around them and the cytoplasm depicted vacuolar degeneration.

In the same eggs which had been treated with 50 μg for 48 hrs, some of the ovarioles depicted a very different picture in which although the oocytes continued to form normal strings each oocyte had some of the other structural aberration. A compartmentalization of each oocyte appeared because of the abnormal development and division of the intercalated interfollicular tissue. The germinal vesicle is not observed in majority of the sections and the coplasm was relatively clear.

Egg 5 days after 50 μg treatment:

The 5 days adults developed from 50 μg treated eggs exhibited extreme degeneration of the ovary. The ovarioles were no longer seen and a few shriveled and mutilated oocytes were observed. Abnormal trophocytes were scattered in between the degenerated oocytic component while the oocyte depicted a brownish coloration, the trophocytes continued to stain eosin positive.

Adult 5 days after normal control:

The 5 days old adult female moth depicted a normal complinent of ovarioles. The number of mature oocytes was relatively small whereas a large number of immature oocytes formed a long chain.

Adult 5 days after 10 μg treatment :

In adults treated with 10 μg, the ovary appeared to be intact after 5 days post-treatment. Some of the oocytes, however, developed thick darkly stained intercalated zones. The cytoplasm in the mature ova appeared to be normal and their epithelial lining was complete with the presence of distinct follicular epithelial cellular component.

Adult 5 days after 25 μg treatment :

25 μg treatment of the adults showed a little effect on the ovary . The strings of oocytes were smaller. The large mature oocytes exhibited clear coplasm devoid of yolk platelets. The middle order oocytes depicted a complete epithelial lining alongwith the cellular component but alternations in their shape and formation of spaces in coplasm were observed. The population of immature oocytes was small and they were highly basophilic and depicted gross structural abnormalities.

At this stage cytoplasmic lysis was evident in many mature oocytes. Some of them depicted cavities because of the lytic processes and such cavities are invariably filled with a large number of eosin positive cells which look like those from the trophocytes and cells of the epithelial linings. Thus there was a possibility that the cellular population from the trophocytes as also the cells from the epithelial covering start phagocytising the mature oocytes.

Adult 5 days after 50 µg treatment :

50 µg treatment resulted in total destruction of the ovary after 5 days post-treatment. Few oocytes were virtually destroyed as a result of the induced fragmentation. The oocytes which do not undergone fragmentation throw out pseudopodia like extensions resulting in the array of shapes. In all such cells, the staining properties had been completely altered. The epithelial lining was abnormal. Infiltration of certain cell types into the oocytes was not uncommon. Finally, the trophocytic component was virtually non-existent and remnants of a few trophocytes could only be identified.

Discussion :

Form the histological studies it becomes quite clear that the compound N-(1-methyl-2-oxo-2-piperidino-ethyl) benzenesulphonamide (2.36) under investigation possesses a distinct chemosterilizing influence on the ovaries of the potato tuber moth. The animals treated with 10 µg/ml depict relatively low or negligible activity of the compound after 48 hrs and even after 5 days. The ovarioles tend to be normal and it is only the immature oocytes which exhibited certain amount of basophilia which is indicative of some metabolic distinctions resulting in the altered pH of the medium. At the rate of 25 µg and 50 µg/ml the damage seen in the ovaries of the potato tuber moth is quite acute and

not a single normal oocyte can be seen in any of the section. Evidently, this appears to be an irreversible change resulting in the permanent sterilization of the insect. Similar conclusions were arrived at by Malhotra and Kaur (232) after treatment of Red Cotton bugs with 1-phenyl - 4, 4, 6-trimethly-1H, 4H -pyrimidine-2-thiol.

As already shown in the observations, the eggs treated with 10 μg/ml of the compound give rise to adults with fully developed ovaries showing strings of mature and immature oocytes in successions. On the other hand the 25 μg and 50 μg/ml treated specimens do not show complete ovarioles. There is a distinct possibility that while at low dose rate the development of ovarioles is either enhanced or maintained, at higher dose rate such a development of the ovarioles is not permitted. Thus the role of this compound as juvenile hormone enhancing the rate of ovarian development cannot be ruled out.

The observations have also revealed that the treatment with 25 μg and 50 μg of the compound also resulted in development of abnormal wings which lends further support for its possible JH like action. However, no firm opinion can be formed without a detailed investigation in this direction.

Finally there was no mortality and almost all eggs remained viable leading to the conclusion that the compound does not have insecticidal action on the potato tuber moth.

It is pertinent to point out that very similar juvenile hormonal and chemosterilizing activity against tuber moth Phthorimea operculella were observed when N-(2-oxo-2-piperidino-ethyl) benzenesulphonamide was earlier tested in our laboratory (206).