

THE EFFECT OF STEROIDAL AZIRIDINES ON DEVELOPMENT AND  
OVIPOSITION OF *TROGODERMA GRANARIUM*

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A B S T R A C T

A series of steroidal aziridines was prepared and their effect on development and fecundity of *T. granarium* was studied.

Stigmaaziridine affects the larval growth and the viability of the eggs but not the oviposition rate of the females. Cholestanone-aziridine and desmoaziridine injected into larvae influenced significantly the oviposition rate of the females. Their effect on egg hatchability was relatively low.

I N T R O D U C T I O N

The Khapra beetle *Trogoderma granarium* is a serious dermestid pest, which infests stored cereals in hot, arid regions of the world. Numerous investigations were recently directed toward control of insects by the use of chemosterilants which include alkylating agents, such as aziridines (ethylenimines), antimetabolites and other compounds (Borkovec, 1966, 1974).

Insects require dietary sources of cholesterol or related sterols for larval growth and adult development. Sterols have several functions

such as being the building stones for cell membranes and precursors of hormones that regulate moulting and metamorphosis. It was of interest, therefore, to test the effect of steroidal aziridines as potential anti-metabolites and chemosterilants for *T. granarium*. For this purpose a series of steroidal aziridines derived from stigmasterol, lanosterol, desmosterol and 5-ketocholestanone were synthesized (Ikan et al., 1972) and their effect on the development and oviposition of *T. granarium* was studied.

## MATERIALS AND METHODS

### Test insects

Test insects were larvae and adults of *T. granarium*, reared at 33°C and 40-45% r.h. Newly hatched larvae were obtained by the technique described by Yinon (1965); Diapausing larvae were produced as reported previously (Ikan et al., 1970). All experiments were conducted at 33°C and 40-45 % r.h., in the dark.

### Test materials

The following steroidal aziridines were tested :

- 1) 2,3-iminocholestan-6-one (cholestanone-aziridine)
- 2) 22,23-iminostigmasteryl acetate (stigmaaziridine)
- 3) 24,25-iminolanosteryl acetate (lanoaziridine)
- 4) 24,25-iminodesmostanyl acetate (desmoaziridine).

#### a. Tests on larval growth

The above listed materials were dissolved in ether and mixed with the food (whole wheat flour) at a concentration of 0.1 % (by weight). The control was wheat flour treated with ether. After evaporation of the ether, for each experimental condition, 1.5 g of food medium was given to 30 one-day old larvae. Mortality and pupation were recorded over a period of 36 days. The cumulative percentage of pupation was calculated from the number of larvae living at the time of appearance of the first pupa.

#### b. Tests on termination or maintenance of the diapause

Tests on effect of steroidal aziridines on termination or maintenance of the larval diapause in *T. granarium* were carried out with diapausing female larvae of an average weight of 6 mg as reported elsewhere (Ikan et al., 1970). The test material was dissolved or suspended in

liquid paraffin at a quantity of 1 mg material in 1 ml solvent, and injected into larvae at a volume of 0.6  $\mu$ l/larva. The control larvae were injected with liquid paraffin. After injection, groups of 30 larvae were kept on 1.5 g of fresh food i.e. they were subjected to diapause-terminating conditions (Ikan et al., 1970). Termination of the diapause was recognized by pupation of the larvae. The number of pupating larvae was expressed as cumulative percentage of pupation at definite time intervals.

#### c) Fertility tests

Newly emerged adults which were descendants from experimental larvae which developed from their first instar on the diet with 0.1% of the test material i.e. treated males and females (see paragraph a), or from diapausing larvae injected with the aziridine suspended in liquid paraffin i.e. only treated females (see b), were tested on their ability to oviposit. Since *T. granarium* adults do not feed and do not need the food for oviposition, each pair of adults was kept in an empty test tube 23 mm diameter for mating and reproduction. Males which died in the course of the experiment were replaced by new ones. Eggs were counted daily till the death of the female. The results were calculated as an average number of eggs laid by female. Statistical analysis was done according to Dunnet's Many One T Statistics (Miller, 1966).

## RESULTS AND DISCUSSION

#### a) Tests on larval growth

The results in Table 1 show that stigmaaziridine retards strongly the development of the larvae, whereas desmo- and lanoaziridines and cholestanone-aziridine were relatively ineffective. Mortality of the larvae ranged between 0-6 %, being the highest in the feed medium containing 0.1 % stigmaaziridine. Higher concentrations of stigmaaziridine were

Compound added to wheat flour *	Number of larvae in the experiment	Cumulative percentage pupation after days:					
		18	20	24	28	32	36
None (Control)	116	4.3	20.6	47.4	72.4	93.1	95.6
Cholestanone-aziridine	90	2.2	10.0	38.8	70.0	84.4	87.7
Desmoaziridine	57	5.3	14.0	45.6	73.6	82.4	85.9
Lanoaziridine	117	1.7	10.2	34.1	58.9	82.0	83.7
Stigmaaziridine	113	0.0	0.0	7.0	15.9	40.7	54.8

\* 0.1% in food medium.

Table 1 Dietary effect of steroidal aziridines on the development of *T. granarium*

toxic to the larvae. Newly emerged adults (only 3 % of adults were malformed) were used in fertility tests (see c). It is interesting to note that similar results were obtained with *Dermestes maculatus*, a carnivorous member of the same family. Markus (1972) stated that 0.2 % of stigmaaziridine mixed with the semisynthetic diet of Levinson et al. (1967) affected the growth and the pupation of the larvae in a high degree, while lanoaziridine had no influence on the larvae in all the concentrations tested.

Compounds injected	Cumulative percentage pupation after days :							Mortality of the larvae, %
	4	6	8	10	12	14	16	
Liquid paraffin (Control)	2.3	29.5	43.1	68.1	86.3	97.7	100.0	4
Cholestanone-aziridine	2.1	34.0	42.5	53.1	65.9	72.3	74.4	6
Desmoaziridine	0.0	14.9	19.1	27.6	48.9	61.7	70.2	6
Lanoaziridine	10.0	19.1	44.6	59.5	72.3	80.8	85.1	6
Stigmaaziridine	0.0	29.5	40.9	56.8	75.0	86.6	97.7	10

Table 2 The effect of steroidal aziridines injected into diapausing female larvae on termination or maintenance of the diapause in *T. granarium*

b) Tests on termination or maintenance of the diapause

From the results of Table 2 it could be seen that about 30% delay in pupation was obtained when desmoaziridine and cholestanone-aziridine were injected into diapausing larvae in comparison to the control (100 % pupation in 16 days). The highest mortality of the treated larvae was observed in stigmaaziridine-injected larvae. Newly emerged females (6 % were pupal-adult intermediates) were used with untreated males in fertility tests (see c).

Compounds added to wheat flour *	No. of pairs in experiment	Average No. of eggs deposited/female	Viability of the eggs, %
None (Control)	30	48.5	95
Cholestanone-aziridine	30	53.3	75
Desmoaziridine	26	51.0	72
Lanoaziridine	33	46.3	90
Stigmaaziridine	30	53.3	49

\* 0.1% in food medium.

Table 3 The effect of steroidal aziridines fed to larvae on oviposition of *T. granarium*

c) Fertility tests

Steroidal aziridines fed to larvae had no effect on the oviposition rate of the insect (Table 3) but some decrease in the viability of the eggs deposited by the above adults was noted. The greatest decrease in egg hatchability was observed in adults which larvae were fed stigmaaziridine (49 % viability), the lowest in those raised on a diet with lanoaziridine (90% viability). Injections of desmoaziridine and cholestanone-aziridine into diapausing female larvae affected significantly the oviposition rate of the females (Table 4). The results concerning egg viability paralleled those reported in Table 3; somewhat lower effect of stigmaaziridine was perhaps due to the fact, that in this series of tests only females were treated.

From the above results one may conclude that of all the steroidal aziridines tested stigmaaziridine exhibited the strongest effect on larval growth by delaying pupation, and on the egg viability when both sexes

were treated. Some improvements of the effect could be expected by applying stigmaaziridine topically on the pupae and/or adults of *T. granarium*.

Compounds injected	Average No. of eggs / female <sup>1)</sup>	Viability of the eggs, %
Liquid paraffin (Control)	52.4	91
Cholestanone-aziridine	35.3*	71
Desmoaziridine	35.8*	69
Lanoaziridine	39.2	90
Stigmaaziridine	41.8	58

1) 25 pairs were employed for each set of experimental conditions.

\*) Significant at the level of 5 %.

Table 4 The influence of steroidal aziridines injected into larvae on the fecundity of *T. granarium*

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