© Triveni Enterprises, Lucknow (India)
ISSN : 0254-8704
http : //www.geocities.com/j_environ_biol/

J. Environ. Biol.
29(6), 951-957 (2008)
j_environ_biol@yahoo.com

New synthetic precocenoids as potential insect control agents

Smita Banerjee*[1], G.P. Kalena[2], A. Banerji*[2] and A.P. Singh[1]

[1]Department of Zoology, Dr. H.S. Gour University, Sagar - 470 003, India
[2]Bioorganic Division, BARC, Mumbai - 400 085, India

(Received: October 17, 2006; Revised received: April 22, 2007 ; Accepted: April 30, 2007)

Abstract: *Ageratochromes or precocenes are known for their insect growth regulating (IGR) activity. The present investigation was taken up with an objective to look for the lead structure in these compounds which can be elaborated synthetically to obtain useful growth regulators for practical purposes. With this in mind, some variants of precocenes were synthesized in the laboratory and tested for their toxicity and growth regulating activity using red cotton bug Dysdercus koenigii as the test insect. Most of the precocenoids showed toxicity of various degree and metamorphic derangements to different extents. Adults emerging from treated nymphs could not complete the normal life span. Among the compounds tested 8-acetyl-7-hydroxy-5-methoxy-dimethylchromene (alloevodinol) was more toxic and also showed developmental defects at very low dose such as 0.5 mg l^{-1}/nymph. Precocene II (6, 7 – dimethoxy-2, 2-dimethylchromene) was used as the standard compound. It was the least toxic and showed effects at 30 mg l^{-1}/nymph.*

Key words: *Dysdercus koenigii, Metamorphic derangements, Precocene*
PDF of full length paper is available with author ***(*bsmitasagar@hotmail.com)***

Introduction

In an attempt to compensate their immobility, plants produce an armoury of chemicals known as allelochemicals which make them unstable for utilization by insects and other predators by imparting repellency, toxicity, unpalatibility or biochemical alienation of vital biochemical or physiological functions (Bowers, 1985, 1991; Koul and Smirle, 1994; Banerji, 1994; Agrawal, 1998; Arimura, *et al.,* 2000). All insects appear to have determinant receptors which interact with certain type of compounds and deter the insects, from ingesting possible toxins. Phytophagous insects which feed on plants containing allelochemicals, can tolerate the presence of these compounds which are otherwise toxic to other insect species. Probably all green plants at least at some stage of life history contain one or more chemicals which are deterrent to at least some insects. We do not know how many different deterrents exist, but they are usually plant secondary compounds and it is usual to assume that all these are potential deterrents (Swain, 1977). Bernays and Chapman (1987) estimated that there may be as many as 400,000 different compounds. Recently Khajagi (2004) has shown effects of chromene compounds on *Microplitis* even when administered *via Spodoptera*.

Ageratochromes from *Ageratum haustanianum* was described by Bowers *et al.* (1976) and Bowers (1992) who named the compounds as precocene I and II (7-methoxy-2, 2-dimethyl chromene and 6, 7-dimethoxy-2, 2-dimethyl chromene, respectively) because of their anti-juvenile hormone activity leading to precocious metamorphosis. This type of activity has recently been shown by Mathai and Nair (2005). However, Bowers (1992) has shown that this activity is manifested only in a selective group of insects.

With a view to enhance the bioactivity profile of such compounds, it was proposed to explore growth regulatory activity of compounds related to precocenes. Keeping this in view, rational synthetic, strategies for 2, 2-dimethyl chromenes (DMC) were developed (Banerji and Goomer, 1984; Banerji and Kalena, 1989) and used for the preparation of precocene analogues. The present paper describes the IGR activities of the following compounds {Fig. 1(3) to (10)} : (3) 6-hydroxy-DMC, (4) 6-bromo-DMC, (5) 7-hydroxy-6-acetyl-DMC (Eupatoriochromene), (6) 6-acetyl-5-hydroxy-DMC, (7) 8-acetyl-7-hydroxy-5-methoxy-DMC (Alloevodinol), (8) 3, 7, 7-trimethyl-pyrano-1, 2-benzisoxazole (Pyranobenzisoxazole), (9) 3-methyl-6-acetyl-7-hydroxy-1, 2-benzisoxazole and (10) 3-methyl-7-hydroxy-8-acetyl-1, 2-benzisoxazole. The last three are heterocyclic compounds but without DMC moieties. The results have been compared with those obtained from treatment with naturally occurring precocene II {Fig.1 (2)} which is known to be more active than precocene I {Fig. 1 (1)} (Bowers *et al.,* 1976; Pener *et al.,* 1978; Vyas and Mulchandani, 1980). Recently effects of precocene II in particular, on different aspects of insect physiology have been studied by Khan and Kumar (2000), Ergen (2001), Kumar and Khan (2004), Chen *et al.* (2005), Mathai and Nair (2005) and Ringo *et al.* (2005). Khajagi (2004), has shown effects of both precocene I and II on the parasite of *Spodoptera* administered *via* the later.

Materials and Methods

The red cotton bug, *Dysdercus* sp. is a serious pest of cotton in and around Sagar district as well as in the whole Bundelkhand region. It is easy to rear it in the laboratory under normal conditions and it is very sensitive to morphogenetic compounds. All these facts make the red cotton bug the insect of choice for investigation presented here.

Eggs and adults of the red cotton bug, *Dysdercus koenigii* were obtained from the culture maintained at BARC. They were then reared in the laboratory (Banerjee *et al.,* 2001).

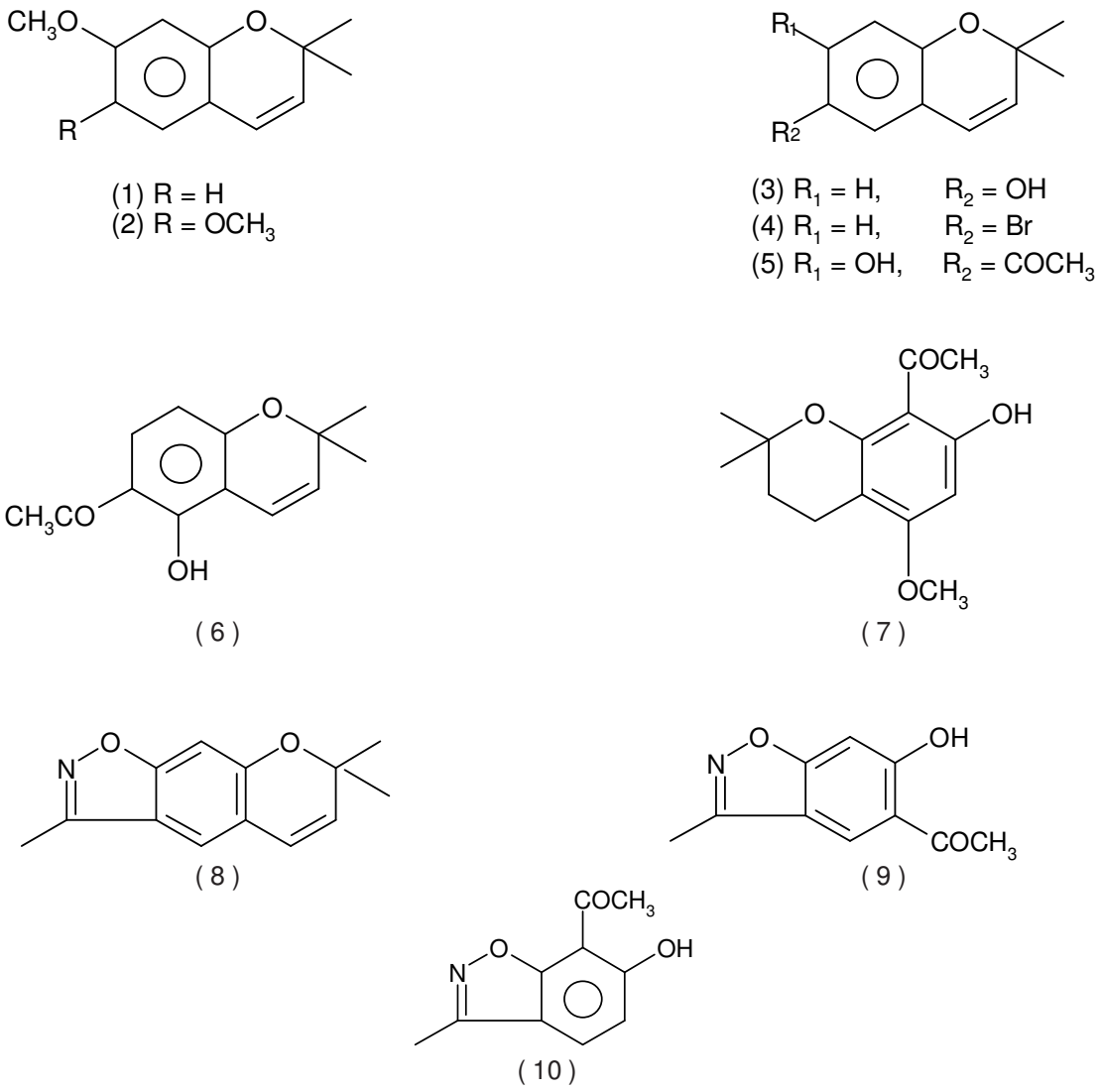

Fig. 1: Precocenoids tested for insect growth regulating (IGR) activities

Precocene II was procured from Aldrich Chemical Company (USA). The precocenoids [compound (3) to (8), Fig.1] were synthesized (Banerji and Goomer, 1984; Banerji and Kalena, 1989; Kalena *et al.,* 1997). Benzisoxazole compounds (9) and (10) were synthesized from 2, 4- and 2, 6- diacetylresorcinol respectively by selective oxidation followed by thermal cyclisation (Kalena *et al.,* 1997).

The test compounds were dissolved in acetone (1 mg ml^{-1}). The required concentrations were then topically applied with the help of a 24-36 hr old 4^{th} and 5^{th} instar nymphs. The treated nymphs were placed back in the jars after the acetone had evaporated. Three replicates were used, with 15 insects/replicate, for each dose (concentration) of a compound. A parallel control group of nymphs treated with acetone alone was set up with each experiment. In addition, another control group of untreated bugs were also observed along with each experimental (chromene treated) and acetone treated controls. The results obtained with both types of control groups were more or less similar and have been presented in the tables as 'control'. The bioactivity of a test compound was determined by its effects on mortality (toxic/insecticidal effect), moulting and growth (growth inhibiting/regulating activity). Benzisoxazole compounds which are also heterocyclic compounds but without dimethyl chromene moiety (DMC) were screened against 5^{th} instar nymphs only.

The results have been derived on a percentage basis from the total number of 45 nymphs in three replicates, therefore, standard deviation has not been mentioned. Standard deviation in case of interecdysial period was invariably between 0.89 to 1.2 in all the experimental groups, hence they have not been mentioned separately in the tables. The 50% lethality dose (LD_{50}) was calculated (Turner, 1965) for all compounds and accordingly treatment was given with other doses.

Table - 1: Toxicity and IGR activity of precocene II (2) in *D. koenigii*

Dose (mgl^{-1})	4th Instar, LD_{50} = 85.46 (mgl^{-1})			5th Instar, LD_{50} = 82.37 (mgl^{-1})		
	% mortality	% moult to 5th instar (days)*		% mortality	% adult emergence (days)*	
	(72 hr)	Normal	Abnormal	(72 hr)	Normal	Abnormal
30	31.1	68.9 (7)	Nil	22.2	77.8 (8)	Nil
50	44.4	55.6 (8)	Nil	51.1	22.2 (8)	26.7 (9)
75	53.3	15.6 (9)	31.1 (9)	55.6	17.8 (8)	26.6 (10)
100	70.0	8.0 (10)	22.0 (11)	64.4	Nil	35.6 (11)
200	82.2	Nil	17.8 (11)	82.2	Nil	17.8 (11)
Control	Nil	100 (7)	Nil	2.0	98.0 (7)	Nil

*Interecdysial period

Table - 2: Toxicity and IGR activity of 6-hydroxy-DMC (3) in *D. koenigii*

Dose (mgl^{-1})	4th Instar, LD_{50} = 15.95 (mgl^{-1})			5th Instar, LD_{50} = 13.35 (mgl^{-1})		
	% mortality	% moult to 5th instar (days)*		% mortality	% adult emergence (days)*	
	(72 hr)	Normal	Abnormal	(72 hr)	Normal	Abnormal
8	35.0	65.0 (8)	Nil	30.0	70.0 (7)	Nil
10	40.0	60.0 (8)	Nil	35.0	65.0 (8)	Nil
15	45.0	45.0 (8)	10.0 (8)	45.0	55.0 (8)	Nil
20	50.0	40.0 (9)	10.0 (10)	60.0	20.0 (8)	20.0 (9)
25	65.0	19.0 (9)	16.0 (10)	75.0	Nil	25.0 (10)
Control	Nil	100.0 (7)	Nil	Nil	100.0 (6)	Nil

*Interecdysial period

Table - 3: Toxicity and IGR activity of 6-bromo-DMC (4) in *D. koenigii*

Dose (mgl^{-1})	4th Instar, LD_{50} = 12.08 (mgl^{-1})			5th Instar, LD_{50} = 12.03 (mgl^{-1})		
	% mortality	% moult to 5th instar (days)*		% mortality	% adult emergence (days)*	
	(72 hr)	Normal	Abnormal	(72 hr)	Normal	Abnormal
5	35.7	64.3 (6)	Nil	37.2	28.3 (7)	34.5
10	44.4	55.6 (8)	Nil	45.5	25.5 (9)	29.0 (9)
15	53.3	46.7 (9)	Nil	55.0	20.0 (9)	25.0 (9)
20	56.6	43.4 (5)	Nil	69.2	10.0 (9)	20.8 (9)
Control	2.0	98.0 (6)	Nil	1.0	99.0 (9)	Nil

*Interecdysial period

Results and Discussion

Effects of precocene II on development of *Dysdercus koenigii:* A dose of 50 mg l^{-1} precocene II led to lack of appetite and with higher doses the nymphs did not feed at all. The LD_{50} has been found to be 85.46 mg l^{-1}, for 4th instar and 82.37 mg l^{-1} for 5th instar nymphs (Table 1). Doses from 30 mg l^{-1} and above caused mortality of both 4th and 5th instar nymphs. The surviving nymphs showed statistically significant ($p < 0.05$) dose dependent delay in metamorphosis at 75 mg l^{-1} and higher dose treated groups as shown in the Table 1. The individuals emerging from 30 and 50 mg l^{-1} treated nymphs were apparently normal although they did not survive the normal life span (7-8 days for 5th instar nymphs and 35-40 days for adults). The 5th instar nymphs emerging from treated 4th instars as well as adults from treated 5th instars in the 75 mg l^{-1} to 200 mg l^{-1} treated groups showed retarded development with small and pale body and underdeveloped wing pads/wings.

Effects of 6-hydroxy-DMC on development of *Dysdercus koenigii*: The LD_{50} of compound (3) on 4th instar nymph was 15.95 and that on 5th instar nymth was 13.35 mg l^{-1}. Upto a dose of 10 mg l^{-1} no abnormality was observed in the 5th instar or adults emerging from treated 4th or 5th instars respectively (Table 2). At a dose between 15 to 25 mg l^{-1} there was an increase in mortality. Some of the surviving nymphs metamorphosed to 5th instars with a delay of 2 to 3 days and showed metamorphogenic abnormalities. The treated 5th instar nymphs (upto 20 mg l^{-1}) showed mortality but the surviving nymphs emerged apparently into normal adults with a delay of 1 to 2 days. At

Table - 4: Toxicity and IGR activity of 6-acetyl-7-hydroxy-DMC (5) in *D. koenigii*

Dose (mg l^{-1})	4th Instar, LD_{50} = 5.09 (mg l^{-1})			5th Instar, LD_{50} = 4.39 (mg l^{-1})		
	% mortality	% moult to 5th instar (days)*		% mortality	% adult emergence (days)*	
	(72 hr)	Normal	Abnormal	(72 hr)	Normal	Abnormal
1	Nil	100.0 (6)	Nil	15.0	85.0 (8)	Nil
2	Nil	100.0 (6)	Nil	25.0	72.5 (8)	2.5 (8)
3	Nil	100.0 (7)	Nil	40.0	45.0 (8)	15.0 (8)
5	37.5	62.5 (7)	Nil	45.0	42.5 (8)	12.5 (8)
6	55.0	20.0 (7)	25.0 (8)	50.0	22.5 (8)	27.5 (9)
7	62.5	20.0 (8)	17.5 (8)	60.0	15.0 (8)	25.0 (9)
Control	Nil	100.0 (6)	Nil	04.0	96.0 (7)	Nil

*Interecdysial period

Table - 5: Toxicity and IGR activity of 6-acetyl-5-hydroxy-DMC (6) in *D. koenigii*

Dose (mg l^{-1})	4th Instar, LD_{50} = 3.75 (mg l^{-1})			5th Instar, LD_{50} = 3.50 (mg l^{-1})		
	% mortality	% moult to 5th instar (days)*		% mortality	% adult emergence (days)*	
	(72 hr)	Normal	Abnormal	(72 hr)	Normal	Abnormal
1	20.0	80.0 (6)	Nil	25.0	75.0 (7)	Nil
2	40.0	60.0 (7)	Nil	25.0	75.0 (8)	Nil
3	45.0	55.0 (7)	Nil	45.0	55.0 (8)	Nil
5	55.0	35.0 (8)	10.0 (8)	55.0	40.0 (8)	5.0 (9)
6	65.0	15.0 (8)	20.0 (9)	60.0	30.0 (9)	10.0 (10)
8	70.0	Nil	30.0 (9)	67.5	7.5 (9)	25.0 (11
Control	2.5	97.5 (6)	Nil	10.0	90.0 (7)	Nil

*Interecdysial period

Table - 6: Toxicity and IGR activity of 8-acetyl-7-hydroxy-5-methoxy-DMC (7) in *D. koenigii*

Dose (mg l^{-1})	4th Instar, LD_{50} = 1.78 (mg l^{-1})			5th Instar, LD_{50} = 1.40 (mg l^{-1})		
	% mortality	% moult to 5th instar (days)*		% mortality	% adult emergence (days)*	
	(72 hr)	Normal	Abnormal	(72 hr)	Normal	Abnormal
0.5	25.0	73.0 (7)	2.0 (7)	30.0	60.0 (6)	10.0 (9)
1.0	28.0	71.0 (7)	1.0 (7)	37.0	20.0 (7)	43.0 (9)
1.5	35.0	30.0 (8)	35.0 (9)	45.0	15.0 (8)	40.0 (10)
2.0	47.0	25.0 (8)	28.0 (10)	55.0	Nil	45.0 (11)
2.5	61.0	Nil	39.0 (10)	68.0	Nil	32.0 (12)
Control	3.0	97.0 (7)	Nil	2.0	98.0 (7)	Nil

*Interecdysial period

the dose of 25 mg l^{-1} all the surviving 5th instar nymphs emerged into abnormal adults which were small and pale with underdeveloped wings. They survived for 2 to 3 days only as against a normal life span of 35 to 40 days in the control.

Effect of 6-bromo-DMC on development of *Dysdercus koenigii:* This analogue of compound (3) showed LD_{50} of 12 mg l^{-1} for both 4th and 5th instar nymphs. No abnormalities were observed in the 5th instar nymphs which emerged from surviving treated 4th instars after a delay of 2 to 3 days. There was precocious moulting only by one day in 20 mg l^{-1}/nymph treated group. However, adults emerging from surviving 5th instar nymphs treated with 10 mg l^{-1} and higher doses showed abnormality (Table 3). There was a prolonged ecdysial period of three days. The abnormal adults survived only for 24 hr (20 mg l^{-1} treated group) to 3 days (10 and 15 mg l^{-1} treated groups).

Effect of 6-acetyl-7-hydroxy-DMC on development of *Dysdercus koenigii*: The LD_{50} value were 5.09 and 4.39 mg l^{-1} for 4th and 5th instar nymphs respectively. Treated 4th instar nymphs showed no mortality upto 3 mg l^{-1} dose and no apparent abnormality upto dose levels of 5 mg l^{-1} but amounts more than this resulted in emergence of small and pale 5th instars without pigmentation of wing pads (Table 4). Similar observations were recorded for 5th instar nymphs. Emerging adults were deformed even a dose of 2 mg l^{-1}/nymph. The moulting was delayed by 1 to 2 days. Such adults survived only 2-4 days with 1-4 mg l^{-1} dose and only few hours with higher doses.

Table - 7: Toxicity and IGR activity of 3,7,7-trimethyl-7-hydroxy-pyrano-3,2-f)-1,2-benzisoxazole (8) in *D. koenigii*

Dose (mgl^{-1})	5th Instar, LD_{50} = 6.83 (mgl^{-1})		
	% mortality (72 hr)	% adult emergence (days)*	
		Normal	Abnormal
2	20.0	8 0.0 (7)	Nil
4	24.0	76.0 (7)	Nil
6	44.0	56.0 (8)	Nil
8	53.0	22.0 (9)	25.0 (10)
12	62.0	11.0 (9)	27.0 (10)
Control	Nil	100.0 (7)	Nil

*Interecdysial period

Table - 8: Toxicity and IGR activity of 3-methyl-6-acetyl-7-hydroxy-1,2-benzisoxazole (9) in *D. koenigii*

Dose (mgl^{-1})	5th Instar, LD_{50} = 37.0 (mgl^{-1})		
	% mortality (72 hr)	% adult emergence (days)*	
		Normal	Abnormal
20	13.0	87.0 (8)	Nil
30	27.0	62.0 (9)	11.0 (9)
40	44.0	29.0 (9)	27.0 (11)
50	60.0	9.0 (9)	31.0 (11)
Control	8.0	92.0 (7)	Nil

*Interecdysial period

With lower doses like 1-3 $mg\ l^{-1}$/nymphs feeding deterrence was not observed. Nymphs treated with doses higher than the above showed inhibited feeding activity. The same was also observed in the subnormal individuals emerging from treated nymphs.

Effects of 6-acetyl-5-hydroxy-DMC on development of *Dysdercus koenigii*: The analogue of eupatoriochromene has been found to be fairly toxic to nymphs showing LD_{50} of 3.75 $mg\ l^{-1}$ for 4th instar and 3.5 $mg\ l^{-1}$ for 5th instars. Treatment of nymphs with this compound showed abnormalities at dose levels of 5-8 $mg\ l^{-1}$ (Table 5). The number of normal moult gradually decreased with increased dosage and interecdysial period was prolonged by 1 to 4 days. In addition to abnormalities like small and pale body, the emerging 5th instars had shrunken abdomens with black spots. These as well as deformed adults from treated 5th instars were short lived than the controls and survived only for 10 to 15 hr in 8 $mg\ l^{-1}$ treated group to 2 to 3 days in the 5 $mg\ l^{-1}$ treated group.

Effects of 8-acetyl-7-hydroxy-5-methoxy-DMC on development of *Dysdercus koenigii:* It was the most toxic amongst the compounds tested in the present investigation with a LD_{50} of 1.78 $mg\ l^{-1}$ for 4th and 1.40 $mg\ l^{-1}$ for 5th instar, respectively. Treatment with this compound caused serious abnormality to the surviving nymphs even at the lowest dose tested (0.5 $mg\ l^{-1}$/nymph, Table 6). In addition to the abnormalities described in previous cases, the legs and antennae were deformed. The wings in the adults were crumpled. Moulting was delayed by 1-5 days. Bugs treated even with 0.5 $mg\ l^{-1}$/nymph avoided soaked cotton seeds given as food.

Table - 9: Toxicity and IGR activity of 3-methyl-8-acetyl-7-hydroxy-1,2-benzisoxazole (10) in *D. koenigii*

Dose (mgl^{-1})	5th Instar, LD_{50} = 72.38 (mgl^{-1})		
	% mortality (72 hr)	% adult emergence (days)*	
		Normal	Abnormal
20	Nil	87.0 (7)	13.0
30	18.0	58.0 (7)	24.0 (7)
40	24.0	42.0 (7)	34.0 (8)
50	49.0	Nil	51.0 (8)
10	60.0	Nil	40.0 (10)
Control	Nil	100.0 (7)	Nil

*Interecdysial period

Effects of 3,7,7-trimethyl-7-hydroxy-pyrano (3,2-f)-1,2-benzisoxazole on development of *Dysdercus koenigii:* This compound has an isoxazole moiety fused linearly to DMC. Its LD_{50} value for 5th instar nymph was found to be 6.83 $mg\ l^{-1}$. It did not show any morphogenic effects upto a dose of 6 $mg\ l^{-1}$. However, at higher doses moulting was delayed by 1-3 days and abnormalities were observed in the adults emerging from surviving 5th nymphs (Table 7). At a dose of 9 $mg\ l^{-1}$, 55% of emerging abnormal adults showed mortality during eclosion, the other 45% within 24 hr of emergence.

Effects of 3-methyl-7-hydroxy-6-acetyl-benzisoxazole on development of *Dysdercus koenigii:* The LD_{50} of this compound was 37 $mg\ l^{-1}$ for 5th instar nymphs. Upto 20 $mg\ l^{-1}$, no metamorphic effects were seen in the surviving adults, but considerable effects were observed at doses 30, 40 and 50 $mg\ l^{-1}$ (Table 8). There was a delay in moulting by 2-4 days. With 30 $mg\ l^{-1}$ dose, emerging small and pale adults with open wings either died while moulting or within a few days. Abnormal adults emerging from 40-50 $mg\ l^{-1}$ treated 5th instars survived only for 10 to 15 hr.

Effects of 3-methyl-7-hydroxy-8-acetyl-benzisoxazole on development of *Dysdercus koenigii:* Amongst the three benzisoxazole compounds tested, this was the least toxic (LD_{50} 72.38 $mg\ l^{-1}$ for 5th instar nymphs) but at doses 20 $mg\ l^{-1}$ and above, serious abnormalities were observed in the adults emerging from the treated 5th instar nymphs after a prolonged interecdysial period of 1-3 days (Table 9). At higher dose like 50-100 $mg\ l^{-1}$ eclosion was not complete and emerging abnormal adults showed mortality in a few hours to a week.

LD_{50} at 72 hr was determined to ascertain the lethal/toxic effects of the compounds and to use a few lower and other higher doses for inhibitory effects on growth, if any. Thus the effective dose (ED) or inhibitory dose (ID) for developmental derangements was mostly lower than LD_{50} as can be seen from Table 1-9.

LD_{50} values showed wide variations and therefore it was not possible to use uniform doses for all the test compounds. Based on the LD_{50} values following order of toxicity was established : 8-Ac-7-OH-5-MeOH-DMC (alloevodinol) > 6-Ac-5-OH-DMC > 6-Ac-7-OH-DMC > Pyrano-BIO > 6-Br-DMC > 6-OH-DMC > 6-Ac-7-OH-

BIO > 8-Ac-7-OH-BIO > 6-7-MeOH-DMC (precocene II). Thus, alloevodinol was the most toxic and precocene II the least toxic to both 4th and 5th instar nymphs. The results show that LD_{50} of precocene II as well as the synthetic chromenes were higher for 4th instar nymphs in comparison to 5th instars with the differences ranging from 3.4 mg l^{-1} in the insects treated with the former to 0.38 mg l^{-1} in those treated with alloevodinol. The reason for this is not known.

In the present investigation, our primary aim was to look for lead structures which can be further elaborated synthetically to obtain useful growth regulators. Since the double bond in the precocene I and II are important for impairing bioactivity (Bowers, 1992), we have synthesized analogous precocene compounds (3) to (7) in which the dimethylchromene (DMC) moiety with double bond has been retained. Since benzisoxazoles are important pharmacophores (Kumari *et al.,* 1973), we have also included a few compounds (8) to (10) of this group. Compound (8) is interesting in having heterocyclic moieties. Although compounds containing dimethylchromene moiety occur widely in nature (Proksch and Rodriguez, 1983). 6-oxygenated compounds are of rare occurrence. The 6-hydroxy-DMC (3) compound was the simplest one among all other compounds tested in the present investigation. Its occurrence has been reported from the marine tunicate, *Alpidium californicum* and it is known to have anti-mutagenic and antitumour activities (Proksch and Rodriguez, 1983).

Depending upon the compounds and dose, some of the nymphs which survived the toxic effects, showed apparently normal metamorphosis while others showed retarded growth and incomplete metamorphosis. It is interesting to note that apparently normal adults emerging from treated nymphs did not live normal life span when compared to controls. They rarely mated and females only occasionally laid very few scattered eggs or none at all. This was in contrast to normal *D. koenigii* females which lay 45 – 55 eggs at a time in a single bunch. This led us to interpret that the insects were sterile. This was further confirmed by histological structure of ovaries (Banerjee and Magdum, 2007). All these abnormalities pointed to a hormonal imbalance as has been also indicated in *Anacridium* by Ergen (2001) a study on the corpus allatum of precocene II treated insects.

Thus the results of the present work have been found to be quite different form those of Bowers (1976) for the same genus of a hemipteran pest. Nevertheless, these results are derived from observations on almost 50 insects per experiment as mentioned under Material and Methods and are in conformity with those of Joshi *et al.* (1990) in the same insect and other authors in some other insects (Cupp *et al.,* 1977; Rembold *et al.,* 1979; Azambuja *et al.,* 1981). Gujar (1994) has also pointed out the possibility of interference with prothoracicotrophic hormone in insects in which precocenes cause delayed moulting.

It has been observed that in general, compounds which show antifeedant activity cause developmental changes suggesting disturbance in the endocrine activity (Rembold, 1989). For example, azadirachtin, which is a strong anti-feedant in most insect species studied, causes growth disruption in insects possibly by interfering with normal hormonal balance (Rembold, 1989; Rembold and Sieber, 1981; Sieber and Rembold, 1983). In adult insects it also retards egg maturation and even leads to sterilization. The most inhibiting effect is a result of changes in haemolymph ecdysteroid and juvenile hormone titers which are mediated by a widespread blockade of trophic factors from the brain (Subrahmanyam *et al.,* 1989; Subrahmanyam and Rembold, 1989; Banerjee and Rembold, 1992; Banerjee *et al.,* 1998). The compounds tested in the present investigation showed feeding inhibition to different extents. Histochemical staining followed by Ewen (1962), has shown accumulation of neurosecretory material in the neurosecretory cells of brain of precocene treated *Dysdercus koenigii.* This may be due to inhibition of release of trophic factors regulating homone secretion necessary for moulting just as in case of azadirachtin treated insects (Subrahmanyam *et al.,* 1989; Subrahmanyam and Rembold, 1989; Banerjee and Rembold, 1992; Banerjee *et al.,* 1998).

Based on the results of the present work it can be interpreted that precocene and its derivatives act as antifeedants and possibly through this, they disturb the endocrine processes and cause metamorphic derangements. At higher doses they are general toxicants. Benzisoxazole derivatives have also been shown as inhibitors of acetylcholinesterase activity in insects just like general toxicants (Villalobos *et al.,* 1994). Further work on optimization of structure-activity is likely to provide promising compounds with relevance in insect control.

Acknowledgments

Award of a BRNS (DAE) research project to S. Banerjee is thankfully acknowledged.

References

Agrawal, A.A.: Induced responses to herbivory and increased plant performance. *Nature*, **270**, 1201-1202 (1998).

Arimura, G.I., R. Ozawa, T. Shimoda, T. Nishioka, W. Baland and J. Takabyshi: Herbivory induced volatiles elicit defence genes in lima bean leaves. *Nature*, **406**, 512-515 (2000).

Azambuja, P.D., E.S. Garcia and J.M.C. Rebeiro: Effects of ecdysone on the metamorphosis and ecdysis prevention induced by precocene II in *Rhodnius prolixus*. *Gen. Comp. Endocntol*., **45**, 100-104 (1981).

Banerjee, S. and H. Rembold: Azadirachtin interferes with control of serotonin pools in neuroendocrine system of locusts. *Naturwissen,* **79**, 81-84 (1992).

Banerjee, S. and S. Magdum: Mode of action of quinones as potential insect pest control agents. *In* : Perspectives in animal ecology and reproduction, Vol. 4 (*Eds.:* V.K. Gupta and A.K. Verma). Daya Publ. House, Delhi. pp. 341-393 (2007).

Banerjee, S., S. Magdum, G.P. Kalena and A. Banerji: Insect growth regulatory activity of naturally occurring quinones and their derivatives in *Dysdercus Koenigii* (Pyrrhocoridae, Hemiptera). *Z. Angew Zool.*, **125**, 25-30 (2001).

Banerjee, S., W. Oberthuer and H. Rembold: Azadirachtin A affects GABA pools in neuroendocrine organs of locusts. *In*: Current concepts in Biopollution research (*Eds.*: N.M. Dutta, S. Gupta-Bhattacharya, S. Mandal and K.N. Bhattacharya). Research Periodicals and Book, Publishing House, USA/UK/Taiwan/India. pp. 173-177 (1998).

Banerji, A.: Insect plant interactions : Natural insect control agents. *In*: Recent advances in insect physiology and toxicology (*Ed*.: G.T. Gujar). Agricole Publ. Acad., New Delhi. pp. 41-47 (1994).

Banerji, A. and G.P. Kalena: Heterocycles from diacetyl phenols: Synthesis of benzodipyrans, acetyl chromenes of pyrano – (1,2) benzisoxazoles. *Heterocycle*, **28**, 711-718 (1989).

Banerji, A. and N.C. Goomer: Synthesis of naturally occurring 6-oxygentaed-2, 2-dimethylchromenes. *Ind. J. Chem*., **23B**, 885-886 (1984).

Bernays, E.A. and R.F. Chapman: Chemical deterrence of plants. *In*: Molecular entomology (*Ed*.: R. Allan). Liss. Inc. pp. 107-116 (1987).

Bowers, W.S.: Phytochemical disruption of insect development and behaviour. (*Ed*.: P.A. Hedin). ACS Symposium series. No. 276. Bioregulation for Pest control (1985).

Bowers, W.S.: Insect hormones and anti-hormones in plants. *In*: Herbivores, their interaction with secondary plant metabolites (*Eds*.: G.A. Rosenthal and D.H. Janzen). Academic Press, New York. pp. 431-456 (1991).

Bowers, W.S.: Insecticidal compounds from plants *In*: Phytochemical resources for medicine and agriculture (*Eds*.: H.K. Nigg and D. Segler). Plenum Press, New York. pp. 227-235 (1992).

Bowers, W.S., T. Ohta, J.S. Cleere and P.A. Marsella: Discovery of insect juvenile hormones. *Science*, **193**, 542-547 (1976).

Chen, Y.R., S.C. Chen, H.W. Chang, G. Sun and R. Kous: Effect of exogenous juvenile hormone III and precocene II on agonistic behaviour and the *Corpora allata in vitro* activity in the male lobster cockroach, *Nauphoeta cinerea* (Dictyoptera : Blaberidae : Oxyhaloinae). *J. Zool. Stud.*, **44**, 409-416 (2005).

Cupp, E.W., J.B. Lok and W.S. Bowers: The developmental effects of 6,7-dimethoxy-2,2-dimethylchromene on the pre-imaginal stages of *Aedes aegypti* (Diptera: Culicidae). *Ent. Exp. Appl*., **22B**, 23-28 (1977).

Ergen, G.: The effects of precocene II on the fine structure of corpus allatum in adult female, *Anacridium aegyptium* L. (Orthroptera : Acrididae). *Turk. J. Zool*., **25**, 95-103 (2001).

Ewen, A.B.: Ani mproved aldehyde-fuchsin staining for neurosecretory products in insects. *Trans. Am. Micr. Soc*., **81**, 94-96 (1962).

Gujar, G.T.: Insect growth regulaors: Action, metabolism and insect control status. *In*: Recent advances in insect physiology and toxicology (*Ed*.: G.T. Gujar). Agricole Publishing Academy. New Delhi. pp. 239-242 (1994).

Joshi, N.K., V. Ramakrishnan and K.M. Lathika: Morphogenetic hormones reduce the precocene induced delay in the metamorphasis of *Dysdercus cingulatus* (Hemiptera). *Acta Entomol. Bohemoslov*, **87**, 157-158 (1990).

Kalena, G.P., A. Jain and A. Banerji: Amberlyst 15 catalysed phenylation of phenols: One step synthesis of benzopyrans. *Molecule*, **2**, 100-105 (1997).

Khajagi, W.E.: Effect of juvenile hormone I, precocene I and precocene II on the progeny of *Microplitis rutinentris* female when administered via its host, *Spodoptera liltoralis. J. Appl. Entomol*., **128**, 6-10 (2004).

Khan, I.A. and K. Kumar: Precocene II acts as IGR in Polyphagous pest *Pericallia ricini* F. (Lepidoptera: Arctiidae). *Proc. Nat. Acad. Sci. India,* **70B**, 279-286 (2000).

Koul, O. and M.J. Smirle: Allomones in insect plant interactions. *In*: Recent advance in insect physiology and toxicology (*Ed*.: G.T. Gujar). Agricole. Publ. Acad. New Delhi. pp. 14-33 (1994).

Kumar, K. and I.A. Khan: Precocene effects on ovarian follicle development in *Sarcophaga. Ind. J. Exp. Biol.,* **42**, 74-80 (2004).

Kumari, S.S., K.S.R.K., Mohanrao and N.V. Subbarao: Synthesis of γ-pyrano (6,7) benz (1,2) isoxazole and their spectral studies. *Ind. J. Chem.,* **11,** 541-544 (1973).

Mathai, S. and V.S.K. Nair: Treatment of larvae with repeated doses of precocene II induces precocious metamorphic changes in *Spodoptena mauritia* (Lepidoptera : Noctuidae). *Arch. Insect Biochem. Phy.,* **I**, 199-203 (2005).

Pener, M.P., L. Orshan and J. Dewilde: Precocene II causes atrophy the corpora allata in *Locusta migratoria. Nature,* **272**, 350-351 (1978).

Proksch, P. and E. Rodriguez: Chromenes and benzofurans of the Asterceae: Their chemistry and biology. *Phytochem.,* **22**, 2335-2348 (1983).

Rembold, H.: Isomeric azadirachtins and their mode of action. *In*: CRC State of the art series in hytochemical pesticides (*Eds*.: M. Jacobson and I. Kubo). Vol. I. The Neem Tree, CRC Press, Boca Raton. pp. 47-63 (1989).

Rembold, H., C. Czoppelt and G. Sharma: Preconene II is no antijuvenile hormone in *Apis mellifera. Zool. Naturforsch,* **34**, 1216-1263 (1979).

Rembold, H. and K.P. Sieber: Inhibition of oggenesis and ovarian ecdysteroid sysnthesis by azadirachtin in *Locusta migratoria. Zool. Naturforsch,* **36**, 466 (1981).

Ringo, J., T. Beaky and M. Brannan: Effects of precocene and low protein diet on reproductive behaviour in *Drosophila melanogaster* (Diptrera : Drosophilidae). *Ann. Entomol. Soc. Am.*, **98**, 601-607 (2005).

Sieber, K.P. and H. Rembold: The effects of azadirachtin on the endocrine control of moulting in *Locusta migratoria. J. Insect Physiol.,* **29**, 523 (1983).

Subrahmanyam, B. and H. Rembold: Effect of azadiractin A on neuroendocrine activity in *Lousta migratoria. Cell and Tissue Res.,* **256**, 513-517 (1989).

Subrahmanyam, B., T. Mueller and H. Rembold: Inhibition of turnover of neurosecretion by azadirachtin in *Locusta migratoria. J. Insect Physiol.,* **35**, 493 (1989).

Swain, T.: Secondary compounds as protective agents. *Ann. Rev. Plant Physiol.,* **28**, 479 (1977).

Turner, R.A.: Screening Methods in Pharmacology. Academic Press, New York/London (1965).

Villalobos, A., J.F. Blake, C.K. Biggers, T.W. Butler, D.S. Chapin, Y.L. Chem, J.L. Ives, S.B. Jones and D.R. Liston: Novel benzisoxazole derivatives as potent and selective inhibitors of acetylcholinesterase. *J. Med. Chem.,* **37**, 2721-2734 (1994).

Vyas, A.V. and N.B. Mulchandani: Biosynthesis of precocenes I and II, anti-juvenile hormones. *Phytochem.,* **19**, 2597-2598 (1980).