Biological activity of precocene analogues on *Locusta migratoria*

I. Kiss*, A. Fodor*, T. Timar, S. Hosztafi, P. Sebok, T. Torok*, E. Viragh and M. Berenyi*

* *Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, P.O.B. 521, H-6701 Szeged (Hungary), and Alkaloida Chemical Company, H-4440 Tiszavasvari (Hungary)*
Received 15 December 1987; accepted 3 June 1988

Summary. Structure-optimization studies of C-5–C-8-substituted precocene (P) analogues revealed that 1) compounds disubstituted asymmetrically at C-6, C-7 had an anti-allatal effect only if the C-6 side-group was shorter than that at C-7, 2) in the case of C-5, C-7 disubstitution, activity was enhanced by Me at C-5 whereas MeO caused inactivity. Effects of various other substitutions are also discussed.
Key words. Precocene; structure-activity relationship; effect of C-5–C-8 substitution and their combination; *Locusta migratoria.*

Bowers and his coworkers [1] were the first to discover that 2,2-dimethyl-chromene compounds of the bedding flower *Ageratum houstonianum* had anti-juvenile hormone (anti-JH) activity on certain Hemipteran and Orthopteran insects. Typical effects were the precocious metamorphosis of treated young larvae, resulting in sterile adultiforms, and sterilization of adult females – hence the compounds were named 'precocenes'. A considerable effort has been made to elucidate the mode of action of these compounds and the details of the chemical structure essential for the anti-JH effect. The results were summarized recently [2–4].

It was soon revealed that the precocenes exerted a special cytotoxic effect on the cells of the corpora allata (CA) in the sensitive species, and the anti-JH effect was evoked by destroying the hormone-producing organ [5]. It is widely accepted that the cytotoxic effect is due to the formation of a highly reactive 3,4-epoxide, catalyzed by the enzyme methyl farnesoate epoxidase, specific for the CA [6], although other models can also be considered [7, 8]. As precocenes seemed to become activated only in the CA, the name 'pro-allatocidins' was suggested as being appropriate for them [3].

Structure-optimization studies [9–14] established that the 3,4-double bond and the 2,2-dimethyl groups are indispensable, and substituents at the C-6 and C-7 positions can also be decisive for the anti-allatal effect. The aim of the present study was to assess the effect of various substituents and their combinations on the precocene activity and toxicity of chromene derivatives as measured on *Locusta migratoria.* While previous observations were generally confirmed, new information was also obtained on the modifying role exerted by substituents at C-5, C-6 and C-8 on the precocene activity of the C-7 substituted chromenes.

Materials and methods. The substituted 2,2-dimethyl-chromenes have been prepared following the general methods described by Timar et al. [15] and Bowers and Ohta [16]; substituted phenols (from Aldrich) reacted with 3-methyl-but-2-enoic acid in a phosphoryl chloride/zinc chloride (water) system to produce in high yield the corresponding hydroxy-4-chromanones [15]. The latter compounds were regioselectively alkylated [17]. Further alkylation, reduction and dehydration according to the procedure published by Bowers and Ohta [16] resulted in the formation of novel precocene derivatives.

Locusts were reared at 28–30 °C under a 12-h daylight/12-h darkness regimen, in crowded populations. Larvae which molted into the 4th instar within 24 h were used for the tests. The substances were dissolved in acetone at 150 mM concentration if not stated otherwise, and this stock solution was diluted in a tenfold series with acetone for the tests. Both the substances and stock solutions were kept at – 20 °C. Locust nymphs were anesthetized in CO_2, and 10 µl of the solution to be tested was applied to the abdominal sternites; application was followed by quick evaporation in a stream of cold air from a hair-dryer. Twenty animals were kept in wire cages under optimal conditions and checked daily for death and molting. Final evaluation was made when all of them had metamorphosed or died. Additional tests were made at intermediate concentrations. Precocene activity was characterized by the frequency of adultiforms expressed as a percentage of the animals surviving up to metamorphosis (i.e. adults and adultiforms together). Toxicity was expressed as the percentage of the treated nymphs which died during the first four days following the treatment. ED50 and LD50 values were calculated after log-logit transformation of the data and expressed as nmol/animal (table).

Results and discussion. The results are summarized in the table. The different chromene derivatives are cited in the text by their serial numbers (in brackets) according to the table.

Precocene effect. 1. Monosubstitution at C-7: Among the 2,2-dimethyl-chromenes substituted at C-7, the highest precocene effect was shown by those in which the C-7 substituent was EtO or nPrO (table, compounds **2** and **3**). If the side-chain was shorter (**1**, Precocene 1), or longer (**5**) than that, the activity decreased. Branching (**4, 6, 7**) or unsaturated substituents (**8, 9**) also decreased the effectiveness. The inactivating effect of a triple bond (**9**) is stronger than that of a double bond (**8**). If the side-chain is branching and unsaturated at the same time, the two effects seem to be additive (**10, 11**). Benzyloxy (**15**), the only aralkyloxy substituent we tried at C-7, also showed a strong inactivating effect. Cycloalkoxy substituents (**12–14**) resulted in complete inactivity. This is in accordance with the data by Brooks et al. [10] referring to some of these compounds.

2. Disubstitution at C-6 and C-7. Of the *symmetrically disubstituted* compounds (**16–19**), only the 6,7-dimethoxy derivative (Precocene 2) showed activity which was in fact comparable to that of the most active molecules in the previous group. The rest of them were totally inactive. It seems that as a rule C-6 alkoxy-substituents with more than one carbon atom efficiently decrease the precocene effect elicited by an appropriate side-chain at C-7.

This 'rule' was further extended by the study of isomeric pairs of molecules in which C-6 and C-7 shared the same pair of substituents of different lengths. Only those compounds showed a considerable precocene activity which had a MeO group at C-6 (**20, 22, 24, 28**). While the precocene activity decreased with longer side-chains at C-7, a MeO substituent was even able to enhance the activity of prop-2-ynyloxy and cyclopentyloxy at C-7. This was not true for a benzyloxy group at the same position (see compounds **9, 12, 15** and **24, 28, 30**). An exception to this was the precocene activity of compound (**26**) which had EtO at C-6 and nPrO at C-7. This can suggest that, at least in the case of alkoxy substituents, precocene activity would be more dependent on a relative one-carbon-atom-difference in the lengths of the substituents at C-6 and C-7 than on their absolute lengths (cf. **1, 20** and **26** as well as **17** and **18**). When their positions were reversed, however, all these substituents resulted in complete inactivity (see **21, 23, 25, 27** and **29**). This effect could be exerted e.g. through changing the electron structure of the

Effect of different substituents on the precocene activity and toxicity of chromene derivatives[a]

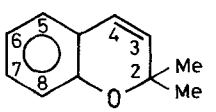
Compound serial No.	C-5	C-6	C-7	C-8	ED_{50}[b]	LD_{50}[b]
1 (Precocene.1)			MeO		305	> 1500
2			EtO		152	642
3			nPrO		147	669
4			iPrO		229	633
5			nBuO		344	400
6			sBuO		258	452
7			iBuO		796	1024
8			AllylO		291	888
9			prop-2-ynylO		1232	943
10			1,1-diMe-prop-2-ynylO		> 1500	> 1500
11			PrenylO		851	344
12			cyclopentylO		> 1500	295
13			cyclohexylO		> 1500	387
14			cycloheptylO		> 1500	1179
15			BenzylO		957	293
16 (Precocene.2)		MeO	MeO		150	554
17		EtO	EtO		> 1500	246
18		nPrO	nPrO		> 1500	416
19		cyclopentylO	cyclopentylO		> 1500	1500
20 (Precocene.3)		MeO	EtO		141	1110
21		EtO	MeO		> 1500	542
22		MeO	nPrO		217	431
23		nPrO	MeO		> 1500	346
24		MeO	prop-2-ynylO		119	147
25		prop-2-ynylO	MeO		> 1500	70
26		EtO	nPrO		183	133
27		nPrO	EtO		> 1500	153
28		MeO	cyclopentylO		364	554
29		cyclopentylO	MeO		> 1500	> 1500
30		MeO	BenzylO		> 1500	> 1500
31		BenzylO	MeO		> 1500	> 1500
32		NO_2	MeO		> 1500	> 1500
33		Cl	MeO		890	> 1500
34	Me		MeO		< 1500	N.T.
35	MeO		MeO		> 1500	N.T.
36	Me		sBuO		< 1500	N.T.
37	MeO		sBuO		> 1500	N.T.
38	Me		iBuO		175	> 1500
39	MeO		iBuO		> 1500	N.T.
40	Me		prop-2-ynylO		657	324
41	MeO		prop-2-ynylO		> 1500	217
42	Me	MeO	prop-2-ynylO		697	> 1500
43	Me	MeO	MeO		> 1500	> 1500
44	Me	MeO	EtO		> 1500	> 1500
45	Me	EtO	MeO		> 1500	> 1500
46	MeO	MeO	MeO		> 1500	> 1500
47			EtO	MeO	> 1500	598
48			MeO	EtO	> 1500	> 1500

[a] Blank space means the position is occupied by H. [b] ED_{50} and LD_{50} values are given as topically applied nanomoles/animal. N.T.: not tested.

heteroatom-containing ring, ultimately leading to a decreased reactivity of the 3,4-double bond and/or the 3,4-epoxide and to a decreased precocene effect[3]. However, the electron-donating or withdrawing nature of the C-6 substituent is clearly not enough alone to explain the precocene effect (cf. compounds **32, 33** and Brooks et al.[10]).

Steric effects could also result in biological inactivity (e.g. **29, 31**).

Taken together, the above observations could equally well be explained by any of the following three mechanisms, or by a combination of them; 1) an inactivating steric effect of the larger substituents at C-6, 2) oxydative dealkylation at C-6 and/or C-7[18,19], 3) opening of the pyran ring[8]. Whatever the basic mechanism is, we feel it is logical to assume that steric effects play a role in this phenomenon. The problem needs further investigation.



3. Disubstitution at C-5 and C-7. These analogues were synthesized in pairs, in which C-7 was occupied by the same substituent while at C-5 there was either Me or MeO. A qualitative test was made with one large dose (1500 nM) so that we can tell whether ED50 is below or above 1500 nM of the compound. As the table shows, only those compounds were active which had a Me group at C-5 (**34, 36, 38** and **40**). In two cases where quantitative measurements were also made, the ED50 values are significantly lower than those for the monosubstituted analogues which have the same alkoxy-groups at C-7 (compare **40** with **9; 38** with **7**). This suggests that the C-5 methyl group can have an activity-enhancing effect in certain combinations.

When C-5 was occupied by MeO, the original activity exerted by the C-7 substituent was completely lost (**35, 37, 39, 41**). This effect is superficially similar to that observed with the C-6, C-7 disubstituted derivatives. However, instead of the difference in length of two substituents, it is the substituent at C-5 alone which is decisive. Whether the presence or absence of the oxygen atom in the substituting group at C-5 exerts its effect through altering the steric conditions or electron density, remains to be seen.

Brooks et al.[10] and Chenevert et al.[11] tested the 5-Me, 7-MeO (**34**) and 5,7-diMeO (**35**) derivatives, respectively. Their data are in agreement with the above results and support our conclusion on the role of the substitutents at C-5 in precocene activity.

4. Trisubstitution at C-5, C-6 and C-7. The tested compounds were generally inactive, even if the related C-6, C-7 or C-5, C-7 disubstituted derivatives showed precocene activity (cf. e.g. **16, 34** and **43, 46** as well as **20** and **44** in the table). We can conclude that trisubstitution at C-5, C-6 and C-7 decreases or eliminates precocene activity as compared to the disubstituted molecules.

5. Disubstitution at C-7 and C-8. The tested molecules (**47, 48**) showed no precocene activity, even if the related C-7-monosubstituted compounds were highly active (cf. **47, 48** and **1** and **2**). As C-8-monosubstitution with MeO did not yield any activity in previous studies[9,12,13], the ineffectiveness of the C-7, C-8 disubstituted derivatives is probably due to the C-8 substitution itself.

Toxic effects. As can be seen in the table, the LD50 values did not follow the rules established for precocene activity, hence no clearcut conclusion on the relationship between toxicity and chemical structure can be reached. If we assume that general toxicity is also based on the oxidative activation of these compounds, it is very likely that the target of the toxic effect is much broader than the CA, and includes other peripheral tissues and organs[3].

Conclusions. According to previous studies[9-11] the C-7 substitutent has a basic role in precocene activity. In our experiments, only the alkoxy substituents showed activity at C-7. The maximum effect was observed with EtO and nPrO while longer or shorter as well as branching and/or unsaturated alkoxy-substituents decreased the activity.

In the case of C-6, C-7 disubstitution, the C-6 alkoxy-group can seriously modify the effect of the C-7 substituent. This was also observed in precocene experiments carried out on nematodes[20-22]. Notable precocene activity can be observed only if both substituents are alkoxy-groups and the C-7 side-chain is longer than that at C-6. In the reverse case, the precocene effect is invariably lost. In absolute terms, the C-6 alkoxy-group should not be longer than EtO.

In the case of C-5, C-7 disubstitution, C-7 substituents remain active or even gain an enhanced activity if C-5 is occupied by Me. When C-5 bears MeO, the precocene effect is invariably lost.

In the tested C-7, C-8 disubstituted compounds, the C-8 substituent seems to eliminate the precocene activity derived from the C-7 substituted derivative.

Compounds bearing more than two substituents or $-NO_2$ and Cl as substituents, have no appreciable activity.

The LD50 values do not follow the rules established for the precocene effect, hence the toxic effect has to have another target/mechanism.

Acknowledgment. The authors would like to express their appreciation to the Alkaloida Chemical Company for its support of the project.
We should like to thank to Dr K. Koczka, Mrs E. Kulcsar-Gavranovics, Mrs A. Rehak-Csendes and Mrs A. Budavary for their valuable technical assistance.

1 Bowers, W. S., Ohta, T., Cleere, J. S., and Marsella, P. A., Science *193* (1976) 542.
2 Bowers, W. S., in: Endocrinology of Insects, pp. 517–523. Alan R. Liss, Inc., New York 1983.
3 Pratt, G. E., in: Natural Products for Innovative Pest Management, vol. 2, pp. 323–355. Ed. T. R. Odhiambo. Pergamon Press, Oxford 1983.
4 Bowers, W. S., in: Comprehensive Insect Physiology, Biochemistry and Pharmacology, vol. 1, pp. 551–564. Eds G. A. Kerkut and L. I. Gilbert. Pergamon Press, Oxford 1985.
5 Unnithan, G. C., Nair, K. K., and Bowers, W. S., J. Insect Physiol. *23* (1977) 1081.
6 Pratt, G. E., Jennings, R. C., Hamnett, A. F., and Brooks, G. T., Nature *284* (1980) 320.
7 Dinya, Z., Timar, T., Hosztafi, S., Fodor, A., Deak, P., Somogyi, A., and Berenyi, M., QSAR Strategies Des. Bioact. Comp. Proc. Eur. Symp. QSAR, 5th (J. K. Seydel VCH). 403–409 (1985) CA: 104, 16510t (1986).
8 Bowers, W. S., in: Insecticide Mode of Action, pp. 403–427. Academic Press, New York 1982.
9 Bowers, W. S., Pontif. Acad. Scient. Scripta Varia *41* (1976) 129.
10 Brooks, G. T., Ottridge, A. P., Jennings, R. C., Mace, D. V., and Alexander, B. A., Pestic. Sci. *16* (1985) 571.
11 Chenevert, R., Perron, J. M., Paquin, R., Robitaille, M., and Wang, Y. K., Experientia *36* (1980) 379.
12 Ohta, T., Kagaku to Seibutsu *17* (2) (1979) 92 CA: *91* (1979) 1318a.
13 Ohta, T., Konchu no Seiri to Kagaku (1979) 63–74 CA: *92* (1980) 107632j.
14 Camps, F., ACS Symp. Ser. No. 276, Bioregulators for Pest Control. Ed. P. A. Hedin. *16* (1985) 238.
15 Timar, T., Hosztafi, S., Jaszberenyi, J. Cs., Kover, K. E., and Batta, Gy., Acta chim. hung. *125* (1988) 303.
16 Bowers, W. S., Ohta, T., Chem. Pharm. Bull. *25* (1977) 2788.
17 Timar, T., and Jaszberenyi, J. Cs., J. heterocycl. Chem. *25* (1988).
18 Bergot, B. J., Judy, K. J., Schooley, D. A., and Tsai, L. W., Pestic. Biochem. Physiol. *13* (1980) 95.
19 McCaffery, A. R., and McDowell, P. G., Pestic Sci. *19* (1987) 185.
20 Fodor, A., Deak, P., and Kiss, I., Gen. comp. Endocr. *46* (1982) 99.
21 Fodor, A., and Timar, T., Gen. comp. Endocr. (1988) submitted.
22 Fodor, A., Timar, T., Kiss, I., Hosztafi, S., Sebok, P., Boromissza-Varga, E., and Soos, J., Gen. comp. Endocr. (1988) submitted.

0014-4754/88/090790-03$1.50 + 0.20/0

Volatiles from clover head *Hypera meles* (Fab.) and alfalfa *H. postica* (Gyllenhal) weevils: search for pheromones

P. A. Hedin, M. M. Ellsbury and R. J. Dysart[a]

Crop Science Research Laboratories, USDA-ARS, Mississippi State (Mississippi 39762, USA) and [a] Northern Plains Soil and Water Research Center, USDA-ARS, Sidney (Montana 59270, USA)
Received 18 April 1988, accepted 9 June 1988

Summary. Volatile fractions of the clover head, *Hypera meles* (Fab.), and alfalfa, *Hypera postica* (Gyllenhal), weevils contained three of four boll weevil, *Anthonomus grandis* (Boh.), pheromone components, (Z)-3,3-dimethylcyclohexane $\Delta^{1,\beta}$-ethanol and (Z)- and (E)-3,3-dimethylcyclohexane-$\Delta^{1,\alpha}$-acetaldehyde. Also found were eight oxygenated monoterpenes, previously identified as precursors and intermediates of the boll weevil pheromones.
Key words. Clover head weevil; alfalfa weevil; *Hypera meles* (Fab.); *Hypera postica* (Gyll.); candidate pheromones, Curculionidae.