Effects of Pharmacological Agents on Ecdysteroid Synthesis *in vitro* in Ovaries and Abdominal Integument from Female Adult Crickets, *Gryllus bimaculatus* de Geer (Ensifera, Gryllidae)

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In female adults of *Gryllus bimaculatus*, ovaries as well as the abdominal integument produce free and conjugated ecdysteroids *in vitro* (Hoffmann *et al.*, 1992). The aim of the current study was to determine the influence of various potential ecdysteroid biosynthesis effectors (RH 5849, KK 42, diflubenzuron, ketoconazole, azadirachtin, acetylenic and allenic cholesteryl derivatives B1, B6, AL2), and also of the protein synthesis inhibitor cycloheximide, on net release of moulting hormones *in vitro* by the adult ecdysteroid sources. All the compounds examined can be divided into four groups due to their different effectiveness on both the ecdysiosynthetic tissues. The non-steroidal ecdysteroid agonist RH 5849 (group 1) enhanced ecdysteroid synthesis in ovaries, but inhibited hormone production in the abdominal integument. Treatment *in vitro* with diflubenzuron (dimilin) and cycloheximide (group 2) strongly reduced ovarian ecdysteroid synthesis whereas they were hardly effective on integumental hormone production. The group 3 compounds (ketoconazole, B1, B6, AL2, azadirachtin A) demonstrated a stronger inhibitory effect on the abdominal integument than on the ovary. The imidazole compound KK 42 (group 4) was a very potent effector of ecdysteroid biosynthesis in both hormone sources.

Introduction

Ecdysteroids and juvenile hormones (JH) are the principal hormones regulating moulting, metamorphosis, diapause and reproduction in insects. These biological events can be artificially controlled by the application of hormone analogs and antihormone agents (Sehnal, 1983; Bowers, 1985). Hormone-mimetics and antihormones which disturb development, metamorphosis and reproduction are commonly known as insect growth regulators (IGR). The effectiveness of IGR action depends largely on the developmental stage of the insect and on its physiological state. Several juvenoids and antijuvenile compounds have been identified which may be of practical importance in insect control (review in Cymborowski, 1992). Less attempts have been made to find ecdysoids (ecdysteroid analogs) and antiecdysteroids. Wing (1988) has found that certain substituted hydrazines (e.g., RH 5849) can act as non-steroidal ecdysteroid agonists. The most efficient natural substance with moult-inhibiting activity is azadirachtin, a tetranortriterpenoid plant (neem tree) limonoid with ecdysteroid-like structure (review in Mordue (Luntz) and Blackwell, 1993). Synthetic compounds interfering specifically with the synthesis of ecdysteroids were also found. The imidazole compound KK 42 acts on prothoracic glands directly (Kadono-Okuda *et al.*, 1987). KK 42 also inhibits the ecdysteroid biosynthetic pathway in ovarian follicle cells of *Locusta migratoria* (Jarvis *et al.*, 1994). Acetylenic (B1, B6) and allenic (AL2) cholesterol derivatives inhibit ecdysone biosynthesis in larval prothoracic glands dose-dependent and irreversible (Roussel, 1994). Diflubenzuron (DFB), a benzoyl phenyl urea derivative is an IGR which is known to inhibit chitin synthesis during the deposition of cuticle (review in Retnakaran *et al.*, 1985), but also affects ovarian DNA synthesis (Soltani-Mazouni and Soltani, 1994). Ketoconazole (KTZ), a synthetic imidazole derivative, inhibits human gonadal and adrenal steroidogenesis at the level of cytochrome P-450-dependent enzymes (Cedeno *et al.*, 1990). Besides that, KTZ is a potent broad-spectrum antimycotic agent and acts

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very effective both *in vitro* and *in vivo* against parasitic protozoa (Lazardi *et al.*, 1990). Cytochrome P-450 monooxygenase enzymes are of critical importance in the biosynthesis of ergosterol in yeasts and fungi, sterol and steroid biosynthesis in fish and mammals, and also in JH and ecdysteroid production in insects. Very recently, Jarvis *et al.* (1994) demonstrated that in *Locusta migratoria* follicle cell incubations 50 μM KTZ completely inhibit the conversion of ^{3}[H]2,22,25-trideoxyecdysone into any other compounds.

In the majority of insects, the prothoracic glands are the major source of ecdysteroid during larval development. The involvement of alternative sites of ecdysteroid production seems to be limited to the imago or to pupal stages (Redfern, 1989; Delbecque *et al.*, 1990). In *Gryllus bimaculatus*, we have recently shown that the ovary and the abdominal integument together with the adjacent segmental fat body represent alternative sites of ecdysteroid production in adult females (Hoffmann *et al.*, 1992), but also in last instar larvae (Gerstenlauer and Hoffmann, 1994). In adult females, the rates of ecdysteroid synthesis and release, as measured *in vitro*, change during vitellogenesis in a characteristic way (Weidner *et al.*, 1992), and both ecdysteroid sources represent primary sources of ecdysteroids. Only a primary source is able to synthesize ecdysteroids (or even an inactive prohormone) from a sterol, e.g. cholesterol or 7-dehydrocholesterol (Delbecque *et al.*, 1990). The very early steps in ecdysone biosynthesis, supposed to lead to 5β-ketodiol (2,22,25-trideoxyecdysone), are not yet well understood. The final steps of ecdysone biosynthesis consist of a sequence of hydroxylations at C-25, C-22 and C-2. The enzymes catalyzing the final hydroxylation steps are suggested to be cytochrome P-450-dependent hydroxylases.

A possible way to evidence a putative ecdysteroid source is to inhibit it specifically, *in vivo* and *in vitro*. In the present paper, we have therefore studied the influence of various potential ecdysteroid biosynthesis effectors (RH 5849, KK 42, DFB, KTZ, azadirachtin A, B1, B6, AL2), and also of the protein synthesis inhibitor cycloheximide, on moulting hormone synthesis *in vitro* from ovaries and abdominal integument of adult females of *G. bimaculatus*.

Materials and Methods

Insects

The crickets, *Gryllus bimaculatus* de Geer, were reared at a constant temperature of 27°C in a 16:8 hr light/dark photoperiod as described earlier (Hoffmann *et al.*, 1992). Under these conditions, the synthesis and release of ecdysteroids by ovaries and abdominal integument *in vitro* reach peak values at days 4/5 after imaginal moult (Weidner *et al.*, 1992).

Inhibitors

Cycloheximide (3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxylethyl]glutarimid) was purchased from Sigma (Deisenhofen, Germany) and diflubenzuron (dimilin; 1-(4-chlorophenyl)-3-(2,6-difluorbenzoyl)urea) was obtained from Laboratory Dr. Ehrenstorfer (Augsburg, Germany). Ketoconazole (*cis*-1-acetyl-4-[4[[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]-methoxy]phenyl]piperazine) was provided by Dr. K. Schellekans, Janssen Research Foundation (Beerse, Belgium). Azadirachtin A was a generous gift of Prof. H. Rembold (Munich, Germany). KK 42 (1-benzyl-5-[(*E*)-2,6-dimethyl-1,5-heptadienyl]imidazole) was kindly supplied by Prof. O. Yamashita (Nagoya, Japan). RH 5849 (1,2-dibenzoyl-1-*tert*-butylhydrazin) was a gift from Rohm & Haas (Philadelphia, USA), and the acetylenic and allenic cholesterol derivatives (B1, B6, AL2; Mauvais *et al.*, 1994; Roussel, 1994) were kindly provided by Dr. Roussel (Strasbourg, France).

Tissue preparation and in vitro incubation

Dissections and *in vitro* incubations were performed as previously described (Hoffmann *et al.*, 1992). Briefly, this consisted of dissection of ovary and abdominal integument from day 4 females if not otherwise stated. For each incubation, one of each pair of ovaries and one half of the abdominal integument, respectively, were washed in sterile Grace's medium and thereafter placed in one well of a 24-well tissue culture plate (Costar, Cambridge, UK) that contained 1 ml sterile Grace's medium (control) or 1 ml Grace's medium with the agent. The contralateral organ/tissue served as 0-time control allowing for the determination of

ecdysteroid content prior to incubation. During the 16 hr incubation period at 27°C, samples were continuously gased with O_2. Agents were dissolved in very small volumes of organic solvents (KK 42 in acetone; KTZ and DFB in chloroform; azadirachtin in 90% ethanol; RH 5849, B1, B6, and AL2 in DMSO; cycloheximide in 100% methanol) and diluted with Grace's medium yielding a stock solution of 10^{-3} M and 10^{-4} M, respectively. The final concentrations of agents in the incubations were as indicated in Results.

Extraction and analysis

After incubation, tissues were lyophilized, homogenized in 800 µl 100% methanol and centrifuged (8000×*g*, 2 min). The pellets were reextracted twice with 100% methanol and 70% (v/v) methanol in water, respectively, and all supernatants were combined, resulting in an 88% methanol solution. The methanolic extracts were partitioned against an equal volume of *n*-hexane before being concentrated to a small volume (ca. 200 µl) and filled up to 4 ml with distilled water (final methanol concentration <5%). Incubation media were extracted with 5 ml 100% methanol and partitioned against the same volume of *n*-hexane.

Fractionation of ecdysteroids was carried out with the Sep-Pak C18 purification procedure as previously described (Bulenda *et al.*, 1986; Espig *et al.*, 1989). Polar and apolar ecdysteroid conjugates were completely hydrolyzed with porcine liver esterase for 16 hr at 37°C and again subjected to C18 Sep-Pak fractionation (Thiry and Hoffmann, 1992).

A radioimmunoassay (RIA) was used for quantification of the ecdysteroids (Hoffmann *et al.*, 1981). Values for cross-reactions of the antiserum were ecdysone = 1.0; 20-hydroxyecdysone, makisterone and inokosterone = 0.9; 3-dehydroecdysone ≤ 0.1; cholesterol = 0.

To calculate the net amount of RIA-positive ecdysteroids released, the amount of ecdysteroids present in the 0-time control tissues was subtracted from post-incubation values (tissue plus incubation medium). 0-Time controls from day 4 females contained 11–36 ng ecdysteroids per single ovary and 3.5–6.0 ng ecdysteroids per $^1/_2$ integument, respectively (Hoffmann *et al.*, 1992; Hoffmann *et al.*, 1994). Ecdysteroid contents in day 2 ovaries and abdominal integument (0-time controls) were 4.1 ng and 0.5 ng, respectively. The inhibitory/activatory activity of the pharmacological agents is indicated by comparisons of the values for the control and the experimental tissues giving the level of inibition/activation in per cent. The U-test of Mann-Whitney was used in statistical treatment of the data.

Results

In the course of the experiments between Dezember 1991 and September 1993 the rate of ecdysteroid biosynthesis *in vitro* of the abdominal integument from 4-days-old adult females varied considerably, even when the experimental animals had been reared under constant laboratory conditions (383 to 3616 pg/hr·animal)(Hoffmann *et al.*, 1994). In general, the synthesis of ecdysteroids was high during spring and summer and low in autumn and winter. High rates of ecdysteroid synthesis co-

Table I. Effects of the ecdysteroid agonist RH 5849 on ecdysteroid synthesis by ovaries and abdominal integument from day 2 and day 4 adult females of *G. bimaculatus*. Values are expressed in per cent of control and represent the mean of 5–6 determinations ± SEM.

	Control	10^{-8} M	10^{-6} M	10^{-4} M RH5849
Ovary d 2	100.0 (130.7 ± 133.2 pg/hr·animal)	n.d.	467.7 ± 119.3	320.0 ± 102.8
Integument d 2	100.0 (253.6 ± 55.4 pg/hr·animal)	136.2 ± 35.2	62.6 ± 11.9	68.4 ± 8.8
Ovary d 4	100.0 (3345.5 ± 281.4 pg/hr·animal)	146.0 ± 30.6	139.2 ± 22.6	113.2 ± 17.8
Integument d 4	100.0 (3616.6 ± 1556.6 pg/hr·animal)	n.d.	4.1 ± 3.0	14.2 ± 4.0

n.d., not determined.

incided with high concentrations of hormones in the integument and vice versa. Rates of ecdysteroid synthesis by the ovary of day 4 females also varied in the course of the year (2072 to 6957 pg/hr·animal). The abdominal integument mainly released free ecdysteroids (Sep-Pak 60% methanol fraction) whereas the ovaries produced free and apolar conjugated hormones in a similar range.

Ecdysteroid agonist RH 5849 (Table I)

Effects of the non-steroidal ecdysteroid agonist RH 5849 on ecdysteroid synthesis by ovaries and abdominal integument of adult female crickets are shown in Table I. Addition of RH 5849 to the incubation medium enhanced ecdysteroid synthesis in ovaries, but inhibited hormone synthesis in the abdominal integument. The increase in ecdysteroid synthesis in the presence of RH 5849 was high in ovaries with a low spontaneous rate of hormone production (day 2 females; $p < 0.001$ at 10^{-6} M) and moderate in already active organs (day 4 females; $p < 0.05$ at 10^{-6} M).

Imidazole derivative KK 42 (Table II)

The 1,5-disubstituted imidazole with a JH-like terpene chain suppressed the secretion of ecdysteroids from ovaries and abdominal integument cultured *in vitro* in a dose-dependent manner. The half inhibition concentration was estimated to be approximately 5×10^{-10} M, whereas a high concentration of KK 42 (10^{-6} M) was less effective.

Imidazole fungicide ketoconazole (KTZ) (Table III)

The cytochrome P-450 inhibitor ketoconazole is a very potent effector on ecdysteroid biosynthesis *in vitro* by the abdominal integument from day 4 crickets. At increasing concentrations of KTZ in the incubation medium, the hormone production decreased in a dose-dependent manner until at 10^{-6} M. A 50 per cent inhibition was observed at about 8×10^{-7} M KTZ.

Benzoyl phenyl urea derivative diflubenzuron (DFB) (Table IV)

Treatment *in vitro* with the IGR diflubenzuron (dimilin) resulted in a significant reduction ($p < 0.001$ at 10^{-9} to 10^{-5} M) in ovarian ecdysteroid synthesis whereas it was less effective on integumental hormone production. In ovaries, 50 per cent inhibition of ecdysteroid synthesis was reached at about 5×10^{-10} M DFB.

Table II. Effects of the imidazole derivative KK 42 on ecdysteroid synthesis by ovaries and abdominal integument from day 4 adult females of *G. bimaculatus*. Values are expressed in per cent of control and represent the mean of 6–12 determinations ± SEM.

	Control	10^{-10} M	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M KK 42
Ovary d 4	100.0 (2249.0 ± 772.0 pg/hr·animal)	68.3 ± 15.6	n.d.	27.5 ± 6.0	n.d.	30.6 ± 16.1
Integument d 4	100.0 (1360.4 ± 69.2 pg/hr·animal)	n.d.	38.3 ± 1.9	37.5 ± 1.6	33.4 ± 2.9	60.3 ± 19.1

n.d., not determined.

Table III. Effects of the imidazole fungicide ketoconazole on ecdysteroid synthesis by ovaries and abdominal integument from day 4 adult females of *G. bimaculatus*. Values are expressed in per cent of control and represent the mean of 6 (ovary) to 12 (integument) determinations ± SEM.

	Control	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-4} M KTZ
Ovary d 4	100.0 (3372.2 ± 457.3 pg/hr·animal)	n.d.	88.6 ± 23.5	n.d.	71.1 ± 14.2	64.5 ± 15.7
Integument d 4	100.0 (1384.8 ± 378.3 pg/hr·animal)	85.3 ± 7.4	66.5 ± 7.5	60.2 ± 13.0	16.4 ± 9.3	45.2 ± 7.1

n.d., not determined.

Table IV. Effects of the insect growth regulator diflubenzuron (dimilin) on ecdysteroid synthesis by ovaries and abdominal integument from day 4 adult females of *G. bimaculatus*. Values are expressed in per cent of control and represent the mean of 6 determinations ± SEM.

	Control	10^{-9} M	10^{-7} M	10^{-5} M DFB
Ovary d 4	100.0 (6957.1 ± 2423.5 pg/hr·animal)	30.2 ± 16.1	14.0 ± 7.3	25.4 ± 6.9
Integument d 4	100.0 (850.8 ± 144.8 pg/hr·animal)	71.6 ± 18.5	64.5 ± 9.5	56.9 ± 23.9

Acetylenic and allenic cholesteryl derivatives (B1, B6, AL2) (Table V)

B1, B6, and AL2 have been synthesized as selective suicide substrate type inhibitors of ecdysone biosynthesis. They are formed of a steroid nucleus on which a short side chain, with an acetylenic or an allenic function closely linked to an hydroxyl group, has been grafted (Roussel, 1994). All the molecules showed a clear inhibitory activity on ecdysteroid biosynthesis *in vitro* by the abdominal integument, whereas they were less effective on moulting hormone synthesis by day 4 ovaries. At least in the abdominal integument, B6 showed an higher inhibitory efficiency (80% inhibition versus control at 10^{-6} M; $p < 0.001$) than the other two compounds.

Table Va. Effects of acetylenic and allenic cholesteryl derivatives B1, B6, and AL2 on ecdysteroid synthesis by ovaries and abdominal integument from day 4 adult females of *G. bimaculatus*. Values are expressed in per cent of control and represent the mean of 6 determinations ± SEM.

	Control	10^{-6} M	10^{-4} M inhibitor
Ovary d 4			
B1	100.0	69.5 ± 3.9	n.d.
B6	100.0	72.3 ± 6.5	n.d.
AL2	100.0 (4322.3 ± 828.1 pg/hr·animal)	61.2 ± 12.6	n.d.
Integument d 4			
B1	100.0	48.8 ± 7.2	43.2 ± 9.0
B6	100.0	22.2 ± 3.3	23.1 ± 6.9
AL2	100.0 (636.1 ± 42.2 pg/hr·animal)	34.3 ± 16.2	27.6 ± 6.0

n.d., not determined.

Table Vb. Effects of B1, B6, and AL2 on ecdysteroid synthesis *(in vitro)* by prothoracic glands (PG) from day 5 last instar larvae of *Locusta migratoria*. Values are expressed in per cent of control. Data from Roussel (1992, 1994).

	Control	10^{-6} M	10^{-4} M inhibitor
PG d 5			
B1	100	58	32
B6	100	60	30
AL2	100	68/69	25/28

Neem tree component azadirachtin A (Table VI)

Azadirachtin, a tetranorterpenoid with an ecdysteroid-like structure, inhibited ecdysteroid synthesis *in vitro* in both sites of ecdysteroid synthesis. The inhibitory effect was more pronounced in the abdominal integument (50 % inhibition at about 10^{-10} M; 75 % inhibition at 10^{-7} M) than in the ovary.

Protein synthesis inhibitor cycloheximide (Table VII)

Cycloheximide is a general protein synthesis inhibitor which also depressed ecdysone secretion *in vitro* of epidermal explants from *Tenebrio molitor* pupae at a concentration of 5×10^{-5} M (J. P. Delbecque, personal communication). At concentrations of 10^{-6} to 10^{-4} M, cycloheximide did not affect ecdysteroid biosynthesis *in vitro* in the abdominal integument from day 4 female adult *G. bimaculatus*, but it significantly inhibited moulting hormone synthesis and release from the ovaries of these crickets ($p < 0.05$ at 10^{-6} M; $p < 0.001$ at 10^{-4} M). Fifty per cent inhibition were observed at about 10 μM cycloheximide in the incubation medium.

Table VI. Effects of azadirachtin A on ecdysteroid biosynthesis by ovaries and abdominal integument from day 4 adult females of *G. bimaculatus*. Values are expressed in per cent of control and represent the mean of 4–6 determinations ± SEM.

	Control	10^{-11} M	10^{-9} M	10^{-7} M	10^{-5} M azadirachtin
Ovary d 4	100.0 (2950.5 ± 542.1 pg/hr·animal)	98.9 ± 12.8	44.5 ± 9.1	61.6 ± 3.5	44.6 ± 10.1
Integument d 4	100.0 (382.5 ± 167.4 pg/hr·animal)	100.7 ± 26.3	36.6 ± 17.4	24.5 ± 10.7	n.d.

n.d., not determined.

Table VII. Effects of the protein synthesis inhibitor cycloheximide on ecdysteroid synthesis by ovaries and abdominal integument from day 4 adult females of *G. bimaculatus*. Values are expressed in per cent of control and represent the mean of 3 determinations ± SEM.

	Control	10^{-6} M	10^{-4} M cycloheximide
Ovary d 4	100.0 (2072.2 ± 442.0 pg/hr·animal)	68.5 ± 23.3	31.6 ± 6.3
Integument d 4	100.0 (741.0 ± 59.5 pg/hr·animal)	85.1 ± 9.8	84.0 ± 7.5

Discussion

In female adult crickets, the ovary and the abdominal integument together with the adjacent segmental fat body synthesize and release ecdysteroids (Hoffmann *et al.*, 1992). Both ecdysteroid sources produce and release mainly ecdysone. Control tissues released more ecdysteroids during the 16 hr incubation *in vitro* than were present in the tissues prior to or after incubation, therefore eliminating just a release of ecdysteroids from storage. The present results on the action of more selective inhibitors of ecdysone synthesis (acetylenic and allenic cholesteryl derivatives, RH 5849, ketokonazole) evidence the ovaries and the abdominal integument as putative ecdysteroid sources in female adult crickets.

There have been a number of reports on the inhibition of ecdysteroid biosynthesis in prothoracic glands from insect larvae by pharmacological agents (Kadono-Okuda *et al.*, 1987; Roussel *et al.*, 1987; Roussel, 1992; Grieneisen *et al.*, 1993). Less information is available about substances being active on alternative ecdysteroid sources in adult insects. Feder *et al.* (1988) studied azadirachtin action on *in vivo* and *in vitro* production of ovarian ecdysteroids in adult females of *Rhodnius prolixus*. From the *in vitro* analysis it was suggested that azadirachtin may directly interfere in ovarian ecdysteroid production. However, *in vivo* experiments with mature females of *Locusta migratoria* let assume that azadirachtin may interfere with the neuroendocrine control of hormone synthesis. Although, its biochemical effects at the cellular level are still unknown, the inhibitory action of azadirachtin on ecdysteroid synthesis *in vitro* by ovaries and abdominal integument from female adult *G. bimaculatus* let suggest a direct effect. Soltani *et al.* (1989) demonstrated that diflubenzuron has no inhibitory effect on ecdysteroid synthesis by prothoracic glands of *Tenebrio molitor*, whereas another site of moulting hormone secretion which replaces prothoracic glands at the end of larval development (pupal epidermis), is sensitive to DFB. More selective inhibitors of ecdysone biosynthesis, acetylenic and allenic cholesteryl derivatives (Burger *et al.*, 1988; 1989) are active *in vitro* on conventional sources, but also on the alternative one(s) in *T. molitor* (Delbecque, 1990). Very recently, Jarvis *et al.* (1994) demonstrated that a series of cytochrome P-450 inhibitors (KK 42, ICI-L-635, ketoconazole) strongly inhibit the ecdysteroid biosynthetic pathway in ovarian follicle cells of *L. migratoria* by inhibition of the C-22 hydroxylase enzyme.

For the present study, the pharmacological agents can be divided into four groups. One substance, the ecdysteroid agonist RH 5849 (group 1) enhanced the synthesis of ecdysteroids by mature ovaries of *G. bimaculatus*, as measured *in vitro*. RH 5849 is a metabolically stable, non-steroidal ecdysteroid agonist which binds to ecdysteroid receptors with moderate affinity and elicits all known biological effects of ecdysteroids (Koolman, 1990). In *Manduca sexta*, RH 5849 failed to

affect ecdysteroid secretion by intact prothoracic glands, but strongly inhibited fat body ecdysone 20-monooxygenase activity (Grieneisen *et al.*, 1993). This supports the concept that RH 5849 acts specifically as a competitive substrate of ecdysone or its product 20-hydroxyecdysone, at least in Lepidoptera, but is not a general inhibitor of P-450 enzymes. The stimulatory activity of RH 5849 in cricket ovaries may indicate a positive feed-back regulation of hormone synthesis by the main secretion product ecdysone (Hoffmann *et al.*, 1992) or its mimic. In contrast to the ovaries, RH 5849 coincubation strongly depressed ecdysteroid synthesis and release by the abdominal integument of mature female crickets.

The group 2 compounds (diflubenzuron, cycloheximide) demonstrated strong inhibitory effects *in vitro* in ovaries, but only a weak depression of ecdysteroid synthesis in the abdominal integument of adult female crickets. DFB is an insect growth regulator which is known to inhibit chitin synthesis during the deposition of cuticle. DFB also inhibits epidermal DNA synthesis (Soltani *et al.*, 1989) and, thereby, interferes with the moulting process (Wright and Retnakaran, 1987). Treatment *in vivo* of adults is generally not lethal but disturbes reproduction. The decrease in fecundity results from inhibition of DNA synthesis in the ovaries by DFB (Soltani-Mazouni and Soltani, 1994). Since ovarian follicle cell mitoses are a necessary condition for the secretion of ecdysteroids by the cricket ovary, the DFB inhibition of moulting hormone production is probably consecutive to the perturbation of its DNA synthesis. Cycloheximide is a general protein synthesis inhibitor which acts on translation. In *T. molitor*, cycloheximide strongly inhibits ecdysteroid secretion *in vitro* by wings explanted from 2-days-old pupae. A similar effect could be shown by the mitosis inhibitor demecolcine (Delbecque *et al.*, 1990). Cycloheximide inhibited ecdysteroid biosynthesis in the vitellogenic, growing oocyte of *G. bimaculatus*, but did not affect hormone secretion by the fully developed abdominal integument.

All the group 3 compounds (ketoconazole, B1, B6, AL2, azadirachtin A) demonstrated a stronger inhibitory effect on the abdominal integument than on the ovary. Ketoconazole is known to inhibit ecdysone 20-monooxygenase and was also very effective in inhibiting the terminal hydroxylation steps of ecdysteroid biosynthesis in ovarian follicle cells of *L. migratoria* (Jarvis *et al.*, 1994). The acetylenic and allenic cholesteryl derivatives B1, B6, and AL2 were not only active on conventional ecdysteroid sources (Table Vb), but also on the epidermal one in pupae of *T. molitor* (Delbecque, 1990). These suicide substrate type inhibitors are suggested to react in the last steps of ecdysone biosynthesis on C-22 and C-25 hydroxylases which are cytochrome P-450-dependent monooxygenases (Roussel, 1994). In contrast to the *in vitro* action, these synthetic molecules barely induced a delayed metamorphosis or adult morphological modifications *in vivo* (Roussel, 1992). The lack of *in vivo* activity could be due to some difficulty of the molecules entering the cells of the ecdysteroidogenic tissues or to the inactivation of the injected compounds in the haemolymph. Azadirachtin does not affect the synthetic capacity of prothoracic glands directly (review in Mordue (Luntz) and Blackwell, 1993), whereas epidermal cells or oenocytes, as potential sites of ecdysteroid production, may well be the target for azadirachtin action.

The imidazole compound KK 42 was a very potent inhibitor of ecdysteroid biosynthesis in both alternative hormone sources of *G. bimaculatus* (group 4). The results are in contrast to those of Roussel *et al.* (1989) who had found no effect of this compound in ovarian follicle cells *in vitro* from *L. migratoria*, whereas Jarvis *et al.* (1994) have recently shown that KK 42 and its analog ICI-L-635 inhibited ecdysone biosynthesis in ovarian follicle cells of this locust species (see above). KK 42 also depressed biosynthesis of ecdysone by prothoracic glands of *L. migratoria*, *in vitro*, but in a non-specific manner (Roussel *et al.*, 1987). Addition of KK 42 to the incubation medium slows down the synthesis of proteins in the glands in parallel to the depression of ecdysone biosynthesis.

The cellular mode of action of several substances used in this study remains unsolved. Nevertheless, all the compounds may be a valuable tool in studies of ecdysone biosynthesis as well as their physiological action in adult insects. With the renewed interest in ecdysteroid agonists and antagonists as possible novel invertebrate pest control agents, it can be expected that this area of research will experience a rapid increase over the next years (Dinan, 1989).

Bowers W. S. (1985), Antihormones. In: Comprehensive Insect Physiology, Biochemistry and Pharmacology (G. A. Kerkut and L. I. Gilbert, eds.), Vol. **8,** Pergamon Press, Oxford, pp. 551–564.

Bulenda D., Stecher A., Freunek M. and Hoffmann K. H. (1986), Ecdysone metabolism in adult crickets, *Gryllus bimaculatus*. Insect Biochem. **16**, 83–90.

Burger A., Colobert F., Hetru C. and Luu B. (1988), Acetylenic cholesteryl derivatives as irreversible inhibitors of ecdysone biosynthesis. Tetrahedron **44**, 1141–1152.

Burger A., Roussel J.-P., Hetru C., Hoffmann J. A. and Luu B. (1989), Allenic cholesteryl derivatives as inhibitors of ecdysone biosynthesis. Tetrahedron **45**, 155–164.

Cedeno J., Mendoza S. G., Velazquez E., Nucete H., Speirs J. and Glueck C. J. (1990), Effect of ketoconazole on plasma sex hormones, lipids, lipoproteins, and apolipoproteins in hyperandrogenic women. Metabolism **39**, 511–517.

Cymborowski B. (1992), Insect Endocrinology. Elsevier, Amsterdam, pp. 193–205.

Delbecque J.-P. (1990), Ecdysteroid secretion by epidermal cells in *Tenebrio molitor*: autocrine control of pupal and adult moults. Invertebr. Reprod. Develop. **18**, 110–111.

Delbecque J.-P., Weidner K. and Hoffmann K. H. (1990), Alternative sites of ecdysteroid production in insects. Invertebr. Reprod. Develop. **18**, 29–42.

Dinan L. (1989), Ecdysteroid structure and hormonal activity. In: Ecdysone – From Chemistry to Mode of Action (J. Koolman, ed.), Thieme, Stuttgart, pp. 345–354.

Espig W., Thiry E. and Hoffmann K. H. (1989), Ecdysteroids during ovarian development and embryogenesis in the cricket, *Gryllus bimaculatus* (de Geer). Invertebr. Reprod. Develop. **15**, 143–154.

Feder D., Valle D., Rembold H. and Garcia E. S. (1988), Azadirachtin-induced sterilization in mature females of *Rhodnius prolixus*. Z. Naturforsch. **43c**, 908–913.

Gerstenlauer B. and Hoffmann K. H. (1994), Ecdysteroid release and ecdysteroid titer during larval adult development of the Mediterranean field cricket, *Gryllus bimaculatus* (Ensifera, Gryllidae). Eur. J. Entomol. **91** (in press).

Grieneisen M. L., Warren J. T. and Gilbert L. I. (1993), Early steps in ecdysteroid biosynthesis: evidence for the involvement of cytochrome P-450 enzymes. Insect Biochem. Molec. Biol. **23**, 13–23.

Hoffmann K. H., Behrens W. and Ressin W. (1981), Effects of daily temperature cycle on ecdysteroid and cyclic nucleotide titres in adult female crickets, *Gryllus bimaculatus*. Physiol. Entomol. **6**, 375–385.

Hoffmann K. H., Weidner K. and Seidel M. (1992), Sites of ecdysteroid biosynthesis in female adults of *Gryllus bimaculatus* (Ensifera, Gryllidae). J. Comp. Physiol. B **162**, 731–739.

Hoffmann K. H., Neuhäuser T., Gerstenlauer B., Weidner K. and Lenz M. (1994), Thermal control of endocrine events in cricket reproduction (*Gryllus bimaculatus* de Geer). In: Perspectives in Comparative Endocrinology (K. G. Davey, R. E. Peter and S. S. Tobe, eds.), National Research Council of Canada, Ottawa, pp. 561–567.

Jarvis T. D., Earley F. G. P. and Rees H. H. (1994), Inhibition of the ecdysteroid biosynthetic pathway in ovarian follicle cells of *Locusta migratoria*. Pesticide Biochem. Physiol. **48**, 153–162.

Kadano-Okuda K., Kuwano E., Eto M. and Yamashita O. (1987), Inhibitory action of an imidazole compound on ecdysone synthesis in prothoracic glands of the silkworm, *Bombyx mori*. Growth and Diff. **29**, 527–533.

Koolman J. (1990), Ecdysteroids. Zool. Sci. **7**, 563–580.

Lazardi K., Urbina J. A. and de Souza W. (1990), Ultrastructural alterations induced by two ergosterol biosynthesis inhibitors, ketoconazole and terbinafine, on epimastigotes and amastigotes of *Trypanosoma* (*Schizotrypanum*) *cruzi*. Antimicr. Agents and Chemotherapy **34**, 2097–2105.

Mauvais A., Burger A., Roussel J.-P., Hetru C. and Luu B. (1994), Acetylenic inhibitors of C-22 hydroxylase of ecdysone biosynthesis. Bioorg. Chem. **22**, 36–50.

Mordue (Luntz) A. J. and Blackwell A. (1993), Azadirachtin: an update. J. Insect Physiol. **39**, 903–924.

Redfern C. P. F. (1989), Ecdysiosynthetic tissues. In: Ecdysone – From Chemistry to Mode of Action (J. Koolman, ed.), Thieme, Stuttgart, pp. 182–187.

Retnakaran A., Granett J. and Ennis T. (1985), Insect growth regulators. In: Comparative Insect Physiology, Biochemistry and Pharmacology (G. A. Kerkut and L. I. Gilbert, eds.), Vol. **12,** Pergamon Press, Oxford, pp. 529–601.

Roussel J.-P. (1992), Activité, in vivo, d'inhibiteurs de la biosynthèse de l'ecdysone chez *Locusta migratoria*. Entomol. exp. appl. **64**, 97–100.

Roussel J.-P. (1994), Synthetic molecules designed as potential inhibitors in ecdysone biosynthesis. Entomol. Exp. Appl. **71**, 193–199.

Roussel J.-P., Kiuchi M., Akai H. and Kuwano E. (1987), Effets d'un dérivé de l'imidazole (KK-42) sur la biosynthèse de l'ecdysone par les glandes prothoraciques de *Locusta migratoria* (Insecte, Orthoptère), in vitro. C.R. Acad. Sci. Paris **305**, Sér. III, 141–144.

Roussel J.-P., Mojtahed K., Lanot R., Kuwano E. and Akai H. (1989), Effects of an imidazole derivative (KK-42) on development and ecdysteroid production in *Locusta migratoria* (Insecta, Orthoptera). Arch. Int. Physiol. Biochem. **97**, 511–514.

Soltani N., Delachambre J. and Delbecque J.P. (1989), Stage-specific effects of diflubenzuron on ecdysteroid titers during the development of *Tenebrio molitor*: evidence for a change in hormonal source. Gen. comp. Endocrinol. **76**, 350–356.

Soltani-Mazouni N. and Soltani N. (1994), Diflubenzuron affected DNA synthesis in the ovaries of *Tenebrio molitor*. Invertebr. Reprod. Develop. **25**, 19–21.

Thiry E. and Hoffmann, K. H. (1992), Dynamics of ecdysone and 20-hydroxyecdysone metabolism after injection and ingestion in *Gryllus bimaculatus* (de Geer). Zool. Jb. Physiol. **96**, 17–38.

Weidner K., Clasz M., Rieck H. and Hoffmann K. H. (1992), Developmental changes in ecdysteroid biosynthesis *in vitro* during adult life and embryogenesis in a cricket, *Gryllus bimaculatus* de Geer. Invertebr. Reprod. Develop. **21**, 129–140.

Wing K. D. (1988), RH 5849, a nonsteroidal ecdysone agonist: effects on a *Drosophila* cell line. Science **241**, 467–469.

Wright J. E. and Retnakaran A. (1987), Chitin and Benzoylphenyl Ureas. Junke, Dordrecht.