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Ecdysteroid biosynthesis and embryonic development are disturbed in insects (*Locusta migratoria*) reared on plant diet (*Triticum sativum*) with a selectively modified sterol profile

(fenpropimorph/cycloeucal*enol*obtusifoliol isomerase/sterols/ecdysteroid biosynthesis)

MARIE F. COSTET*, MOSTAFA EL ACHOURI†, MAURICE CHARLET†, RENE LANOT†, PIERRE BENVENISTE*, AND JULES A. HOFFMANN†

*Laboratoire de Biochimie Végétale de l'Université Louis Pasteur, Unité Associée au Centre National de la Recherche Scientifique 1182, 28 rue Goethe, 67 000 Strasbourg, France; †Laboratoire de Biologie Générale de l'Université Louis Pasteur, Unité Associée au Centre National de la Recherche Scientifique 672, 12 rue de l'Université, 67 000 Strasbourg, France

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ABSTRACT **Wheat seedlings germinating in the presence of the systemic fungicide fenpropimorph accumulate 9β,19-cyclopropylsterols (95% of total sterols) in place of Δ^5-sterols, which are normally produced in these plants. Adult females of the phytophagous insect *Locusta migratoria* show a dramatic decrease in their cholesterol content when reared on fenpropimorph-treated wheat. These females lay eggs with the ecdysteroid concentration reduced by up to 80% as compared to controls. Injection of fenpropimorph to the insects or feeding them on wheat coated with the fungicide (normal sterol composition) does not affect their sterol or ecdysteroid profiles; addition of cholesterol to fenpropimorph-treated wheat prior to feeding restores normal ecdysteroid titers in the insects. The severe reduction of the ecdysteroid content in eggs laid by females reared on fenpropimorph-treated wheat is associated with a series of developmental arrests and/or abnormalities. The results show that the dietary 9β,19-cyclopropylsterols cannot be used by *Locusta* in place of Δ^5-sterols for ecdysteroid biosynthesis. They suggest that the selective inhibition of specific enzymes in the sterol biosynthetic pathway of the plants can be used as a strategy to control insect development.**

Insects are unable to synthesize *de novo* the steroid nucleus and depend on an exogenous source for their steroid economy. In particular, the biosynthesis of the molting hormone family, the ecdysteroids, which are polyhydroxylated steroids, is dependent on the uptake of sterols from the diet. The phytophagous grasshopper *Locusta migratoria* uses phytosterols such as sitosterol, stigmasterol, and campesterol, which it dealkylates to cholesterol, the most common precursor for ecdysone biosynthesis (Fig. 1) (1, 2).

We have recently shown that long-term treatment of maize seedlings with low concentrations of the systemic fungicides tridemorph or fenpropimorph leads to an almost complete replacement in these plants of the Δ^5-sterols by the 9β,19-cyclopropylsterols [>95% of total sterols (3–5)]. This replacement could be traced back to a selective inhibition by the fungicide of two enzymes in the biosynthetic pathway of plant sterols: cycloeucal*enol*obtusifoliol isomerase and $\Delta^8 \rightarrow \Delta^7$-sterol isomerase (6).

We have investigated the effect of abnormal sterol diet on the sterol economy of *Locusta* and have focused on possible interferences with ecdysteroid titers. This study was prompted by previous reports showing that lanosterol could not meet the sterol requirements of *Dermestes vulpinus* (7) and that some species of *Drosophila* were unable to complete development when raised on yeast mutants lacking $\Delta^8 \rightarrow \Delta^7$-sterol isomerase (8).

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MATERIALS AND METHODS

Chemicals. Fenpropimorph {4-[3-(4-*t*-butylphenyl)-2-methyl]propyl-2,6-dimethylmorpholine} was a gift of H. Pommer (BASF, Limburgerhof, F.R.G.). The cyclopropyl sterols used as standards were extracted from maize seedlings treated with tridemorph (3).

Plant Material and Insects. Wheat (*Triticum sativum*) caryopses were purchased locally. The caryopses were allowed to germinate in vermiculite and the seedlings were watered daily with a solution of fenpropimorph in water (5 mg/liter). The plants were presented to the insects after 14 days. An aliquot fraction was analyzed to determine the sterol profile.

Locusta migratoria migratorioides were reared as described (9).

Isolation of Sterols. An identical procedure was applied to the roots and leaves from wheat seedlings and to the insects and their eggs. This procedure has been detailed in previous articles (3, 4, 10). Each sterol was identified as described (3–5, 10). The mass spectrometry and ^{1}H NMR data are given in previous publications (4, 10).

Extraction and Separation of Ecdysteroids. Eggs and animals were homogenized in ethanol/water (60:40), heated to 60°C for 15 min, and centrifuged at 800 × *g* for 10 min. The supernatant was dried under reduced pressure or under N_2. The dried extracts were dissolved in ethanol/water (60:40).

Ecdysteroids were separated by TLC on precoated Silica Gel 60 F 254 plates (Merck) in a chloroform/methanol (80:20) solvent system. The plates were developed twice and the products were eluted by 5-mm bands with ethanol/water (95:5), except for the three bands close to the deposit, which were eluted with the more polar solvent system ethanol/water (60:40).

Aliquots of the polar fractions were always submitted to enzymatic hydrolysis for 18 hr at 37°C in 50 mM acetate buffer (pH 5.3) containing semipurified β-glucuronidase (5 mg/ml) (including sulfatase and phosphatase activities) from *Helix pomatia* (Sigma G 0751). After hydrolysis, the free ecdysteroids were extracted with ethanol/water (95:5) and separated by TLC in chloroform/ethanol (80:20).

The molecules comigrating with reference 2-deoxyecdysone and ecdysone were eluted with ethanol/water (95:5) and subjected to RIA measurements.

RIA. The dried extracts were suspended in 0.1 M citrate buffer (pH 6.2), and [23,24-^{3}H]ecdysone (9000 cpm; 40 Ci/mmol; 1 Ci = 37 GBq) was added to each sample. This solution was dialyzed against the antibody "black" (cf. ref.

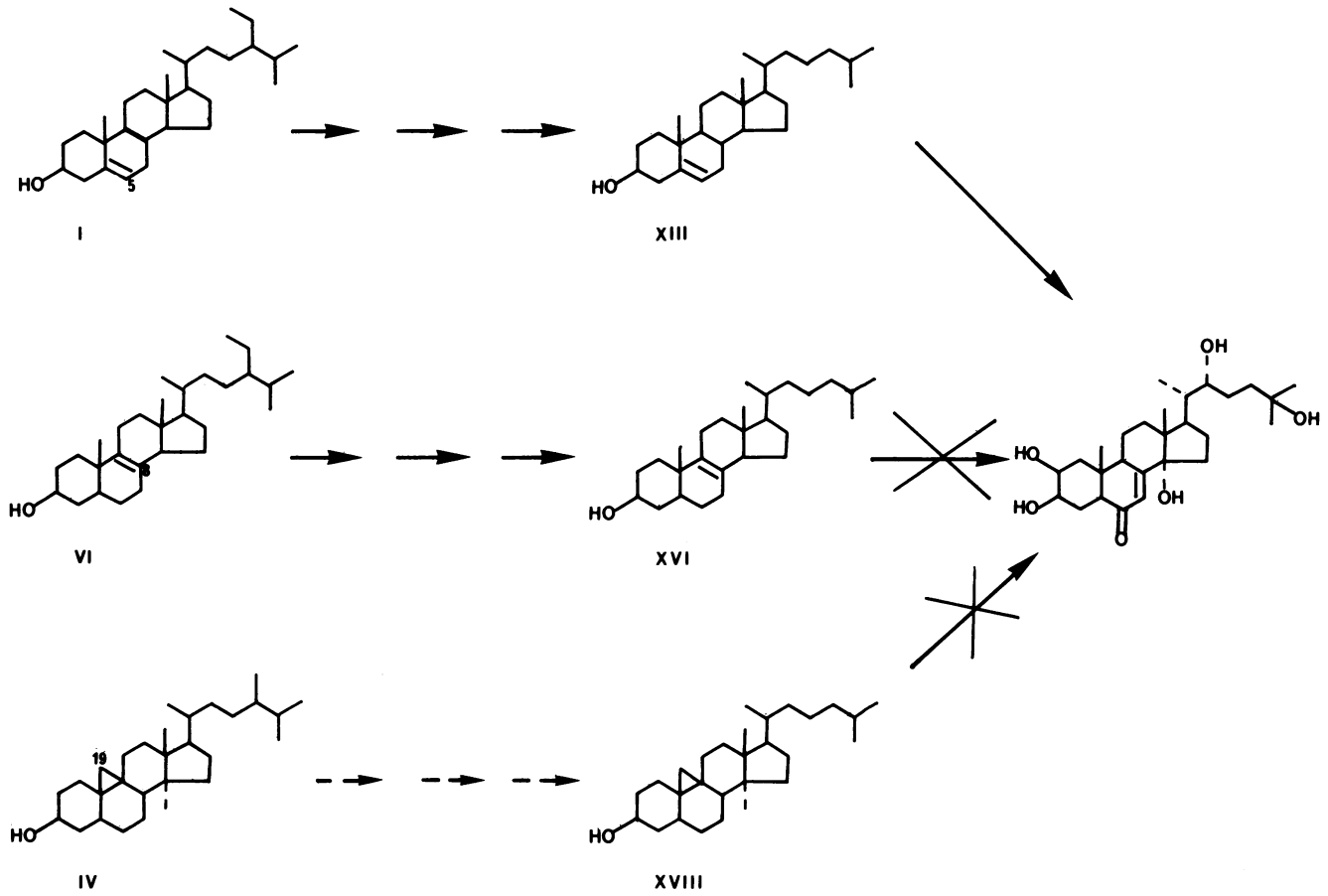

FIG. 1. Metabolism of Δ^5-, Δ^8-, and 9β,19-cyclopropylsterols in *L. migratoria*. The conversion of compound **IV** to compound **XVIII** (dotted arrows) is probably very slow. Compounds: **I**, sitosterol; **XIII**, cholesterol; **VI**, 5α-stigmast-8-en-3β-ol; **XVI**, 5α-cholest-8-en-3β-ol; **IV**, 24-methyl pollinastol; **XVIII**, pollinastanol.

11) diluted 1:4000. The technique was devised by De Reggi *et al.* (12). In our present conditions, the concentration of ecdysone required for 50% inhibition of [23,24-^{3}H]ecdysone binding was 10 nM. Cross-reactions between 2-deoxyecdysone, ecdysone, and 20-hydroxyecdysone were 1:1:0.05.

Quantification of Fenpropimorph. The biological material was extracted with CH_2Cl_2/CH_3OH (2:1). After evaporation of the solvent, the residue was acidified with 0.1 M HCl, washed with hexane, and alkalinized with NaOH to pH 10, after which fenpropimorph was extracted with chloroform. After solvent evaporation, the residue was submitted to TLC (in acetone). Fenpropimorph was eluted from the silica gel and submitted to GLC on the capillary column used for sterol separation. The temperature program included a fast increase from 60°C to 120°C (30°C/min) followed by a slow increase from 120°C to 180°C (2°C/min). Quantification was performed according to a standard curve.

RESULTS

Sterol Composition of Experimental Wheat. Sterol composition of treated wheat was determined by analyzing 1-g (dry weight) samples. In experimental plants (Table 1), the 9β,19-cyclopropylsterols make up 90% of the total sterols and consist essentially of 24-methyl pollinastanol, cycloeucalenol, 24-dihydrocycloeucalenol, and 31-norcyclobranol. The Δ^8-sterols, primarily 5α-stigmast-8-en-3β-ol and 5α-ergost-8-en-3β-ol, represent 9% of the total sterols. No cholesterol was detectable in either control or experimental plants.

Sterol Composition of Insects Reared on Experimental Wheat. Sterol content in treated females was analyzed after their first egg laying (Table 2). The total amount of sterols was similar to that of controls; however, experimental females had a markedly decreased content in Δ^5-sterols (essentially cholesterol) and high titers of Δ^8-sterols and 9β,19-cyclopropylsterols, which are undetectable in controls. Separate determinations in the gut (which contained wheat) and the carcass of the insects showed no major differences in the sterol profiles (namely as regards the ratio of cholesterol to sitosterol), indicating that these profiles were not significantly affected by the sterols present in the gut.

Table 3 compares the sterol composition in the hemolymph of females reared on experimental and normal diets. The major difference is the 90% reduction of the titer of cholesterol in the experimental hemolymph. The percentages of the other sterols in the hemolymph are consistent with the data obtained from total insects.

Sterol and Ecdysteroid Compositions in Eggs of Experimental Females. Rearing females exclusively on experimental wheat after their imaginal molt neither prevents nor delays the successive cycles of egg laying, although the egg pods contain 10–20% fewer eggs than normal. The sterol composition of these eggs (Table 4) differs from that of controls. Δ^5- and Δ^0-sterols decrease from 95.5% of the total in normal eggs to 38% in experimental eggs, while Δ^8-sterols and 9β,19-cyclopropylsterols, which are undetectable in controls, reach 34.5% and 25.5%, respectively. Whereas the Δ^8-sterols appear predominantly as 24-dealkylated forms, the cyclopropylsterols carry a methyl group at C-24.

The experimental diet also induces a marked reduction of the ecdysteroid titer in the eggs (Table 5). Females normally

Table 1. Sterol composition of control (A) and experimental (B) wheat

Compound	Percentage of total sterols A	B	RRT
(24ξ)-24-Methyl-cholest-5-en-3β-ol (III)	24	0.3	1.29
(24ξ)-24-Methyl-5α-cholest-8-en-3β-ol (VII)	—	2.5	1.32
(24*S*)-24-Ethyl-cholesta-5,22-*E*-dien-3β-ol (II)	8	0.1	1.34
(24ξ)-24-Ethyl-5α-cholesta-8,*E*-22-dien-3β-ol	—	1.5	1.35
(24ξ)-24-Methyl-9β,19-cyclo-5α-cholestan-3β-ol (IV)	—	40	1.38
9β,19-Cyclo-5α-ergost-24(28)-en-3β-ol	—	tr	1.38
4α,14α-Dimethyl-5α-ergosta-8,24(28)-dien-3β-ol (IX)	3.5	tr	1.38
(24*R*)-24-Ethyl-cholest-5-en-3β-ol (I)	54	0.5	1.43
β-Amyrin	0.1	tr	1.43
Stigmasta-5,*Z*-24(28)-dien-3β-ol (XIV)	7	—	1.44
4α,14α-Dimethyl-9β,19-cyclo-5α-ergost-24(28)-en-3β-ol (V)	4	26	1.44
4α,14α(24ξ)-Trimethyl-9β,19-cyclo-5α-cholestan-3β-ol (VIII)	—	9	1.45
4,4,14α-Trimethyl-9β,19-cyclo-5α-cholest-24-en-3β-ol (XII)	0.1	3	1.45
(24ξ)-24-Ethyl-5α-cholest-8-en-3β-ol (VI)	—	5	1.45
(24ξ)-24-Ethyl-9β,19-cyclo-5α-cholestan-3β-ol	—	1	1.49
4,4,14α-Trimethyl-9β,19-cyclo-5α-ergost-24(28)-en-3β-ol	0.1	2	1.50
9β,19-Cyclo-5α-stigmasta-*Z*-24(28)-en-3β-ol	—	1	1.51
4α,14α-Dimethyl-9β,19-cyclo-5α-ergost-24(25)-en-3β-ol (X)	—	10	1.51
4α,14α-Dimethyl-9β,19-cyclo-5α-stigmast-*Z*-24(28)-en-3β-ol (XI)	—	3	1.62
Total			
Δ^5-Sterols	93	1	
Δ^8-Sterols	3.5	9	
9β,19-Cyclopropylsterols	3.5	90	

RRT, relative retention time of steryl acetates relative to cholesterol. Compounds: **I**, sitosterol; **II**, stigmasterol; **III**, campesterol; **IV**, 24-methyl pollinastanol; **V**, cycloeucalenol; **VIII**, dihydrocycloeucalenol; **IX**, obtusifoliol; **X**, 31-nor-cyclobranol; **XI**, cyclofontumienol; **XII**, cycloartenol; **XIV**, isofucosterol.

fed up to the first egg laying were reared exclusively on experimental diet for five successive gonotrophic cycles. The ecdysteroid content was measured by RIA (after hydrolysis) in the eggs laid during these cycles and a rapid decline in the values was recorded; in the last cycle, <25% of the normal ecdysteroid content per egg was present (Table 5).

In newly laid eggs of *Locusta*, up to 98% of the ecdysteroids are conjugated. This held true for the experimental eggs (separate measurements of the free and conjugated forms). In the free ecdysteroid fraction of normal eggs, ecdysone exceeds 2-deoxyecdysone by a factor of 2, whereas in the large conjugated fraction, the ratio of 2-deoxyecdysone to ecdysone is 1:2. The same ratios were found in the experimental eggs (data not shown). The effect of the experimental diet on ecdysteroid economy is therefore at the quantitative rather than at the qualitative level.

The hypothesis that the drastic alterations in sterol contents and ecdysteroid titers observed in insects reared on experimental wheat might reflect a toxic effect of fenpropimorph is eliminated by the following results.

Table 2. Sterol composition of normal (A) and experimental (B) female adults of *L. migratoria*

Compound	Percentage of total sterols A	B	RRT
Cholest-5-en-3β-ol (XIII)	70	41.5	1.16
5α-Cholestan-3β-ol (XVII)	12	4	1.17
5α-Cholest-8-en-3β-ol (XVI)	—	21	1.18
Cholesta-5,7-dien-3β-ol	0.5	—	1.20
5α-Cholest-7-en-3β-ol (XV)	3.5	2	1.22
14α-Methyl-9β,19-cyclo-5α-cholestan-3β-ol (XVIII)	—	1.5	1.23
(24ξ)-24-Methyl-cholest-5-en-3β-ol (III)	3.5	2	1.29
(24ξ)-24-Methyl-5α-cholestan-3β-ol	0.5	0.5	1.30
(24ξ)-24-Methyl-5α-cholest-8-en-3β-ol (VII)	—	1.5	1.31
(24ξ)-24-Methyl-5α-cholest-7-en-3β-ol	0.5	—	1.33
(24*S*)-24-Ethyl-cholesta,5,*E*-22-dien-3β-ol (II)	1.5	1	1.34
(24ξ)-24-Ethyl-5α-cholest-*E*-22-en-3β-ol	0.5	tr	1.345
14α-Methyl-9β,19-cyclo-5α-ergostan-3β-ol (IV)	—	18	1.38
(24*R*)-24-Ethyl-cholest-5-en-3β-ol (I)	6	3	1.43
(24ξ)-24-Ethyl-5α-cholestan-3β-ol	0.5	tr	1.435
Stigmasta-5,*Z*-24(28)-dien-3β-ol (XIV)	0.5	tr	1.44
(24ξ)-24-Ethyl-5α-cholest-8-en-3β-ol (VI)	—	2	1.45
14α-Methyl-9β,19-cyclo-5α-stigmastan-3β-ol	—	1.5	1.49
(24ξ)-24-Ethyl-5α-cholest-7-en-3β-ol	0.5	—	1.59
Total			
Δ^5-Sterols	81.5	47.5	
Δ^0-Sterols	13	4.5	
Δ^7-Sterols	4.5	2	
Δ^8-Sterols	—	24.5	
9β,19-Cyclopropylsterols	—	21	
All sterols	2.5*	2.7	

Insects reared on experimental wheat from fledging and sacrificed after the first egg laying. RRT, relative retention times. Compounds: **I**, sitosterol; **II**, stigmasterol; **III**, campesterol; **IV**, 24-methyl-pollinastanol; **XIII**, cholesterol; **XIV**, isofucosterol; **XV**, lathosterol; **XVIII**, pollinastanol; **XVII**, cholestanol.
*Sterols are expressed as mg per g of dry weight.

(*i*) Normally grown wheat was watered with highly con-

Table 3. Sterol composition of the hemolymph of normal (A) and experimental (B) female adults of *L. migratoria*

Compound	Percentage of total sterols A	B
Cholesterol (XIII) + cholestanol (XVII)	81	70
Lathosterol (XV)	1.5	—
Campesterol (III)	4.5	tr
Stigmasterol (II)	1.5	—
Sitosterol (I)	11.5	tr
5α-Cholest-8-en-3β-ol (XVI)	—	18
(24ξ)-24-Methyl-5α-ergost-8-en-3β-ol (VII)	—	tr
24-Methyl pollinastanol (IV)	—	12
Total sterols	350	55
Cholesterol	285	40

Insects reared on experimental wheat from hatching to the adult stage and sacrificed after the first egg-laying. Total sterols and cholesterol are expressed as μg per ml of hemolymph.

Table 4. Sterol composition of control (A) eggs and of eggs laid by females reared on experimental wheat (B)

Compound	Percentage of total sterols A	B
Cholesterol (XIII) + cholestanol (XVII)	84.5	36
Desmosterol (XIX)	0.5	—
Isofucosterol (XIV)	0.5	—
Campesterol (III)	3.5	1
Stigmasterol (II)	1	tr
Sitosterol (I)	5.5	1
Lathosterol (XV)	4	2
(24ξ)-24-Methyl-5α-cholest-7-en-3β-ol	tr	—
(24ξ)-24-Ethyl-5α-cholest-7-en-3β-ol	0.5	—
(24ξ)-24-Methyl-5α-cholest-8-en-3β-ol (VII)	—	4
(24ξ)-24-Ethyl-5α-cholesta-8,E-22-dien-3β-ol	—	3
(24ξ)-24-Ethyl-5α-cholest-8-en-3β-ol (VI)	—	4.5
5α-Cholest-8-en-3β-ol (XVI)	—	24
Pollinastanol (XVIII)	—	1.5
24-Methyl pollinastanol (IV)	—	23
Total		
Δ^5 + Δ^0-Sterols	95.5	38
Δ^7-Sterols	4.5	2
Δ^8-Sterols	—	35.5
9β,19-Cyclopropylsterols	—	24.5

centrated fenpropimorph 24 hr before being presented to the insects. This wheat was actually found to contain a high concentration of fenpropimorph (75 μM) but the contact between the plant and the fungicide had not been long enough to alter significantly the sterol composition. Insects reared daily on this special wheat laid eggs that exhibited a normal sterol composition (data not shown); moreover, their ecdysteroid content was unaffected (Table 5).

(*ii*) We injected into normal insects amounts of fenpropimorph (20 μl of a 40 μM solution in ethanol/water, 25:75) similar to those found in the modified wheat with abnormal

Table 5. Ecdysteroid content in eggs of *L. migratoria*

	RIA measurement
Control eggs (first egg pod)	1.62 ± 0.08
Eggs laid by insects exclusively reared on modified wheat after fledging (first egg pod)	0.51 ± 0.09
Eggs laid by insects reared on modified wheat after first laying:	
First egg pod	1.68 ± 0.09
Second egg pod	0.91 ± 0.09
Third egg pod	0.63 ± 0.05
Fourth egg pod	0.51 ± 0.08
Fifth egg pod	0.46 ± 0.08
Sixth egg pod	0.40 ± 0.03
Eggs laid by insects reared on wheat watered with a fenpropimorph solution 24 hr before being presented to the insects*	1.59 ± 0.07
Eggs laid by insects injected with fenpropimorph†	
During one gonotrophic cycle	1.71 ± 0.10
During two gonotrophic cycles	1.68 ± 0.30

RIA measurement values are expressed in nanomole equivalents of synthetic ecdysone per egg. Each value represents the mean ± SEM of at least four separate measurements.

*This wheat was shown to contain a concentration of fenpropimorph (75 μM) higher than that (30–40 μM) present in the wheat watered daily with the standard fenpropimorph solution.

†Daily injections after the first laying of 20 μl of an ethanol/water (25:75) solution of 40 μM fenpropimorph; eggs of the second or third pod were analyzed.

Table 6. Ecdysteroids in eggs laid by female adults of *L. migratoria* reared on normal (A) wheat and on experimental wheat supplemented with exogenous cholesterol (B)

	RIA measurement A	B
First egg pod; experimental wheat nonsupplemented	1.57 ± 0.07	0.42 ± 0.06
Second egg pod; experimental wheat supplemented with cholesterol after the first egg laying	1.68 ± 0.04	1.38 ± 0.18
Third egg pod; experimental wheat supplemented with cholesterol after the first egg laying	1.70 ± 0.05	1.60 ± 0.18
Fourth egg pod; experimental wheat supplemented with cholesterol after the first egg laying	1.54 ± 0.08	1.57 ± 0.22

RIA measurements are expressed in nanomole equivalents of synthetic ecdysone per egg. Each value represents the mean ± SEM of at least four separate measurements. The experimental wheat was sprayed with a solution of cholesterol (1 mM) in chloroform/ethanol (1:2).

sterol composition. The injections were performed on females after the first egg laying and were repeated daily up to the second laying. Neither the sterol profiles (data not shown) nor the ecdysteroid titers (Table 5) seemed affected in the eggs.

Complementation Experiments of Experimental Wheat with Exogenous Cholesterol. Females were reared on experimental wheat immediately after emergence and were kept on this diet until the first egg laying. The eggs were collected and their ecdysteroid titer was determined. During the following gonotrophic cycle, the insects were reared on experimental wheat that was supplemented by spraying the seedlings with a solution of 1 mM cholesterol in chloroform/ethanol (1:2). The eggs laid during the subsequent cycles were collected and the ecdysteroids were measured by RIA. The results (Table 6) show that addition of cholesterol to the experimental wheat restores the normal ecdysteroid level after two additional gonotrophic cycles (i.e., 7–8 days).

Developmental Changes in Eggs Laid by Females Reared on Experimental Diet. Eggs laid by females reared on experi-

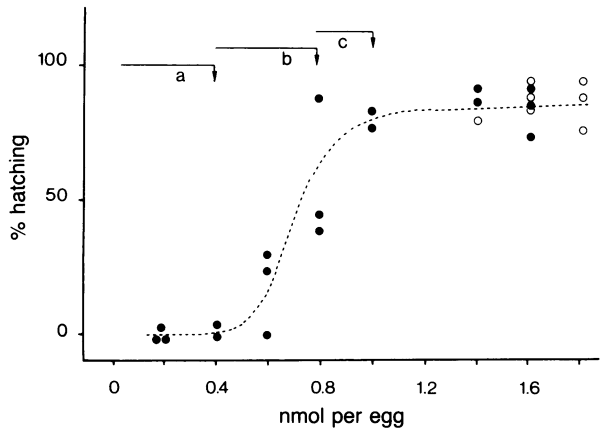

FIG. 2. Fluctuation of the hatching rate of eggs laid by females reared on modified wheat (●) and normal wheat (○) in relation to egg ecdysteroid titers. Ordinate, percentage of hatching. Abscissa, concentration of ecdysteroids in the eggs at oviposition. RIA measurements are expressed in nanomole equivalents of synthetic ecdysone per egg. Arrows a, b, and c are discussed in the text.

mental wheat exhibit ecdysteroid titers significantly lower than those of controls (cf. Table 5). We have compared the rate of depression in the various (≈30) eggs of one egg pod and noted that it was roughly similar for all the eggs. We have followed the destiny of the eggs in each pod after sacrificing three or four newly laid eggs for ecdysteroid measurements. Therefore, the effect of the abnormal titers on development could be analyzed as a function of the level of depression of the ecdysteroid titer. We observed differential effects according to the level of depression of the ecdysteroid titers in the eggs at the time of egg laying. Some of the results are illustrated in Fig. 2.

When the ecdysteroid titer is 70–80% below that of controls in the eggs, no development occurs and apparently meiotic reinitiation has not taken place (Fig. 2, arrow a). When the titers are reduced by 50% and 70% (arrow b), embryonic development is initiated but various abnormalities are observed after 2–3 days (normal duration of embryonic development, 12–13 days), in particular the serosal cuticle is not or is only partly laid down and the normal swelling of the eggs does not occur. When the level of depression is between 40% and 50% (arrow c), many eggs develop but often show complex malformations; some of these eggs hatch normally. In eggs with a depression of 40% or less, embryogenesis is normal and viable larvae hatch.

When cholesterol was added to the experimental wheat and the ecdysteroid titers of the eggs were partly or totally restored to normal values, embryonic development was also restored; some disturbances occurred when restoration was minimal.

DISCUSSION

In the present study, a sequence of events were induced by treatment of wheat seedlings with fenpropimorph. First, the repeated treatment of growing wheat dramatically reduces the relative importance of Δ^5-sterols with the concomitant appearance of 9β,19-cyclopropylsterols and Δ^8-sterols. The replacement of a large proportion of Δ^5-sterols by cyclopropylsterols and Δ^8-sterols could be expected from the data previously obtained (3–5) on the mode of action of fenpropimorph in maize seedlings, which clearly showed an inhibition of the enzymes involved in the cyclopropane ring opening (cycloeucal*enol*-obtusifoliol isomerase) and $\Delta^8 \rightarrow \Delta^7$-sterol isomerization. Second, insects reared on this experimental wheat show a strongly modified sterol profile as compared to normal insects; however, this profile does not merely reflect the sterol composition of the diet, suggesting that a differential mechanism of uptake of the dietary sterols exists in the insects. The overall sterol uptake in the insects is probably not affected, as total sterol contents are similar in insects reared on normal or experimental diets; we therefore may conclude that insects can discriminate between the dietary sterols and preferentially absorb and concentrate the very small amounts of Δ^5-sterols present in the diet (2).

The modification of the sterol composition does not profoundly affect the sequence of gonotrophic cycles, although females laid a reduced number of eggs. However, the sterol composition in the eggs and the ecdysteroid titers are modified. As regards the sterol composition, it roughly reflects that of the hemolymph of the adult female, suggesting that the various sterols pass in equivalent amounts into the growing oocyte. The ecdysteroid titer is in most cases reduced; when insects have been reared for a sufficiently long period, only 20–30% of normal titers are found in newly laid eggs. The data available show that the ratio of the various free and conjugated ecdysteroids in experimental eggs is similar to that of normal eggs; however, a careful chemical analysis will have to be undertaken to decide whether or not abnormal ecdysteroids have been produced in this system with cyclopropylsterols or Δ^8-sterols as precursors. Such compounds might escape radioimmunological detection. Pending such a study, it appears at present most probable that the reduction (up to 70–80%) in ecdysteroid titers in the eggs laid by experimental females primarily reflects the drastic depression (90%) of the blood cholesterol level in these females. Indeed, when the fenpropimorph wheat is supplemented with cholesterol, normal ecdysteroid titers are restored.

The last event in this sequence concerns the modifications of embryonic development. A toxic effect of fenpropimorph on embryonic development can be eliminated in view of the various control experiments. The disturbances observed certainly reflect a deficiency in ecdysteroids, all the more so as supplementation experiments with cholesterol, which restore normal ecdysteroid titers, also restore normal embryonic development. However, we emphasize that the eggs laid by females reared on experimental diet contain not only lower ecdysteroid titers, but also a markedly reduced content of cholesterol. Future studies will have to discriminate between effects resulting from hormonal deficiency and/or the depletion of the available cholesterol pool for which the cyclopropyl- and Δ^8-sterols may not substitute in satisfying certain structural and physiological functions (7).

In experimental eggs, two periods of arrest seem predominant: (*i*) when the ecdysteroid titer is drastically reduced, no development occurs and even nuclear maturation is not observed; (*ii*) when the titer is strongly reduced but somewhat less than in the previous case, development is initiated but does not extend over the time of serosal cuticle deposition and concomitant swelling of the egg. These results suggest, although they do not strictly demonstrate, that ecdysteroids are involved in the control of onset of embryonic development and in the control of the events linked to serosal cuticle deposition. From results obtained in separate investigations (11–13), we know that increased titers of free ecdysone in the eggs correspond to these two periods.

The biological model described in the present study shows that an inhibitor of plant sterol biosynthesis acting on a clearly defined enzyme target leads to a deficiency in normal sterols and to an accumulation of sterols that insects cannot use for normal ecdysteroid biosynthesis (Fig. 1). It also provides the insect physiologist with another tool to investigate the role of ecdysteroids in a system in which the tissues producing these messenger molecules cannot be eliminated by surgery.

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