Evidence for the presence of makisterone A in Drosophila larvae and the secretion of 20-deoxymakisterone A by the ring gland

(ecdysteroid secretion/phytosterols)

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ABSTRACT Ring glands or brain–ring gland complexes from third-instar Drosophila melanogaster larvae secreted ecdysone [(22R)-2β,3β,14α,22,25-pentahydroxy-5β-cholest-7-en-6-one] and two less polar ecdysteroids (LP1 and LP2) in vitro. Radioimmunoassay with different antisera indicated that LP1 and LP2 are side-chain-modified analogues of ecdysone. In high-performance liquid chromatography, the retention time of LP2 was equivalent to that of a precursor of makisterone A [(20R,22R)-2β,3β,14α,20,22,25-hexahydroxy-24-methyl-5β-cholest-7-en-6-one] secreted by Drosophila fasciatus prothoracic glands in vitro. LP2 was metabolized in vitro by the fat body of Drosophila larvae to a product with the characteristics of makisterone A when analyzed by gas chromatography/mass spectrometry (selected ion monitoring). Evidence was obtained for the presence of makisterone A in Drosophila larvae. These data suggest that LP2 is 20-deoxymakisterone A (24-methylecdysone) and that makisterone A could function as an additional moulting hormone in Drosophila, although 20-deoxymakisterone A production is apparently dependent on the sterol composition of the diet. LP1 has not been identified.

The insect steroid ecdysone, a 27 ecdysteroid [(22R)-2β,3β,14α,22,25-pentahydroxy-5β-cholest-7-en-6-one], is secreted by the prothoracic glands (1) and, in cyclorrhaphous Diptera, by the ring gland (2). Ecdysone is hydroxylated at C-20 by monooxygenase enzyme systems in the fat body, gut, and Malpighian tubules to give the biologically active moulting hormone 20-hydroxyecdysone [(22R,22R)-2β,3β,14α,20,22,25-hexahydroxy-5β-cholest-7-en-6-one] (3). Recently, the moulting hormone in Dysdercus fasciatus (4) and some other species of phytophagous Hemiptera (5) has been identified as a 29 ecdysteroid, makisterone A [(20R,22R)-2β,3β,14α,20,22,25-hexahydroxy-24-methyl-5β-cholest-7-en-6-one], which differs from 20-hydroxyecdysone in the presence of an additional methyl group at C-24 of the ecdysoid side chain. By analogy with other insects (1), the prothoracic glands of these hemipterans would be expected to secrete the 24-methylated analogue of ecdysone, 20-deoxymakisterone A (24-methylecdysone).

In a previous study, it was shown that Drosophila ring glands secrete ecdysteroids in vitro and that the rate of secretion reaches a maximum towards the end of the last larval instar (6). Sarcophaga ring glands secrete ecdysone in vitro (2), and the experiments reported here were initially designed to ask whether or not this is also true of Drosophila melanogaster ring glands. However, in addition to ecdysone, explanted Drosophila ring glands secreted apparently significant quantities of two other ecdysteroids. Further analysis has indicated that one of these ecdysteroids is 20-deoxymakisterone A and that its production is apparently dependent on the sterol composition of the diet.

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MATERIALS AND METHODS

Animal Culture. Unless stated otherwise, the Oregon R strain of D. melanogaster was cultured on cornmeal/yeast medium: 118.54 g of whole cornmeal, 21.43 g of dried yeast (heat killed), 107.15 g of sucrose, and 6.43 g of agar per liter of water containing 0.003% Nipagin, added as a 10% solution in 95% ethanol. The medium was sprinkled lightly with live yeast before use. For culturing Drosophila axenically on defined media, the recipe and methods of Sang (7) were used. Yeast RNA was replaced with uridine and inosine, and lecithin was replaced with choline chloride (7). The medium constituents were purchased from Sigma (uridine, nicotinic acid, thiamine-HCl, calcium pantothenate, pyridoxine, biotin, and ergosterol [ergosta-5,7,22E-trien-3β-ol], BDH (sucrose, choline chloride, inosine, fat- and vitamin-free casein, and folic acid), Koch-Light Laboratories (Bucks, England) [riboflavin and "sitosterol" (stigmasta-5-en-3β-ol)], Oxoid (Basingstoke, England; technical agar no. 3), and Steraloids (Wilton, NH) [stigmasterol (stigmasta-5,22E-dien-3β-ol)]. Dietary sterol was either cholesterol (cholest-5-en-3β-ol; 0.03%), ergosterol (0.03%), or a mixture of 0.015% stigmast-3-ol, 0.01% ergosterol, and 0.02% sitosterol/campesterol [(24R)-24-methylcholest-5-en-3β-ol] (ca. 7:3, wt/wt; H. H. Rees, personal communication), according to the experiment.

Fourth-instar D. fasciatus were obtained from May & Baker and maintained as described (4).

Organ Culture. Ring glands or brain–ring gland complexes from third-instar Drosophila larvae with partially or fully bloated larval salivary glands were dissected and cultured (three to six ring glands or brain–ring gland complexes per culture) in Grace’s medium (Flow Laboratories) for 4–8 hr as described (6). Prothoracic glands dissected from late fifth-instar D. fasciatus were cultured under similar conditions. After culture, the tissue was pelleted by centrifugation, and the supernatant was added to methanol to a final methanol concentration of 70% vol/vol.

Fat body from five late-wandering, third-instar Drosophila larvae (selected according to salivary gland morphology as above) were dissected in Grace’s medium, washed by gentle agitation in three successive 0.5-ml aliquots of fresh medium (1–2 min per wash), and cultured in 20 μl of Grace’s medium for 3 hr at 28°C. After culture, the medium was withdrawn, and the tissue was washed with 20 μl of Grace’s medium. The combined supernatants, after pelleting the tissue, were added to 200 μl of methanol and processed for analysis. The conversion of [23,24-3H]ecdysone to 20-hydroxy[23,24-3H]ecdysone was approximately 45% under these conditions.

Abbreviation: GC/MS(SIM), gas chromatography/mass spectrometry (selected ion monitoring).

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Sample Processing. Culture medium samples (stored at
-20°C) were purified by reversed-phase chromatography
using C18 Sep-Pak cartridges (Waters Associates) (8). Free ec-
dysteroids, eluted with 4 ml of 60% (vol/vol) methanol/
water, were retained for analysis. Under these conditions,
recovery of (23,24-3H)ecdysone is >90% (8).

Extraction of Ecdysteroids from Drosophila Larvae. Wan-
dering, third-instar Drosophila larvae were washed, blotted
dry, and stored at -20°C until extracted. The larvae (245 mg,
wt weight) were extracted three times by homogenization in
4 ml of 70% (vol/vol) methanol/water (on ice) and finally
with 4 ml of methanol. The extract, made up to 25 ml with
70% methanol/water, was partitioned against an equal vol-
ume of hexane. The aqueous phase was dried under vacuum,
and the residue was redissolved in 5 ml of methanol and ad-
sorbed onto Celite (Koch-Light Laboratories) (9). After silic-
ic acid chromatography of the adsorbed extract (9), free ec-
dysteroids were eluted with methanol/chloroform, 30:70
(vol/vol), dried under nitrogen, redissolved in 2 ml of 10%
(vol/vol) methanol/water, and further purified by using a
C18 Sep-Pak cartridge as above; the 60% methanol/water frac-
tion was retained for analysis by HPLC. In a control ex-
periment, ca. 75% of 20-hydroxy[3H]ecdysone added to a
batch of larvae at the initial homogenization was recovered
(verified by reversed-phase HPLC) from the final Sep-Pak
purification.

HPLC. Reversed-phase HPLC was carried out by using a
Waters instrument (Waters Associates) (8) with either an Al-
tex (Berkeley, CA) Ultrasphere ODS column (spherical C18;
particle size, 5 μm; column dimensions, 4.6 mm i.d. by 15
cm) or a Waters Associates Resolve column (spherical C18;
5 μm; 3.9 mm i.d. by 15 cm) and by eluting with a linear gradi-
ent of methanol/water, increasing from 35:65 (vol/vol) to
80:20 (vol/vol) over 30 min at a flow rate of 1 ml-min-1.
Except where stated, only fractions (0.5 or 1 min) that were
eluted between 12 and 22 min after injection were assayed
by RIA. A blank or control injection was performed before each
analysis, and ecdysone and 20-hydroxyecdysone standards
were run immediately after each group of samples. The sus-
ceptibility of unidentified ecdysteroid peaks to enzymic hy-
drolysis was tested by using esterase (Sigma), α-glucosidase
(Boehringer Mannheim), and Helix pomatia "arylsulfatase"
(Sigma; type H1, with phosphatase and β-glucuronidase ac-
itivity) (8, 10), followed by HPLC/RIA analysis.

Gas Chromatography/Mass Spectrometry (Selected Ion
Monitoring) (GC/MS(SIM)). Culture medium samples (after C18
Sep-Pak chromatography) and selected HPLC fractions were
dried under dry nitrogen and derivatized with trimeth-
ysilylimidazole (8). Before derivatization, ponasterone A
[5 ng; (20R,22R)-2β,3β,14α,20,22-pentahydroxy-5β-chol-
est-7-en-6-one] was added to all samples and standards as
an internal standard for the GC/MS(SIM) analysis. Subsequent
sample preparation and GC/MS(SIM) analysis were as de-
scribed by Mendis et al. (8). Fragment ions m/z 567 and m/z
566 were monitored.

RIA. HPLC fractions were assayed by using the Horn 12
(16 wk) antiserum (11), a gift of J. D. O’Connor, or the ICT-1
antiserum (12) (supplied by H. H. Rees) by K.-D. Spindler.
The radioimmunoassay method was as described (6). At the
antiserum dilutions used (1:300 and 1:3000, for the Horn 12
and ICT-1 antisera, respectively, unless stated otherwise)
ca. 50% of the radiolabeled ecdysone was bound in the ab-
ance of competing ligand. Where necessary, background
values (blank injection) have been subtracted.

Two further antisera were used for dose–response exper-
iments: the DHS 1-15 antiserum (13), a gift of J. D. O’Connor
to H. H. Rees (used at a dilution of 1:500) and an antiserum
(MAS 7) generated in mice by using as antigen 20-hydroxy-
edysone conjugated through C9 to bovine serum albumin
(Sigma) (14, 15). Five mice (CBA/Ca strain) were each in-
jected i.p. with 200 μg of the antigen (hapten:carrier molar
ratio, 3.3:1) emulsified in 0.5 ml of 1% Tween 80/0.45% NaCl/25% Freund’s complete adjuvant. Booster injections
(200 μg of antigen per mouse) were given 7 and 19 wk later.
The resulting antisera (pooled from five mice) was used at a
dilution of 1:550 and had a Kd for ecdysone of 4.9 × 10-10
M; the affinity for 20-hydroxyecdysone was approximately
half for ecdysone.

RESULTS

HPLC Separation of Ecdysteroids Secreted by Drosophila
Ring Glands. With the culture conditions used here, brain–
ring gland complexes from Drosophila larvae, dissected
from animals shortly before puparium formation, secreted
ecdysteroids at a rate of about 90 pg ecdysone equivalents
per hr per complex (6). To identify the ecdysteroids secre-
ted, the culture media from 11 brain–ring gland cultures
and 15 ring gland cultures were analyzed separately by re-
versed-phase HPLC. RIA activity corresponding to ecdysone rep-
resented 33–42% of the total for all fractions (Fig. 1). Less-
polar cross-reacting material, eluted 1–4 min after ecdysone,
represented 45–49% of the total RIA activity. The results with
brain–ring gland complexes or ring glands alone were sim-
ilar, indicating that the less-polar material did not result
from ecdysteroid secretion or metabolism by the brain tissue
of brain–ring gland complexes. In two subsequent HPLC ex-
periments, the less-polar material (LP in Fig. 1) represented
52% and 67% of the total RIA activity that was eluted be-
 tween 12 and 22 min after injection; ecdysone (confirmed
by GC/MS(SIM)) comprised the remainder.

With fractionation every 0.25 or 0.5 min, the less-polar
ecdysteroid material was clearly resolved into two com-
ponents: these are referred to as LP1 and LP2 (e.g., see Fig. 3
Upper). LP2 was the major component, and in two analyses
represented approximately 75% of the less-polar ecdys-
oids and 30–50% of the RIA activity detected in ring gland
incubations. The retention times of LP1 and LP2, relative to
edysone, are given in Table 1.

Evidence that brain–ring gland complexes of Calliphora
erythrocephala are capable of synthesizing esters and glyco-
sides of hormonally active steroids has been reported (10).
LP1 and LP2 were eluted in the free ecdysteroid fraction
after C18 Sep-Pak chromatography and were unaffected by
incubation with esterase, α-glucosidase, or Helix pomatia
arylsulfatase; therefore, it seems unlikely that they are ec-
dysteroid conjugates. Furthermore, incubating ring glands
with [23,24-3H]ecdysone did not result in any detectable
conversion to less-polar ecdysteroids.

![Fig. 1. HPLC/RIA analysis of ecdysteroids secreted by Drosophila ring glands cultured in vitro for 4 hr at 25°C. One third of the sample from 15 ring gland cultures (3 glands per culture) was injected onto the column (Ultraphase ODS), and 0.25 ml of each 1-min fraction of the 30-min gradient was assayed by RIA (Horn 12 (16 wk) antiserum). The positions of authentic ecdysone (I) and 20-hydroxy-
edysone (II) are marked. LP, less-polar ecdysteroids. Peak III was not present in subsequent analyses and is assumed to be an artefact.](image-url)
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Table 1. HPLC retention times of ecdysteroids relative to ecdysone

<table>
<thead>
<tr>
<th>Ecdysteroid</th>
<th>Column: Resolve</th>
<th>Ultrasphere ODS</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-Hydroxyecdysone</td>
<td>0.71</td>
<td>0.74</td>
</tr>
<tr>
<td>Makisterone A</td>
<td>0.91</td>
<td>ND</td>
</tr>
<tr>
<td>LP1</td>
<td>1.22</td>
<td>1.21</td>
</tr>
<tr>
<td>LP2</td>
<td>1.15</td>
<td>1.12</td>
</tr>
</tbody>
</table>

ND, not determined.

Cross-Reaction of LP1 and LP2 with Ecdysteroid Antisera.
Structural information on unknown ecdysteroids may be obtained by using antisera differing in their specificity towards different parts of the ecdysteroid molecule. Four different antisera were used for dose-response studies with samples of LP1 and LP2, purified by two successive reversed-phase HPLC separations. Both the Horn I2 [16 wk; a batch related to the H218 antiserum (11)] and the ICT-1 antiserum are ecdysone nucleus specific (16): the ecdysteroids used in their preparation were conjugated to the carrier via the ecdysteroid side chain (12, 16). Conversely, the DHS 1-15 antiserum, prepared by conjugating through C2 on the steroid nucleus, is relatively side chain specific (13). The MAS-7 antiserum has not been fully characterized but was prepared by conjugating through C2 and, like the DLW antiserum (16) prepared similarly, should be side chain specific.

Dose-response curves with the Horn I2 (16 wk), DHS I-15, and MAS-7 antisera are shown in Fig. 2. By comparison with unlabeled ecdysone, LP2 competed relatively inefficiently with [23,24-3H]ecdysone for binding to the DHS I-15 and MAS-7 antiserum (Fig. 2). A similar result with respect to the MAS-7 antiserum was obtained by using LP1 (DHS I-15 was not tested). The ICT-1 antiserum appeared to show a greater affinity towards LP2 than for ecdysone: 190 pg ecdysone equivalents of LP2 (as measured with the Horn I2 antiserum) or 370 pg of ecdysone were required to effect a 50% reduction in the binding of [23,24-3H]ecdysone to the ICT-1 antiserum (1:1000 dilution). With ecdysone as a reference, the marked difference in specificity for LP1 and LP2 between the nucleus-specific and side-chain-specific antisera suggests that LP1 and LP2 are ecdysteroids with modified side chains.

Ecdysteroid Secretion by D. fasciatus Prothoracic Glands in Vitro. In view of indications that LP1 and LP2 differ from ecdysone with respect to side-chain modifications, the possibility that LP1 and LP2 are methyl-substituted derivatives was investigated by HPLC analysis of ecdysteroids secreted by D. fasciatus prothoracic glands. The major ecdysteroid (presumably 20-deoxymakisterone A) secreted by D. fasciatus prothoracic glands in vitro had a retention time (reversed-phase HPLC) equivalent to LP2 (Fig. 3). This suggests that LP2 is 20-deoxymakisterone A (24-methylecdysone).

In dose-response RIA experiments with the ICT-1 antiserum (1:1000 dilution), 580 pg of makisterone A or ca. 1000 pg of 20-hydroxyecdysone were required to reduce [23,24-3H]ecdysone binding by 50%. Thus, the presence of a C-24 methyl group on the ecdysteroid side chain apparently confers greater affinity for this antiserum, perhaps by mimicking the chemical bridge of the original antigen. That ICT-1 also shows greater affinity towards LP2 than ecdysone is additional evidence supporting the identification of LP2 as a 24-methyl-substituted analogue of ecdysone.

20-Hydroxylation of LP2 in Vitro. If it is correct that LP2 is 20-deoxymakisterone A, makisterone A should be present in third-instar Drosophila larvae and should also result from the metabolism of LP2 by larval fat body in vitro. Fat bodies from five, late-wandering, third-instar larvae of Drosophila were incubated in Grace's medium (control) or Grace's medium containing 4 ng ecdysone equivalents of LP2. The culture media from the control and experimental incubations were analyzed by GS/MS(SIM), together with samples of LP1 and LP2.

After derivatization with trimethylsilylimidazole both LP1 (not shown) and LP2 (Fig. 4) gave peaks with ion m/z 567. This ion is characteristic of fully silylated ecdysone and ecdysteroids with an ecdysone nucleus and lacking a C-20 hydroxyl group (8, 17). Retention times relative to the ponasterone A internal standard are given in Table 2. In addition to the internal standard, four peaks resulted from the incubation of LP2 with larval fat body (Fig. 4): two of these represent unknown compounds that were eluted or secreted from the fat body and present in the control sample. The remaining two peaks have relative retention times corresponding to fully silylated makisterone A (peak C, m/z 567) and LP2.

![Fig. 2. RIA dose-response curves of ecdysone and LP2 with the Horn I2 (16 wk), DHS I-15, and MAS-7 antiserum. LP2 is expressed as pg ecdysone equivalents as measured with the Horn I2 (16 wk) antiserum. Solid lines: LP2 was assayed in duplicate with the Horn I2 (16 wk) (•), MAS-7 (●), and DHS I-15 (▲) antisera. Lines for these latter two antisera were superimposed. Dashed lines: ecdysone was assayed with the Horn I2 (○), MAS-7 (▲), and DHS I-15 antiserum (□). % bound (ordinate) refers to the amount of [23,24-3H]ecdysone bound expressed as a percentage of label bound in the absence of competing ligand.](image1)

![Fig. 3. HPLC/RIA analysis (Ultrasphere ODS column, Horn I2 (16 wk) antiserum) of ecdysteroids secreted by brain-ring gland complexes from Drosophila (Upper) or prothoracic glands from D. fasciatus (Lower) cultured in vitro. LP1 and LP2 and the positions of authentic ecdysone (I) and 20-hydroxyecdysone (II) are marked.](image2)
hydroxylation of late-wandering Drosophila met.: culture medium

The makisterone droxylated in vitro corresponds to the major ion. For most (peak E, m/z 567), respectively. Ion m/z 561 is the major ion characteristic of ecdysteroids with a C-20 hydroxyl group (8, 17), and these data therefore indicate that LP2 was 20-hydroxylated in vitro to produce makisterone A.

Analysis of Ecdysteroids Extracted from Larvae. A reversed-phase HPLC analysis of ecdysteroids extracted from wandering, third-instar larvae of Drosophila revealed RIA activity with retention times equivalent to 20-hydroxyecdyson (20 pg ecdysone equivalents/mg of wet weight), makisterone A (8 pg ecdysone equivalents/mg of wet weight), and ecdysone (120 pg ecdysone equivalents/mg of wet weight). The makisterone A fraction was analyzed by GC/MS(SIM), and the result (Fig. 5) is further evidence that makisterone A is present in third-instar Drosophila larvae. LP1 and LP2 were not detected by either HPLC/RIA or GC/MS(SIM) in this extract.

Ecdysteroid Secretion by Ring Glands of Drosophila Larvae Grown on Defined Media. D. fasciatus does not dealkylate phytosterols and utilizes C29 phytosterols directly for molting hormone production (4). Similarly, the production of 20-deoxymakisterone A (and LP1) by Drosophila ring glands cultured in vitro may be due the direct utilization of C29 and C28 phytosterols, which account for a major proportion of the sterols in corn (18) and yeast (19). This is supported by the finding that brain–ring gland complexes from Drosophila larvae grown axenically on a defined medium containing cholesterol as the sole dietary sterol only secreted ecdysone in vitro. However, the same result was obtained when cholesterol was replaced by ergosterol (Fig. 6 Upper) a major sterol of yeast (19). The inclusion in the Drosophila food of sitosterol, campesterol, and stigmasterol together with ergosterol consistently (three experiments) resulted in the production of small amounts of LP2 as shown by HPLC/RIA analysis (Fig. 6 Lower).

DISCUSSION

Together, the GS/MS(SIM) data and RIA dose–response curves with different antisera are clear indications that LP1 and LP2 are side-chain-modified analogues of ecdysone. The HPLC retention time of LP2 was equivalent to that of a major ecdysteroid secreted in vitro by D. fasciatus prothoracic glands, and this suggests that LP2 is the 24-methyl-substituted analogue, 20-deoxymakisterone A, since in this species

Table 2. GC/MC(SIM) retention times of fully silylated trimethyl-ecdyysteroid ethers relative to the ponasterone A internal standard

<table>
<thead>
<tr>
<th>Ecdysteroid</th>
<th>Relative retention time</th>
<th>Major ion m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecdysone</td>
<td>1.28</td>
<td>567</td>
</tr>
<tr>
<td>20-Hydroxyecdysone</td>
<td>1.56</td>
<td>561</td>
</tr>
<tr>
<td>Makisterone A</td>
<td>1.98</td>
<td>561</td>
</tr>
<tr>
<td>LP1</td>
<td>1.56</td>
<td>561</td>
</tr>
<tr>
<td>LP2</td>
<td>1.51</td>
<td>567</td>
</tr>
</tbody>
</table>

*On 1% OV-1 column.

![Fig. 4](image_url)  
**Fig. 4.** GC/MS(SIM) analyses. (a) The product(s) of in vitro 20-hydroxylation of 4 ng ecdysone equivalents of LP2 by fat body from late-wandering Drosophila larvae. (b) Four nanogram ecdysone equivalents (Horn I2 antiserum) of purified LP2. (c) Control experiment: culture medium after incubation with fat body from late-wandering third-instar Drosophila larvae. Peak A (m/z 561) is the ponasterone A internal standard; peaks B (m/z 561; retention time relative to ponasterone A (RRT), 1.32) and D (m/z 567; RRT, 0.74) are unknown compounds present in the control; peak C (m/z 561; RRT, 1.97) in a has a retention time, relative to ponasterone A, which corresponds to fully silylated makisterone A (Table 2); peak E (m/z 567) corresponds to LP2. Ordinates: ion intensity relative to the most abundant ion. For this experiment, LP2 was purified from the culture medium of 49 brain–ring gland cultures by two successive HPLC separations.

![Fig. 5](image_url)  
**Fig. 5.** Ecdysteroids extracted from Drosophila larvae: GC/MS(SIM) analysis of the HPLC fraction corresponding to makisterone A. Peak A is the internal standard, ponasterone A; peak C (m/z 561) has a relative retention time (1.98) corresponding to fully silylated makisterone A. The identity of peak B (m/z 561) is unknown. The sample used in this analysis contained ca. 2 ng makisterone A equivalents by RIA. The area of peak C represents approximately 1 ng of makisterone A.
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![Graph](image)

Fig. 6. HPLC/RIA analysis (Resolve column, ICT-1 antiserum) of ecdysteroids secreted in vitro by brain–ring glands from *Drosophila* larvae grown on defined media containing either ergosterol (Upper) or a mixture of sitosterol, campesterol, stigmasterol, and ergosterol (Lower). The positions of LP1, LP2, authentic ecdysone (I), and 20-hydroxyecdysone (II) are marked. A distinct peak of material corresponding to LP2 is visible (Lower).

The corresponding 20-hydroxylated ecdysteroid, makisterone A, is the major ecdysteroid at peak ecdysteroid titer (4). The apparent conversion of LP2 to makisterone A by 20-hydroxylation in vivo supports the identification of LP2 as 20-deoxymakisterone A. That makisterone A is detectable in *Drosophila* larvae is additional corroboration and is evidence that the ring gland secretes 20-deoxymakisterone A in vivo. LP1 is also likely to be a side-chain-modified ecdysone analogue, but its identity is at present unknown.

The C28 phytosterol campesterol is likely to be the precursor of makisterone A in *Dysdercus fasciatus* (4). Dealkylation of the C28 sterol, ergosterol, apparently occurs in *Drosophila*, as has been suggested (20), since only ecdysone is secreted by ring glands from larvae grown on an ergosterol-containing medium. The mechanism leading to the production of 20-deoxymakisterone A by ring glands from larvae grown on cornmeal/yeast medium or on a medium containing a mixture of C28 and C29 sterols is uncertain. Current evidence indicates that dealkylation of C29 sterols by insects does not proceed via a stepwise reduction in carbon atoms of the C-24 ethyl group (21). Thus, C29 sterols are unlikely to be precursors of 20-deoxymakisterone A. Campesterol (24-methylcholesterol) bears a greater structural similarity to cholesterol than does ergosterol and could possibly be an effective substrate for ecdysteroid biosynthesis without prior dealkylation.

The presence of both C27 and C28 ecdysteroids in an arthropod is not unprecedented: Faux *et al.* (22) have identified 20-hydroxyecdysone and makisterone A in the crustacean *Callinectes sapidus*. With respect to insects, makisterone A has been reported to be 10 times more active than 20-hydroxyecdysone in *Oncopeplus* bioassays (23) and at least as active as 20-hydroxyecdysone in the *Calliphora* bioassay (24). Thus, this ecdysteroid may contribute to physiological moultng hormone levels in *Drosophila* grown on cornmeal/yeast media. Clearly, 20-hydroxyecdysone may not necessarily be the only functional moultng hormone in laboratory-grown *Drosophila*.

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