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THE EVALUATION OF ANTI JUVENILE HORMONES USING LAST STADIUM LARVAE OF THE CABBAGE LOOPER, *TRICHOPLUSIA NI* (HÜBNER)*

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Abstract—Treatment of prepupae of the cabbage looper, *Trichoplusia ni* (Hübner), with the anti juvenile hormone (AJH) fluoromevalonolactone (FMev) reduces juvenile hormone esterase (JHE) activity and either delays or inhibits the normal larval–pupal ecdysis in a dose-dependent manner, resulting in the formation of pupae with a suite of characteristic abnormalities. A variety of putative AJHs were evaluated using these teratogenic effects and changes in JHE activity as indicators of AJH activity. Like FMev, the compactin analog L-643,049-01K01 and, at very high doses, 3,3-dichloro-2-propenyl hexanoate (DPH) behaved as AJHs. FMev and L-643,049-01K01 were most effective when larvae were treated at gut purge, while DPH was the most effective after the initiation of spinning behavior.

Key Word Index: *Trichoplusia ni*, anti juvenile hormone, juvenile hormone, juvenile hormone esterase

INTRODUCTION

During the first day of the last larval stadium of the cabbage looper, *Trichoplusia ni* (Hübner), the hemolymph juvenile hormone (JH) titer declines to currently undetectable levels and then briefly reappears when feeding has ceased and wandering behavior has begun (Jones, 1983). Associated with these declines in the hemolymph JH titer is the appearance of juvenile homone esterase (JHE) (Sparks and Hammock, 1979; Sparks *et al.*, 1979).

In *T. ni*, the peak of JH that appears during the wandering phase is necessary for the proper formation of the pupa (Sparks, 1984; Jones and Hammock, 1985) and yet must be removed rapidly to ensure the future development of the pupa to the adult (Jones and Hammock, 1983, 1985; Sparks, 1984). The presence of JHE at this stage of development appears to be induced by JH, which in turn facilitates the removal of JH (Sparks and Hammock, 1979; Jones and Hammock, 1983; Hammock, 1985).

Based on available information concerning JH and JHE regulation in *T. ni*, any disruption of the prepupal JH peak by a xenobiotic should prevent the proper formation of the pupa, as well as reduce the size of the prepupal peak of JHE activity (Sparks and Hammock, 1980; Sparks, 1984; Jones and Hammock 1985). This concept provides the basis for a relatively rapid bioassay that can potentially be used to detect anti juvenile hormone (AJH) activity (Sparks *et al.*, 1985). Using this bioassay, we have evaluated a variety of compounds for AJH activity toward *T. ni*.

MATERIALS AND METHODS

Insects

Larvae of *T. ni* were reared on an artificial diet at 28 ± 2°C, on a 14L:10D photoperiod (lights on at 5 a.m.) with 40% r.h. Last (fifth; L5) stadium feeding (day 1; L5D1) and wandering (day 3; L5D3) stage larvae were selected as descibed previously (Sparks *et al.*, 1979).

Bioassay: In vivo *bioassay* (*part one*)

Larvae were bioassayed as described previously (Sparks *et al.*, 1985). Wandering (L5D3; 3–5 hr after lights on [ALO]) stage larvae, with a minimum of three groups of 10 larvae each (30 larvae total) were treated with the candidate compound, topically (in 1–2 μl ethanol or acetone) on the dorsum of the first two abdominal segments, or in the case of L-643,049-01K01 and A-7041 by injection (in 1–2 μl distilled water) along the mid-dorsal line just behind the thorax. Appropriate solvent controls were also run. Treated larvae were examined for changes in behavior, development, incomplete ecdysis and intoxication (Sparks *et al.*, 1985). Larvae were examined the next day (L5D4) at 1 hr intervals for time of ecdysis to the pupa, or for tanning on the dorsum of the thorax and abdomen when ecdysis to the pupae failed and the pupae remained trapped in their old cuticle. The time for 50% of the larvae to undergo ecdysis to the pupa or (failing proper ecdysis) tan on day 4 (T_{50}) was derived from the cumulative percent response vs time analyzed by probit analysis (Finney, 1971).

Effects on JHE activity (*part two*)

Feeding (L5D1) and wandering (L5D3) stage larvae were treated as above and then assayed for hemolymph JHE activity using JH-III (^{3}H at C-10, 11 Ci/mmol; New England Nuclear) as substrate (5×10^{-6} M) as described previously (Hammock and Sparks, 1977; Sparks *et al.*, 1979). The L5D1 larvae were assayed for JHE activity 1 hr post-treatment, while the L5D3 larvae were assayed at the time of the prepupal peak of JHE activity (approx. 24 hr post-treatment; on early L5D4, 4–5 hr ALO) (Sparks *et al.*, 1979).

RESULTS AND DISCUSSION

Bioassays (*part one*)

A variety of compounds shown to possess AJH activity either *in vivo* (Nos 1–12, 16) or *in vitro* (Nos 13–15) (Menn, 1985; Staal, 1986) were examined in the present study (Table 1, Fig. 1). Treatment with 100 nmol of L-643,049-01K01 prevented ecdysis to

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Table 1. Results of testing compounds for AJH activity

No. Compounds*	Dose (nmol /larva)	Time of treatment (hr ALO)	Part one: Percent abnormal pupae	Part one: T_{50} (hr)**	Part two: % Control JHE act. L5D3†	Part two: % Control JHE act. L5D1‡
1. FMev	200	4	100	6.2−	—	—
	200	10	100	4.4−	—	—
	676	4	100	—	45 ± 21§	—
	676	10	100	—	58 ± 10	99 ± 7§
2. Ethoxy-precocene II	200§	4	0	1.1−	—	—
	1709§§	4	0	1.0−	—	—
3. A_{11}	200	4	0	0.1+	—	—
4. KK-22	200	4	0	2.0−	—	—
5. L-643,049-01K01	100§	4	89	5.7−	56 ± 12§	—
	100	10	100	6.3−	79 ± 16	103 ± 17§
6. DPH	200	4	8	0.4−	—	—
	893	4	0	0.5−	—	—
	1794§§	4	32	4.1−	96 ± 33	—
	1794	10	62	8.0−	37 ± 14	93 ± 14
7. J-2710	100	4	0	1.2−	—	—
	2000	4	0	1.5−	—	—
8. J-2711	100	4	0	0.8−	—	—
9. J-2581	100	4	0	1.3−	—	—
10. J-2922	66	4	13	0.9−	—	—
	1000	4	0	1.3−	—	—
11. J-3230	50	4	0	1.9−	—	—
12. J-3263	66	4	0	1.6−	—	—
13. A-7041	200	4	10	3.1−	—	—
14. Thiobencarb	200	4	0	0.0	—	—
	1556§§	4	15	1.4−	—	—
15. TCPPE	200	4	0	0.1−	—	—
	1709§§	4	20	0.5−	—	—
16. Piperonyl butoxide	200§	4	0	0.1−	—	—
	1183§§	4	18	1.1−	—	—

*Excluding L-643,049-01K01 and A-7041 which were injected, all compounds were applied topically. L5D3 larvae were treated 4–5 hr ALO and then evaluated the next day for time of ecdysis to the pupa and production of abnormal tanned pharate pupae the next day. Sources of the compounds are as follows. 1, 3, 6, G. Quistad, D. Schooley and G. Staal, Zoecon Corp.; 2, Sigma; 4, Dr Kuwano, Dept of Agricultural Chemistry, Kyushu University, Japan; 5, R. Dybas, Merck and Company Inc.; 7–12, Dr L. Jurd, Western Regional Research Center, Berkeley, CA; 13, Abbott Laboratories; 14, 16, Chem Service; 15, R. Feyereisen, Dept of Entomology, Oregon State University.

**Time before (+) or after (−) the controls for 50% of the insects to undergo ecdysis to the pupa or (for animals unable to cast the old cuticle) tan.

†Larvae were assayed on L5D4, 4–5 hr ALO. Values are means ± SD.

‡Larvae were treated on L5D1, 10 hr ALO and assayed 1 hr later. Values are means ± SD.

§Data adapted from Sparks *et al.* (1985).

§§400 μg/larva.

the pupa, resulting in the formation of animals that appeared to be tanned prepupae (pupae that retain the old cuticle and partially tan), a teratogenic effect that can result from treatment with a variety of compounds including JHE inhibitors, JH and FMev (Sparks and Hammock, 1980; Sparks, 1984; Jones and Hammock, 1985) (Table 1, part one; Fig. 2). These abnormal insects all eventually died. Of the other putative AJHs tested in the present study, none displayed this type of teratogenic acitivity in our bioassay (at the dose tested) that was comparable to FMev. At the dose tested, only FMev, L-643,049-01K01 and A-7041 resulted in a large (>3 hr) delay in the time of pupal ecdysis or (for animals that were unable to cast the old cuticle) tanning (Table 1, part one). All other compounds tested had relatively little effect on development or behavior. Thus, on the basis of the first part of the bioassay, only L-643,049-01K01 exhibited the necessary responses, relative to FMev, to be considered for further evaluation.

Although the dosages used in the initial screen were either the highest possible (given limited amounts of material) or reasonable (using FMev as a standard), it was possible that some of the compounds failed to display activity due to either the dose or time of application being suboptimal. To investigate this possibility further, several of the compounds were tested at higher dosages and/or different times. Higher dosages of ethoxy-precocene II, J-2710, J-2922, thiobencarb, TCPPE and piperonyl butoxide had little or no effect (Table 1, part one). At a dose of 893 nmol (200 μg) DPH also had no effect; however, increasing the dose to 1794 nmol (400 μg) resulted in 32% of the treated larvae displaying abnormalities similar to those of FMev, which was associated with a 4.1 hr delay in the time of ecdysis to the pupa or (for those unable to cast the old cuticle) tanning (Table 1, part one). Thus, at a dose of 1794 nmol DPH appeared to possess teratogenic activity similar to that of FMev. Bioassaying DPH at a later time (10 hr ALO) on L5D3 greatly improved its activity (Table 1, part one), while it either had little effect or caused a reduction in AJH activity for L-643,049-01K01 and FMev, respectively.

Effects on JHE activity (part two)

Since a variety of compounds can potentially cause developmental abnormalities (Sparks *et al.*, 1985), the second part of the AJH bioassay involves effects on JHE activity in L5D3 and L5D1 larvae, respectively,

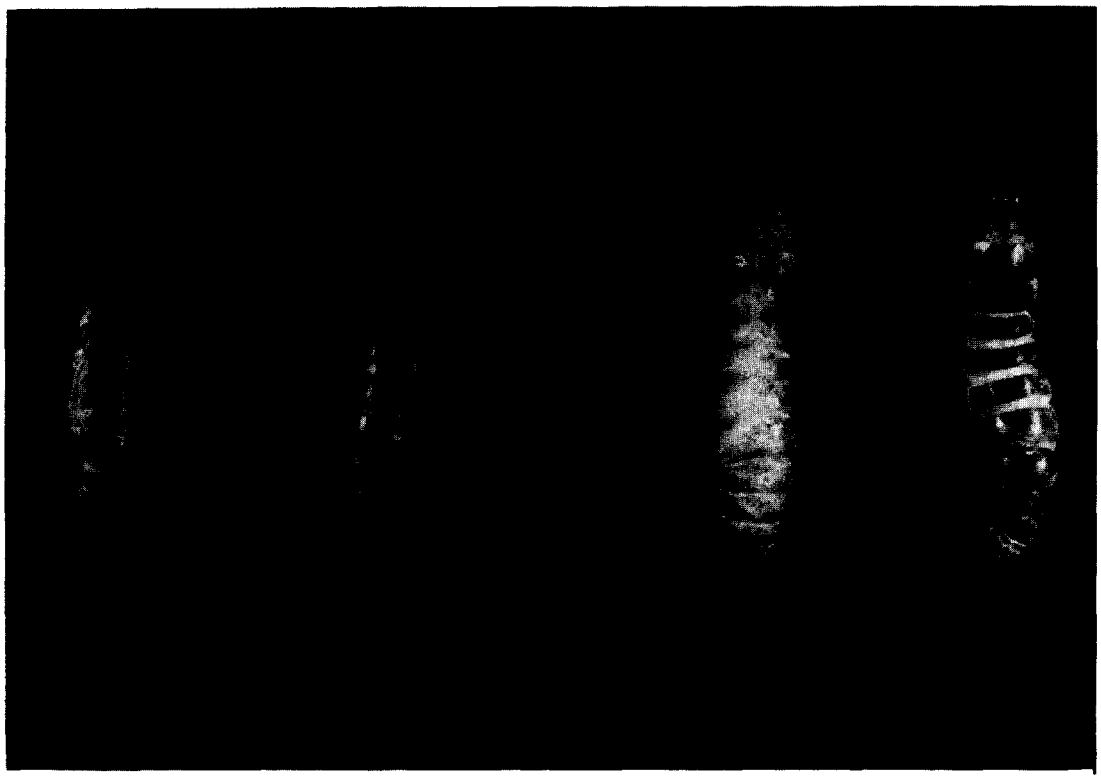

Fig. 2. Lateral and dorsal views of *T. ni.* Right: Animals showing typical abnormalities resulting from AJH treatment, in this case L-643,049-01K01 (100 nmol/larva, injected) on L5D3 (4 hr ALO). Left: Controls which received 1 μl of distilled water.

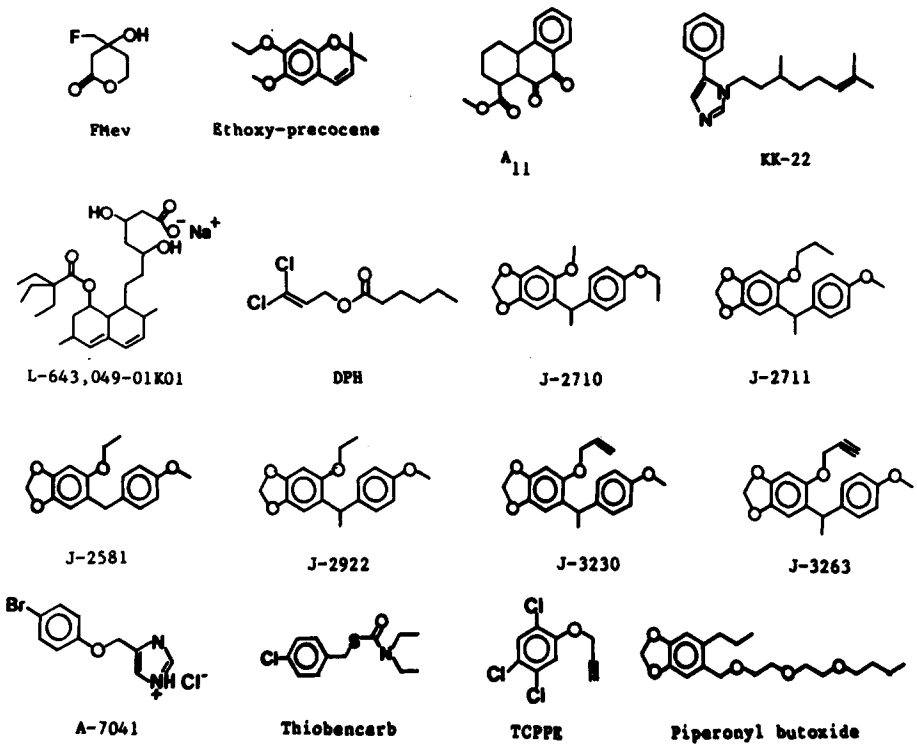

Fig. 1. Structures of the putative AJHs examined.

and is used to validate AJH activity. JHE activity in wandering stage larvae (L5D3, 4–5 hr ALO) treated with FMev (100 nmol) was relatively unaffected when assayed 24 hr post-treatment (L5D4, 4–5 hr ALO; data not shown). However, L-643,049-01K01 (100 nmol) or a higher dose of FMev (676 nmol) caused a reduction in JHE activity by 44% and 55%, respectively (Table 1, part two), becoming slightly less effective when larvae were treated later in the day (10 hr ALO). While DPH (at 1794 nmol) had little effect on the prepupal JHE peak when L5D3 larvae were treated at 4 hr ALO, it was significantly ($P < 0.05$) more effective when larvae were treated 6 hr later (Table 1, part two). An estimation of the ability of these compounds to act directly as JHE inhibitors (and allowing for possible *in vivo* activation) was obtained by treating feeding stage (L5D1) larvae and then monitoring JHE activity *in vivo* 1 hr post-treatment. In these experiments JHE activity is not affected by the JH titer. None of the three compounds (FMev, L-643,049-01K01 and DPH) had any effect on the JHE activity (Table 1, part two). Therefore, the reduction observed for JHE activity in the prepupal stage is most likely due to effects on JH biosynthesis or action and is not the result of these compounds acting directly on the JHE.

3-Hydroxy-3-methylglutaryl coenzyme A reductase, one of the enzymes involved in JH biosynthesis, is inhibited by compactin in both mammals and insects (Monger *et al.*, 1982; Staal, 1986). Although compactin is inactive in *T. ni* (Sparks *et al.*, 1985), L-643,049-01K01, a compactin analog, produced effects similar to those of FMev suggesting that analogs of compactin may warrant further research as potential AJHs. Unlike FMev and L-643,049-01K01, DPH was much more effective when applied slightly later in development. This information suggests that the mode of action of DPH is such that it functions best nearer the time of the prepupal JH release (approx. 10–16 hr ALO on L5D3; Sparks and Hammock, 1979; Jones and Hammock, 1983; Jones 1983). Since it is likely that the dichloroallylic alcohol is the active form of DPH (Quistad *et al.*, 1985), perhaps once the alcohol is produced it must act immediately before it is eliminated. Thus, timing of the application for DPH may be of critical importance to its action in *T. ni.*

Although the lack of activity observed for some of the compounds tested may be the result of suboptimal timing or dose, the results obtained in the present study are not entirely unexpected. For *Manduca sexta* FMev is more active than DPH (Quistad *et al.*, 1985), and other potential AJHs such as compactin, KK-22, piperonyl butoxide and J-2710 are inactive on *Heliothis virescens* and either only marginally active or inactive on *M. sexta* (Monger *et al.*, 1982; Menn, 1985). Thus, the results produced by our AJH bioassay using *T. ni* are consistent with AJH activity observed in other Lepidoptera. Compounds such as FMev, that are effective on a variety of species including *M. sexta*, show a very clear response with this extremely rapid bioassay. Since these data also suggest that AJH effects can also be very species specific, in a commercial screen it will be important to use a target species in the bioassay. The rapid bioassay described in this paper should be readily adaptable to a variety of pest Lepidoptera.

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