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Summary

In our experience, the insecticidal activity of the δ-endotoxin of *Bacillus thuringiensis* is remarkably decreased when suspended in or dissolved with alkaline solutions. Nonetheless, it is widely believed that the alkaline conditions present in the midgut of lepidopterous larvae are indispensible for activity of the crystalline endotoxin. Moreover, strong alkaline solutions are often employed for the solubilization and extraction of this toxin. Such beliefs and procedures appear contradictory to the observed loss of activity in alkaline solutions. Sodium hydroxide, potassium hydroxide, and acetic acid were chosen as representative alkali and acid respectively, and the effect of these solutions on the insecticidal activity of *Bacillus thuringiensis* var. *aizawai* was measured, using larvae of *Bombyx mori* as test animals. Concentration of solutions, duration of treatment, and pH were combined in various ways. The following points were demonstrated:

1. Insecticidal activity was unaffected by exposure to acetic acid.

2. NaOH and KOH decreased insecticidal activity in a manner dependent on dose and duration of exposure. A decrease in toxicity could be demonstrated following long exposure to as low a concentration as 0.01M NaOH.

3. The loss of toxicity could not be reversed by subsequent neutralization or dialysis.

4. The loss of toxicity was dependent on pH rather than NaOH concentration. The critical pH was found to be pH 11-12.

Effects of Some Juvenile Hormone Analogues on the Last Instar Larvae of *Cephonodes hylas* L. (Lepidoptera) Hajime Ikemoto (Tokyo Prefectural Isotope Research Station, Fukasawa, Setagaya, Tokyo) Received April 16, 1975. *Botyu-Kagaku*, 40, 102 1975.

19. オオスカシバの終令幼虫におよぼす幼若ホルモン類縁体の影響

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ZR 512 (ethyl-3, 7, 11-trimethyl-2, 4-dodecadienoate) はオオスカシバ幼虫の終令末期にみられる暗赤色化，造繭および蛹化を阻害した．ZR 512 の影響は施用時期によって異なる．終令脱皮後4日目に施用したときがもっともいちぢるしい効果（過剰脱皮）をもたらした．効果は施用量によってもことなる．なお，合成 *Cecropia* JH および 1-(3, 4-methylenedioxyphenyl)-7-epoxy-4, 8-dimethyl-nona-1, 3-diene (CT 5) の発生などにおよぼす影響を ZR 512 のそれと比較検討した．

Effects of synthetic juvenile hormone (JH) or its analogues on the growth and metamorphosis have hitherto been studied in many insect species. These effects vary with the age of larvae treated and the amount of substances applied[1,2].

Green color of larvae of the larger pellucid hawk moth, *Cephonodes hylas*, turns to heavy dark red prior to cocoon formation. After a short time they become into pale green prepupae, red pigments being disappeared gradually. Such depigmentation is induced by a considerable high titer of the molting hormone and inhibited by

JH[3]. The larvae of *Cerura vinula* (Lepidoptera) exhibit a body color change similar to that of *Cephonodes* larvae. The effects of two JH analogues on the body color change and the metamorphosis in *Cerura vinula* have been tested by using the last instar larvae[4].

In this paper, the effects of a JH analogue, ethyl-3, 7, 11-trimethyl-2, 4-dodecadienoate (ZR 512) on the body color change, the growth and the metamorphosis of *Cephonodes hylas* were examined by means of the topical application to the last instar larvae at various ages. The effects of synthetic *Cecropia* JH and 1-(3, 4-methylenedioxyphenyl)-7-epoxy-4, 8-dimethyl-nona-1, 3-diene (CT 5) were also tested in order to compare with that of ZR 512, by employing 4 days old larvae of the last instar.

Material and Methods

Larvae of *Cephonodes hylas* were reared under a condition of about 25°C as described in the previous paper[3]. The last instar larvae were used for the experiments.

Cecropia JH (methyl-12, 14-dihomojuvenate), ZR 512 (ethyl-3,7,11-trimethyl-2,4-dodecadienoate, purity: 75%) and CT 5(1-(3, 4-methylenedioxyphenyl)-7-epoxy-4, 8-dimethy-nona-1, 3-diene, purity: 70%) were supplied by the courtesy of the Laboratory of Applied Zoology, Tokyo University of Education. They had been donated to the laboratory from Prof. K. Mori, University of Tokyo, Ohtsuka Corporation, Tokushima and Prof. S. Tamura, University of Tokyo, respectively. These compounds, mixed isomers, dissolved in 1 μl of acetone were applied topically to the dorsal integument of the thorax of the larvae. As the control 1 μl of acetone was applied.

Results and Discussion

Twenty μg of ZR 512 was applied topically to the last instar larvae at various ages.

As shown in Table 1, the effects of ZR 512 were related to the time of development at which the chemical was applied. When ZR 512 was applied to 1 to 3 days old larvae of the last instar, the reddening was delayed 2-3 days and the cocoon spining 3-4 days. The time required for red pigmentation varied with the age of the larvae treated. In the older larvae, the longer period was required for development after the last larval ecdysis. But the body weight of matured red larvae did not increase with the length of the time required for development of pigmentation after the last larval ecdysis, if anything, it increased very slightly. Though the red coloring was suppressed by the application of ZR 512, the degree of suppression was not related to the age of larvae at which they were treated. These larvae pupated normally with a delay of 3-4 days.

When ZR 512 was applied to the 4 days old larvae of the last instar, inhibition of cocoon spining, a raising of larval-pupal intermediates, and super larvae were induced, in addition to the suppression of reddening and prolongation of larval period.

However, application of ZR 512 to the 5 days old larvae of the last instar just after green integument turned into heavy dark red did not give any effect on the development, excepting the inhibition of disappearance of the red pigments from epidermal cells[3]. It appears that the sensitivity of the tissue to the chemical is decreased between the 4th and 5th days in the last instar adversely with an increase of ecdysone titer.

To ascertain the effect of ZR 512 on the development, various concentrations of sample were applied on the 4 days old larvae of the last instar. As shown in Table 2, the suppression of red pigmentation and raising of super larvae were brought about with increasing of concentration. The duration between application of the chemical and appearance of red pigments in the integument, or that between the last larval ecdysis and the next molting (duration of the last larval instar), including extra larval ecdysis or normal pupal ecdysis, were prolonged with the increase of concentration. It appears that 4 days old larvae are commited for synthesis of pupal cuticle and that only in the presence of sufficient quantity of ZR 512 do these larvae reprogram to deposit a larval cuticle.

Effects of the other two chemicals on the development were compared with those of ZR 512. *Cecropia* JH was active as ZR 512. But CT 5 was inactive up to the dosage of 50 μg.

Table 1. Effects of ZR 512 at a dosage of 20 μg on the last instar larvae of *Cephonodes hylas*

Time of application (days after the last larval ecdysis)	Weight (g)	Body color at the time ZR 512 was applied (score for dark red pigmentation)	Intensity of dark red pigmentation[b]						Cocoon formation[c]	Duration from last larval ecdysis to reddening (days)[d]	Weight of reddening larvae[d] (g)	No. of larval-pupal intermediates	No. of super larvae	No. of normal pupae	Duration from last larval ecdysis to next molting (days)[d]
			0	1	2	3	4	5							
1	1.5	Bright green (0)		1	3		2		+	6~9 (7.5)	4.0~4.8 (4.3)	0	0	6	10~13 (11.6)
2	2.6	Bright green (0)				2	1	1	+	7~9 (8.0)	4.1~4.9 (4.5)	0	0	4	10~13 (11.3)
3	3.4	Bright green (0)		2	1		1		+	8~9 (8.5)	4.1~4.9 (4.6)	0	0	4	12~13 (12.5)
4	3.7	Bright green (0)	2	4					−	6~11 (7.0)		3	2	1	9~15 (11.1)
5[a]	3.1	Heavy dark red (5)							+	4~6 (5.0)		0	0	9	6~8 (7.6)
Controls							1	9	+	4~6 (5.0)	2.7~3.4 (3.1)	0	0	10	6~9 (8.1)

a) Effect of ZR 512 on the depigmentation has already been reported (see, Ikemoto 1975).
b) Intensity of dark red pigmentation was estimated in score on a scale of 0 to 5.0, no reddening; 5, heavy dark red; 1-4, intermediates between 0 and 5.
c) +, not inhibited; −, inhibited.
d) The number in brackets indicates the mean value.

Table 2. Effects of some juvenile hormone analogues on the development of *Chephonodes* larvae[a]

Sample	Dosage	Intensity of dark red pigmentation[b]						Cocoon formation		Days from application to reddening	Duration from last larval ecdysis to next molting (days)[b]	No. of larval-pupal intermediates	No. of super larvae	No. of normal pupae
		0	1	2	3	4	5	+	−					
ZR 512	40 μg	5						0	5		10~16 (12.3)	0	5	0
	20	2	4					0	6	1~6 (2.0)	9~15 (11.1)	3	2	1
	10		2			3	2	4	3	1~2 (1.1)	7~11 (8.4)	3	0	4
CT 5	50						10	10	0	1	6~ 9 (8.0)	0	0	10
Cecropia JH	20	1	3					1	3	1	9	3	0	1
	10		1			3		4	0	1	8	0	0	4

a) Chemicals were applied topically to the 4 days old larvae of the last instar.
b) Symbols are as in Table 1.

A larva keeping a green color without reddening and failed in cocoon formation was found among the larvae treated with *Cecropia* JH. This larva pupated normaly. This result means that the absence of red pigments in the epidermis or the failure of cocoon formation is not correlated with the absence of larval or pupal ecdysis.

In the studies with larvae of the silkworm, *Bombyx mori*, Akai *et al.*[5] showed that the last instar larvae are less sensitive during the first 3 days to *Cecropia* JH than those of 4-5 days old, and the 6 days old larvae which are wandering and devoting themselves to find a place suitable for pupation are exactly insensitive. The larvae of *Cephonodes hylas* showed a similar response cycle to ZR 512 to the case of silkworm mentioned above. Hintze-Podufal and Fricke[4] showed that wandering larvae of *Cerura vinula* were insensitive to two JH analogues. The larvae of the most endopterygote insects such as Lepidoptera and Coleoptera were less sensitive to JH or its analogues during the first half of the last instar[1,2]. It seems likely that this pattern of sensitivity to JH in the last instar larvae of *Bombyx mori* may well be established extensively among the larvae of many other lepidopterous species.

Although further detailed experiments on the effects of ZR 512 on the *Cephonodes* larvae are necessary to perform, it seems likely that with the progress of age at which the larvae are treated, or by increasing the concentration of ZR 512, the effects induced by the chemical may be progressed in the process: Normal larvae→ prolongation of larval period→suppression of red pigmentation → induction of larval-pupal intermediates and inhibition of cocoon formation→ induction of extra larval ecdysis→inhibition of elimination of red pigments from epidermal cells. A similar conclusion was obtained in respect to the effects of *Cecropia* JH on the change in the growth and pupation process of *Bombyx* larvae[5].

Such a sequential pattern of sensitivity to ZR 512 in *Cephonodes* larvae is probably dependent on the concentration of both kinds of hormone, juvenile hormone and especially molting hormone in the body. It is supposed that the concentration of molting hormone increases rapidly and that of juvenile hormone decreases rapidly toward the end of the larval stage in the larger pellucid hawk moth, *Cephonodes hylas*, as in the case of the silkworm, *Philosamia cynthia*[6]. It is well known that implantation of corpora allata into the last instar larvae prior to a certain critical period causes a prolongation of larval period, raising of extra larval instar or larval-pupal intermediates[7].

The last instar larvae of *Cephonodes hylas* are characterized with impressive change, for instance, in behavior and morphological external characters[3]. These processes are probably controlled by the balance between ecdysone, the hormone of the prothoracic glands and the juvenile hormone of the corpora allata. The red pigmentation, ellimination of red pigments from the epidermal cells and pupation are induced by injection of molting hormone. Its effective concentrations vary with the step of metamorphosis[3,8]. But, on the other hand, JH or its analogue ZR 512 prevents the depigmentation[3]. The results of the experiments showed that JH and its analogue ZR 512 suppress the red pigmentation, cocoon spining and pupation. Furthermore, it appears that JH was responsible in keeping the green color in the integument of *Cephonodes* larvae.

The positive or negative geotactic behavior of larvae of the hawk moth, *Mimas tiliae*, is determined by the concentration of JH[9,10]. Prior to the pupation, larvae of *Cephonodes hylas* leave grass food, crowl down a tree and dig the ground where they spin a loose cocoon and pupate, as in the case of *Mimas* larvae. Detailed experiments on these subjects in *Cephonodes* larvae will be carried out in the future.

Summary

To ascertain the effects of a JH analogue, ethyl-3, 7, 11-trimethyl-2, 4-dodecadienoate (ZR 512) on the body color change, growth and metamorphosis of *Cephonodes hylas* were studied by the topical application of the chemical to the last instar larvae at various ages. The larvae showed a low response to the chemical during a period from the 1st to the 3rd day after the last larval ecdysis and maximum sensitivity appeared on the 4th day in the last instar. Five days old larvae were almost insensitive to the chemical at least within the range of concentrations tested

in the present experiment, being effective to prevent from disappearance of the red pigment from epidermal cells.

The effects of the other two chemicals, *Cecropia* JH (methyl-12, 14-dihomojuvenate) and 1-(3, 4-methylenedioxyphenyl)-7-epoxy-4, 8-dimethylnona-1, 3-diene (CT 5) on the developmental process were tested in order to compare with those of ZR 512, on the 4th day in the last instar. *Cecropia* JH was active as ZR 512, but CT 5 was inactive up to the dosage of 50 μg.

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Quinol Phosphate as a Metabolite of Triphenyl Phosphate. Morifusa Eto, Hiroto Miyamoto* and Yasuaki Hashimoto** (Department of Agricultural Chemistry, Kyushu University, Fukuoka, Japan). Received April 28, 1975. *Botyu-Kagaku*, 40, 106, 1975.

20. リン酸トリフェニルの代謝産物としてのキノールリン酸エステル

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マラソンの共力剤である triphenyl phosphate (TPP) をイエバエに投与し，代謝産物として diphenyl *p*-hydroxyphenyl phosphate (TPP-OH) を得た．TPP-OH はカルボキシエステラーゼ阻害活性の弱い TPP よりもさらに弱い活性しか示さなかった．TPP のマラソン共力作用はエステラーゼ阻害によらず，他の機構によるものではないかと推察される．

With a few exceptions, triaryl phosphates which manifest synergistic activity with malathion have at least one alkyl group on the ortho position[1]. They may be biotransformed into active metabolites, i.e. cyclic phosphate esters, which have a high antiesterase activity, as demonstrated with tri-*o*-tolyl phosphate and some related esters[2,3]. The exceptions are tri-*p*-ethylphenyl phosphate and triphenyl phosphate. The metabolic activation of the former has been shown by finding of the formation of α-oxo derivatives[4]. The bioactivation of all these alkylaryl phosphates is initiated by the hydroxylation of the α-carbon of the alkyl group followed by such a subsequent reaction as intramolecular transphosphorylation or dehydrogenation[5]. This paper deals with the metabolic ring-hydroxylation of triphenyl phosphate (TPP) in houseflies to give a quinol phosphate.

Materials and Methods

Syntheses

Triphenyl phosphate was prepared by heating the mixture of phenol and phosphorus oxychloride under reflux. It was distilled under reduced

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