JUVENILE HORMONE AND SYNTHETIC ANALOGUES: EFFECTS ON LARVAL MOULT OF SILKWORM, *BOMBYX MORI*

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SUMMARY: Morphogenetic activities of three synthetic compounds, *dl*-C₁₈-*Cecropia* juvenile hormone and its two analogues, on larval-larval moult of *Bombyx* silkworms were evaluated. Allatectomized 4th instar larvae moulted into 5th instar after injection or topical application of a certain amount of each compound. *dl*-C₁₈-*Cecropia* juvenile hormone was most active; the dosage required for 50 % larval moult induction was only 0.12 µg per insect by topical application. No defect was seen in the adults developed from 5th instar larvae induced by the treatment with these compounds. The results mean that a single treatment with each compound satisfactorily substituted the juvenile hormone secreting organ, corpus allatum.

INTRODUCTION

Following purification and identification of the juvenile hormone (JH) from *Cecropia* male moths (Röller et al., 1967; Meyer et al., 1968), morphogenetic activities of the extracted JH and synthetic analogues were tested on many hemimetabolous (Wigglesworth, 1969; Hopkins et al., 1970) and holometabolous (Röller and Dahm, 1968; Sehna and Meyer, 1968; Srivastava and Gilbert, 1968; Hsiao and Hsiao, 1969; Meyer et al., 1970) insects. In these experiments the activities were evaluated on unnatural morphogenesis such as excess larval moult of the last instar larvae or the formation of second pupae. Moreover, corpora allata of test insects often remained intact.

Therefore, to evaluate the activities of JH and JH analogues correctly, we chose allatectomized 4th instar larvae of the silkworm as the experimental animal. It enabled us to study the natural action of JH and to know whether JH could really substitute the JH secreting organ, corpus allatum.

This paper deals with the activities of synthetic C₁₈-*Cecropia* JH and two synthetic JH analogues upon larval-larval moult of silkworm and the relationship between the chemical structure and the activity is discussed.

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MATERIALS AND METHODS

Preparation of compounds: In the present experiments, three synthetic compounds, methyl 10, 11-oxido-*trans, trans*-7-ethyl-3, 11-dimethyl-2, 6, 10-dodecatrienoate (Compound I), stereoisometric mixture of C_{17} -*Cecropia* juvenile hormone (Compound II) and *dl*- C_{18} -*Cecropia* juvenile hormone (Compound III), were tested. The skeleton formulae of these three compounds are shown in Fig. 1.

Fig. 1. Skeleton formula of compounds assayed for JH activities.

Procedures for syntheses of Compound I and II were already described in detail by one of the authors (Mori *et al.*, 1969 a, b). Compound III was synthesized by Loew *et al.* (1970).

Test animals and treatment of compounds: Fourth instar larvae of the silkworm, *Bombyx mori*, of F_1 hybrid between two races, females of J. 122 and males of C. 115, were used. Twenty-four hr after the 3rd moulting at 25 C, the corpora allata were extirpated with sharpened forceps under a dissecting microscope. Three hr after the operation, a certain amount of each test compound dissolved in 0.5 μ l of peanut oil was injected with a 100- μ l microsyringe controlled by a micrometer. The needles was pierced through the base of 4th abdominal leg into the haemocoel near the base of 1st abdominal leg. By this manner, no injected oil leaked out from the puncture.

In another series of the experiments, peanut oil solution of the compound was diluted 10-fold with redistilled acetone, and 5 μ l of the diluted solution was topically applied on the whole dorsal surface of each allatectomized larva.

After the treatment, the animals received immediately a plenty of fresh mulberry leaves, and were reared at 25 C. The body weights of the larvae at the time of the operation were 0.25 to 0.30 g.

Evaluation of results: Effects of the compounds were evaluated after the next moulting. The most affected animals moulted into normal 5th instar larvae without any pupal characters. Less affected animals moulted into wide variety ranging from larval-pupal intermediate type to perfect precocious pupae. The effects were classified and scored as shown in Table 1.

Further observations were made until the treated animals became adults, and mating behavior and oviposition were also recorded.

Table 1. Classification of the effects on metamorphosis

Score	States of metamorphosis
—	Perfect precocious pupae
+	Pupal moult with larval character on cuticle or with imperfect pupal development of mouthpart, wing and leg or with both of them
++	Larval moult with partially pupal cuticle
###	Perfect 5 th instar larvae without pupal character

RESULTS AND DISCUSSION

The results of the tests with three compounds are shown in Table 2. The most active substance was *dl*-C₁₈-*Cecropia* JH (Compound III); the other two compounds also possessed high JH activities. As compared with the results on *Galleria* (Röller and Dahm, 1968), sensitivity of the silkworm to Compound III was almost 10 times higher in terms of dose per body weight. The difference may have resulted mainly from the development stage of the test animals; the last instar larvae were used in the *Galleria* test, while 4th instar larvae in the present experiments. In our preliminary experiments, much larger amount of JH was required to cause supernumerary larval moult on the last instar *Bombyx* larvae.

Compound I was less active than Compound II or III. This means that terminal ethyl group at C-11 is important for such high JH activity. Replacement of the ethyl group at C-7 with a methyl group did not so strongly influence the activity; the activity of Compound II was half of that of Compound III on injection though the former is a stereoisometric mixture.

The activity of Compound III was expressed higher by topical application than by injection (Table 3). Similar results were reported by Bowers et al. (1965) in the assay of the JH activity of synthetic epoxy sesquiterpenoids on *Tenebrio*. They interpreted that the lower activity by injection was due to rapid metabolic inactivation of injected substance, and that the higher activity by topical application to the slow partition of active material through the integument. By determining the activities of more than 30 of JH and JH analogues on *Rhodnius*, Wigglesworth (1969) stated that all but one epoxide sesquiterpenoids showed higher activity by injection than by topical application. In the present experiments, Compound I and II were shown to be less active by topical application as was the case on *Rhodnius* (Table 3). Such discrepancy in the assay of JH activity may have been due to the different species used, in which different rates of penetration of the active substances through cuticle or inactivation in insects or both might occur. Physical properties and localization of injected oil drops in haemocoel should also be considered.

The adults developed from 5th instar larvae, which were produced from the allatectomized 4th instar larvae treated with a sufficient amount of the active substances, did not show any detectable abnormality on both external morphology and behaviour. They displayed normal courtships and laid fertilized eggs.

Allatectomized 4th instar *Bombyx* larvae were used as test animals for the first

Table 2. Effects of JH and JH analogues on *Bombyx* larvae:

Injection						
Compound	Dose (μg)	No. of animal tested	No. of animal		transformed into	
			—	+	††	‡‡
I	20	19	4	4	7	4
	10	20	9	3	3	5
	5	20	20	0	0	0
II	10.0	17	0	0	0	17
	1.0	20	8	3	2	7
	0.1	18	18	0	0	0
III	1.0	20	4	3	1	12
	0.5	20	9	2	5	4
	0.25	17	13	2	1	1
	0.10	19	19	0	0	0
Control (Peanut oil 0.5 μl)		20	20	0	0	0
Topical application						
I	100	13	9	3	1	0
	60	16	15	1	0	0
	30	17	16	1	0	0
II	10.0	21	0	0	4	17
	5.0	25	6	3	1	15
	2.5	13	6	2	3	2
III	0.5	16	1	0	1	14
	0.25	15	0	0	2	13
	0.125	16	7	0	0	9
	0.063	20	19	0	1	0
	0.031	20	20	0	0	0
Control (Peanut oil 0.5 μl)		43	43	0	0	0

Table 3. Comparison of JH activity by injection with that by topical application

Compound	Dose in μg /animal to give 50 % larval moult of allatectomized 4 th instar <i>Bombyx</i> larvae	
	Injection	Topical application
I	14	>100
II	1.2	2.7
III	0.6	0.12

time by Fukuda (1962) to demonstrate the endocrine activity of the transplanted corpora allata.

This test method was adopted by us for quantitative assay of JH activity and for obtaining basic knowledge on the biological effects of the synthetic substances. Performing the experiments, we realized that this method is very useful for its short duration, ease of determination and high sensitivity although producing a number of allatectomized larvae requires painstaking work.

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