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Utilization of 20-Hydroxyecdysone Extracted from a Plant in Sericulture

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Abstract

20-Hydroxyecdysone, which is one of the hormone active substances in insects, was found to be contained in an African native plant, and a method of extraction and purification was developed by a chemical company. Then, studies were carried out in order to apply it to sericulture to promote larval maturation. The toxicity of 20-hydroxyecdysone to the 5th instar larvae was low, and experimental results showed that it never affected the survival when orally administered. The administration to newly ecdysed larvae prolonged the larval duration while the administration in the latter half of the 5th instar led to the shortening of the larval duration and growth was synchronized thereafter. The quantitative parameters of the cocoon, especially cocoon shell weight, tended to increase when the substance was administered within 2 days after the 4th ecdysis. The cocoon shell weight tended to decrease when the duration of the 5th instar was shortened by excessive administration of the substance. Thus, it was concluded that 20-hydroxyecdysone was safe and effective for sericulture. Then, the compound was approved by the Japanese Government as an agricultural chemical for use in mounting in sericulture in February 1994.

Discipline: Sericulture
Additional key words: cocoon, maturation, mounting, silkworm

Introduction

Insect hormone active substances have been studied mostly for application as a pesticide in the last 20 years. In sericulture for silk production, studies on their utilization for silkworm rearing were promoted in Japan, since these substances were extracted from some plants[2,7]. For instance, synthesized juvenile hormone active substances were studied for utilization for rearing[1,11], and Methoprene which is one of the juvenile hormone analogues was disseminated as an agricultural chemical for the increase of cocoon shell weight[8]. On the other hand, β-ecdysone active substance, such as Inokosterone extracted from some plants, was studied[12] and attempts were made to use it for growth control as an accelerator of larval maturation[3]. It became available on the market, although it was not widely used by farmers.

Recently 20-hydroxyecdysone, which is similar to β-ecdysone as insect molting hormone, has been detected in large amounts (5% or more) in the root bark of an African native plant, *Vitex strikeri*, and methods for extraction were developed[4,13]. Then studies on application to silkworm rearing as an accelerator of larval maturation were initiated in our Institute[5,9]. We tried to collect essential data for application as an agricultural chemical. Finally the 20-hydroxyecdysone from *Pfaffia iresinoides*[10] was approved as an agricultural chemical for sericulture by the Japanese Government in February 1994.

In this paper, the effect of 20-hydroxyecdysone administered in the 5th instar on larval growth such as maturation and synchronization, cocoon production and survival is reported.

Materials and methods

The silkworm race used in this experiment was C 146 × N 137. The sex of the hybrid race can be distinguished by the shade of body markings. Ten female and 10 male larvae in the 5th instar were used for the experiment.

Ten mg of 20-hydroxyecdysone (95% grade purity) given by Daicel Chemical Industries, Ltd. was

dissolved in 0.1 ml of ethanol and 0.9 ml of sterilized water was added. The solution, 10 μg/μl, was used as initial solution.

20-Hydroxyecdysone was administered by injection or oral ingestion with the initial or diluted solution in various periods of the 5th instar. The micro-injector manufactured by Kiya Seisakusho Ltd. was used. After treatment, maturation, survival and quantitative characters of the cocoon were examined in each individual.

Results

1) Effect of 20-hydroxyecdysone on the acceleration of larval maturation and survival

Five (5.08) and 6 (5.88) days old larvae were injected with or forced to swallow 0.04 to 20 μg of 20-hydroxyecdysone per individual. The effects on mature larvae are shown in Figs. 1 and 2. When the product was injected at 5 days, rapid maturation at 5.96 days after the first feeding in the 5th instar was observed following the injection of 10 to 20 μg per larva, as compared with the control which received 1 μl of 10% ethanol, and in which maturation occurred at 6.88 to 8.50 days. Maturation at 6.63 to 7.5 days was observed when 0.04 to 2.5 μg were injected. It was observed that the shortening of the larval duration was correlated with the dose of 20-hydroxyecdysone administered (Fig. 1 upper). In the injection at 6 days, synchronization of growth was carried out in all the groups to which 0.04 to 20 μg were injected, though the shortening of the duration was not drastic (Fig. 1 lower).

When drops were swallowed at 5 and 6 days (Fig. 2), the effect on the acceleration of maturation was evident in the experiments where 2.5 to 20 μg were administered. The effect of the amount of 20-hydroxyecdysone administered on the maturation and synchronization was evident for a dose of 2.5 to 20 μg per larva as in the case of injection.

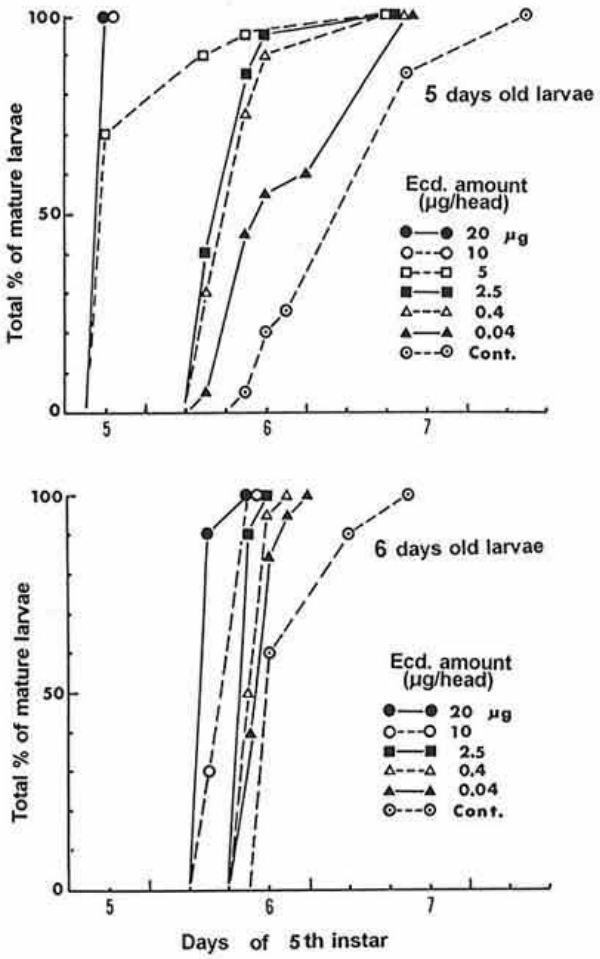

Fig. 1. Promotion of larval maturation by injection of 20-hydroxyecdysone

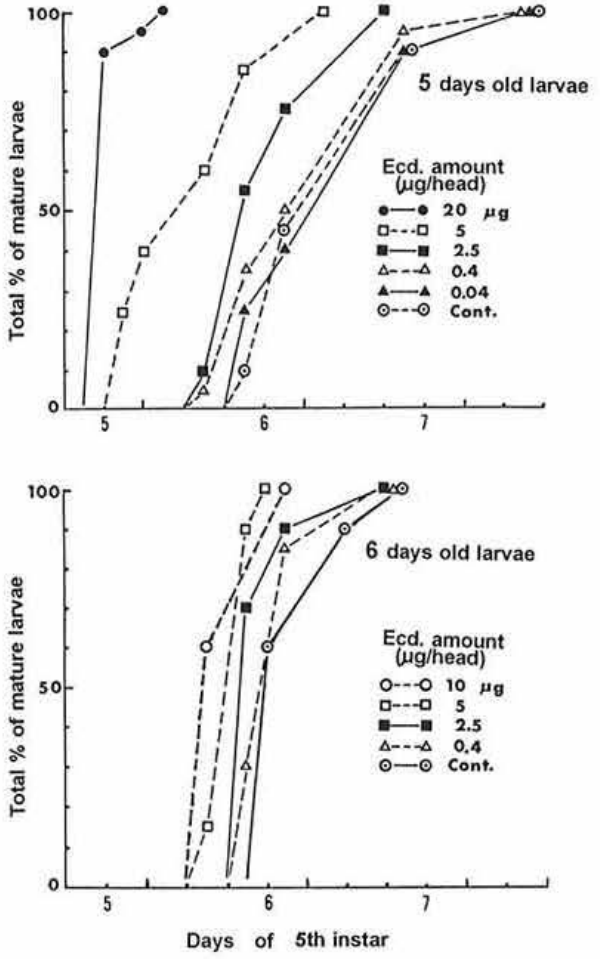

Fig. 2. Promotion of larval maturation by oral administration of 20-hydroxyecdysone

Table 1. Effect of 20-hydroxyecdysone administered to 5th instar larvae on pupal survival

Time of administration		5 days old 5th instar larvae			6 days old 5th instar larvae		
Method	Amount (μg)	No. of larvae	No. of pupae	Survival ratio	No. of larvae	No. of pupae	Survival ratio
Injection	20	20	1	5	20	1	5
	10	20	17	85	20	14	70
	5	20	20	100	20	20	100
	2.5	20	20	100	20	20	100
	0.4	20	20	100	20	19	95
	0.04	20	19	95	20	18	90
	10% ethanol	20	19	95			
Oral administration	20	20	20	100	–	–	–
	10	–	–	–	20	19	95
	5	20	20	100	20	20	100
	2.5	20	20	100	20	20	100
	0.4	20	19	95	20	20	100
	0.04	20	19	95	20	20	100
	10% ethanol	20	20	100	20	20	100
Control		20	19	95			

Survival of the pupae was examined 7 days after maturation, as all the larvae used in these experiments produced a cocoon, although the cocooning level was different. The results on pupation are shown in Table 1. Only one pupa was observed in the groups of 5 and 6 days old larvae to which 20 μg of 20-hydroxyecdysone was injected. Survival ratio reached 85 and 70% in both groups injected with 10 μg per larva, respectively. Almost all the larvae in which less than 5 μg of 20-hydroxyecdysone was injected pupated normally, though the cocoon size tended to increase in several groups. On the other hand, a dose of 20-hydroxyecdysone was not lethal in the experiments with oral administration.

2) Effects of administration of 20-hydroxyecdysone on the duration of the 5th instar

Twenty larvae (♀: 10, ♂: 10) were forced to swallow 1 to 10 μg of 20-hydroxyecdysone per larva every day from 1 day and 5 h (1.21 days) after the first feeding in the 5th instar to 1 day before maturation (6.29 days). The average duration of the 5th instar in the male and female is shown in Fig. 3. The duration of the 5th instar was prolonged

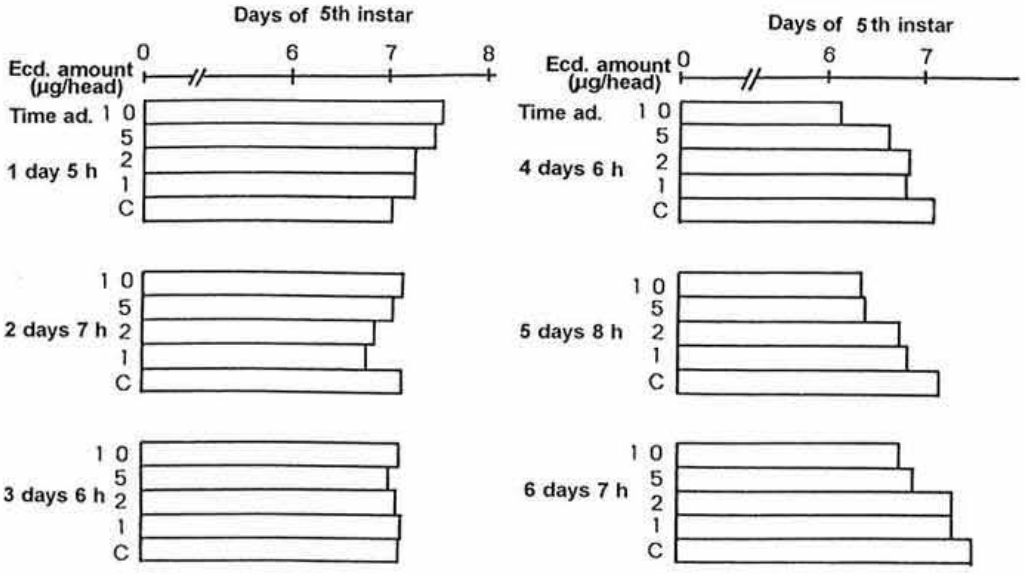

Fig. 3. Effect of 20-hydroxyecdysone administered at 5th instar on larval duration

in the groups which received the compound 1 day after the first feeding in contrast to the control which swallowed 1 μl of 10% ethanol. The delay in the growth was maximum by 0.5 day in the group which received 10 μg. It was suggested that the prolongation of the duration of the 5th instar depended on the amount of 20-hydroxyecdysone administered.

The administration on and after 4 (4.25) days shortened the duration of the 5th instar, though the treatment at 2 and 3 days did not result in appreciable differences from the control. The shortening of the duration of the 5th instar was maximum in the case of administration at 4 days by 1 day approximately. Furthermore, there was a correlation between the amount of 20-hydroxyecdysone administered and the shortening of the duration of the 5th instar.

3) Effects of time and amount of 20-hydroxyecdysone administered on the quantitative characters of the cocoon

The cocoons which were obtained in the experiments shown in Fig. 3 were investigated for several characters. The averages of total cocoon weight and cocoon shell weight from 10 individuals of both sexes are shown in Figs. 4 and 5. The total cocoon weight tended to increase in the groups treated with 20-hydroxyecdysone at 1 day after the first feeding. The tendency was maximum for 10 μg and was not recognized for 1 μg. The administration at 2 and 3 days after the first feeding did not result in any

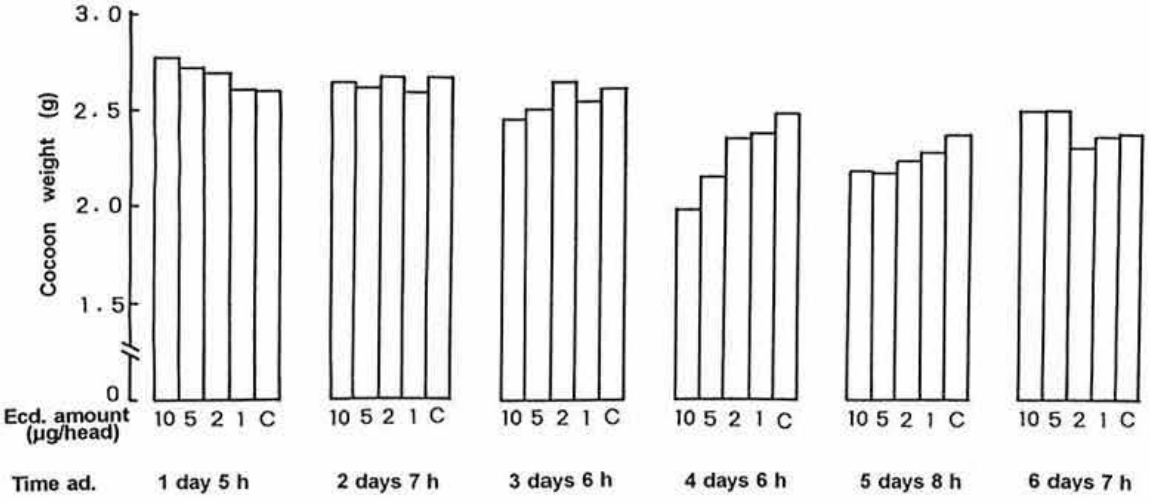

Fig. 4. Effect of 20-hydroxyecdysone administered at 5th instar on total cocoon weight

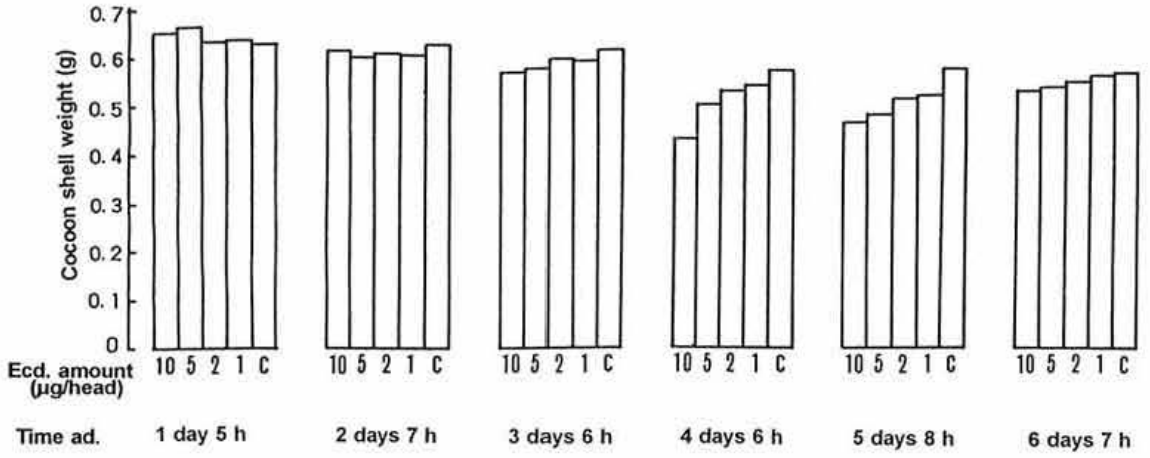

Fig. 5. Effect of 20-hydroxyecdysone administered at 5th instar on cocoon shell weight

obvious effect. The cocoon weight of the groups in which the duration of the 5th instar was shortened by the treatment at 4 and 5 days decreased conspicuously and the tendency of the decrease was maximum for 10 μg. On the contrary, the cocoon weight increased when 5 to 10 μg of the compound was administered at 6 days after the first feeding in the 5th instar just before maturation, though it did not appear to increase for a dose of 1 to 2 μg (Fig. 4).

The cocoon shell weight tended to decrease when the compound was administered on and after the 4th day, though no significant differences were observed in the treatment on and before the 3rd day after the first feeding in the 5th instar. When the compound was administered on and after the 4th day, the decrease of the cocoon shell weight also depended on the amount of 20-hydroxyecdysone administered. Especially the decrease in the cocoon shell weight was conspicuous, when the compound was administered at 4 days (Fig. 5).

Four similar experiments were repeated from the spring of 1990 to late autumn 1991, and the results were identical.

Discussion

The main purpose of these studies was to collect basic data to apply for the registration of 20-hydroxyecdysone as an agricultural chemical. Therefore, the test on the toxicity of 20-hydroxyecdysone was conducted in detail. As the concentration of 20-hydroxyecdysone in the initial solution was 10 μg/μl (10,000 ppm) at which the compound showed maximum solubility in water, it was not toxic for the 5th instar larvae of the silkworm when orally administered. Therefore, the experiments on administration by injection were conducted additionally. In the experiments by injection, a dose of 20 μg led to larval death without pupation in 95% of the cases. However, oral administration of 20 μg did not affect the survival. It was assumed that the effect of the oral administration was less than one half of the administration by injection, based on the results from other experiments. Accordingly, it was concluded that the use of 20-hydroxyecdysone was safe if 4 l of a 10 ppm solution was sprayed on fresh mulberry leaves for 20,000 larvae[5,9].

To confirm the validity of the results of this report, some of the results on the duration of the 5th instar in the experiment where the compound was administered orally were compared with the data on injection reported by Moroboshi[6]. Fig. 6 shows that the administration at about 1 day after the first feeding in the 5th instar prolonged the duration of the 5th instar afterwards, while the administration on and after the 4th day shortened the duration. The pattern shown in Fig. 6 was similar to Moroboshi's data, although the methods of administration were different.

For use in sericultural farms, the response of silkworms to 20-hydroxyecdysone is summarized in Table 2. The use of 20-hydroxyecdysone in these experiments was effective due to the high purity of the compound (90 to 95%). Consequently the effects on maturation and synchronization of growth enable to use the compound in the field, although the quantitative parameters of cocoon may decline

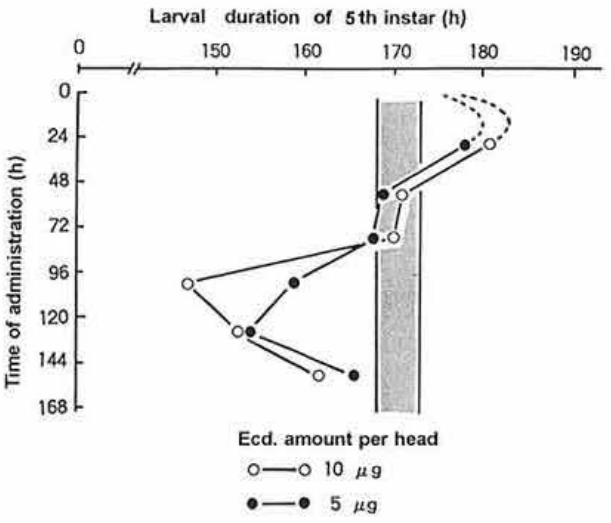

Fig. 6. Effect of time of oral administration of 20-hydroxyecdysone on larval duration

Table 2. Time of administration of 20-hydroxyecdysone in the latter half of 5th instar and effects

Time of administration	Shortening of duration	Synchronization of growth	Cocoon weight	Cocoon shell weight
Early	Less than 1 day	Not-expected	Slight decrease	Decrease
Normal	Adequate	Good	Less decrease	Less decrease
Late	Not appreciable	Good	No change	Small decrease

depending upon the dose and time of administration.

References

1) Akai, H. & Kobayashi, M. (1971): Induction of prolonged larval instar by the juvenile hormone in *Bombyx mori* L. *Appl. Entomol. Zool.*, **6**, 138–139.
2) Bowers, W. S. et al. (1966): Juvenile hormone: identification of an active compound from Balsam fir. *Science*, **154**, 1020–1021.
3) Ito, T. et al. (1968): Acceleration of maturation of larvae of the silkworm by feeding of insect-molting hormone from plants. *Tech. Bull. Sericul. Exp. Stn.*, **92**, 21–40 [In Japanese with English summary].
4) Kubo, I. & Matsumoto, T. (1988): A plant, *Vitex strickeri*, including high amount of insect molting hormone. *Jpn. Agric. Biochem. Abstr.*, **62**, 329 [In Japanese].
5) Mizusawa, H. et al. (1991): Studies on rearing and mounting in the silkworm race adapted to the low-cost artificial diet. *Bull. Natl. Inst. Sericul. Entomol. Sci.*, **3**, 77–88 [In Japanese with English summary].
6) Moroboshi, S., Hugo, H. & Shimada, J. (1975): The control of growth and development in *Bombyx mori*. 14. Ecdysone analogue titer in the fourth instar and combination experiments with allatectmy and injection of ecdysterone during the fifth larval instar. *Proc. Jpn. Acad.*, **51**, 56–61.
7) Nakanishi, K. et al. (1966): Insect hormones: the structure of ponasterone A, an insect-molting hormone from the leaves of *Podocarpus nakaii* HAY. *Chem. Comm.*, **1966**, 915–917.
8) Nihmura, M. et al. (1974): Effect of novel analogues of the juvenile hormone. I. Effect of juvenile hormone analogues with different alkyl substituents at C-7 and C-11 on the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). *Appl. Entomol. Zool.*, **9**, 34–40.
9) Ninagi, O. et al. (1993): Automatic machine for silkworm rearing and use of β-ecdysone for mounting. *Bull. Natl. Inst. Sericul. Entomol. Sci.*, **9**, 7–17 [In Japanese with English summary].
10) Nishimoto, N. et al. (1987): Ecdysteroids from *Pfaffia iresinoides* and reassignment of some CNMR chemical shifts. *Phytochemistry*, **26**, 2505–2507.
11) Otaki, T., Takeuchi, S. & Mori, K. (1971): Juvenile hormone and synthetic analogues; effects on larval molt of silkworm, *Bombyx mori*. *Jpn. J. Med. Sci. Biol.*, **24**, 251–255.
12) Takemoto, T. et al. (1968): Isolation of insect molting substances from *Pteridium aquilinum* var. *latiusculum*. *Chem. Pharm. Bull.*, **16**, 762.
13) Zhang, M., Stout, M. J. & Kubo, I. (1992): Isolation of ecdysteroids from *Vitex strickeri* using RLCC and recycling HPLC. *Phytochemistry*, **31**, 247–250.

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