Juvenile Hormone Activity of Terpenoid Esters and Phenyl-Geranyl-Ethers with Different Alkyl Substituents against the Silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae)

Kenji KIGUCHI, Tetsuya OHTAKI*, Hiromu AKAI and Kenji MORI**

Silkworm Physiology Division, The Sericultural Experiment Station, Sugincmi-ku, Tokyo, 166 Japan

**Department of Entomology, The National Institute of Health, Shinagawa-ku, Tokyo, 141 Japan*

***Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo, 113 Japan*

(Received August 18, 1973)

A total 37 of terpenoid esters and phenyl-geranyl-ethers with different alkyl substituents were newly synthesized as stereoisomeric mixtures, and their biological activities were assayed using allatectomized fourth instar larvae of *Bombyx mori*. The chain length of some alkyl substituents of these compounds was highly correlated with the biological activity. The relationship between the chemical structure and the biological activity of these compounds was discussed.

INTRODUCTION

Our efforts to find the compounds with high juvenile hormone (JH) activity have been continuing, as the JH and its derivatives have the possibility of being developed not only as insecticides, but also as growth control agents of the commercial silkworm to increase the silk productivity (VINSON and WILLIAMS 1967, AKAI et al. 1971, CHANG et al. 1972, MURAKOSHI et al. 1972, NIHMURA et al. 1972).

Recent studies on systematic survey directed toward the elucidation of structure-activity correlation (MORI et al. 1971, OHTAKI et al. 1972, MITSUI et al. 1973) has shown that there is intimate relationship between the length of some of the side chains of the *Cecropia* JH molecule and the biological activity. This relationship suggested to us that further modification of some alkyl substituents in the terpenoid esters might bring out more active compounds. Similar modifications have been conducted on the aromatic terpenoid ethers, which have been shown to display excellent JH activity (BOWERS 1969, PALLOS et al. 1971, REDFERN et al. 1971). In the present paper, the activities of compounds varying with respect to their alkyl groups upon allatectomized fourth instar larvae of the silkworm will be reported. As described in the previous paper (OHTAKI et al. 1971, 1972), this method of bioassay has been proved to be sensitive and satisfactory for the evaluation of JH activity.

MATERIALS AND METHODS

Preparation of the compounds. Basic skeleton of the compounds tested in this experiment is illustrated in Fig. 1, and their alkyl substituents are shown in Tables 1 and 2. All

Fig. 1. Skeleton formula of compounds assayed for juvenile hormone activity.

the compounds are mixtures of stereoisomers. The compounds Nos. 1, 2, 7 and 8 were synthesized following the method described by MORI (1971). The procedure of synthesis of the other compounds will be published elsewhere by MORI and others.

Biological assay. Larvae of the F_1 hybrid crossed between two varieties, J. No. 124 and C. No. 124, were used as test animals. The larvae were reared on mulberry leaves at 25 °C. Twenty-four hours after the third molt the corpora allata were extirpated with fine forceps under a dissecting microscope. Immediately after the operation, allatectomized larvae were injected with different doses of various compounds dissolved in 0.5 μl of peanut oil. After the test animal recovered from the anaesthesia, it was reared under the same condition. The effect of each compound was evaluated with the test animals after their next molt. The most affected animals molted into normal fifth instar larvae without any pupal characters. Less affected animals molted into larval-pupal intermediates, while unaffected ones became perfect precocious pupae. The JH activity was expressed in terms of the percentages of larval molt induction. Regarding to the calculation, it was assumed that the intermediate larvae can be divided into precocious pupae and larval molted ones in the ratio of one to one.

Table 1. JH ACTIVITY OF TERPENOID ESTERS WITH DIFFERENT ALKYL SUBSTITUENTS^a

Compound			Dose (μg)						
No.	Side chain		10	1	0.1	0.01	0.001	0.0003	0.0001
	R	R'							
I (m=0)									
1	Pr ⁿ	Me	100	95	75	0	—	—	—
2	"	Et	100	100	45	0	—	—	—
3	"	Pr ⁿ	100	40	0	—	—	—	—
4	Bu ⁿ	Me	100	100	100	16	0	—	—
5	"	Et	100	100	60	10	0	—	—
6	"	Pr ⁿ	100	20	0	—	—	—	—
I (m=1)									
7	Pr ⁿ	Me	—	100	63	20	0	—	—
8	"	Et	—	100	100	50	0	—	—
9	"	Pr ⁿ	—	100	100	5	0	—	—
10	Bu ⁿ	Me	—	100	100	50	10	0	—
11	"	Et	—	100	100	100	90	5	0
12	"	Pr ⁿ	—	100	100	60	5	0	—
13	Am ⁿ	Me	—	100	60	20	0	—	—

^a Percentages of larval molt inductions are shown in each column. Ten to twelve individuals were used in each test.

RESULTS AND DISCUSSION

Results of the bioassay of the terpenoid esters with various alkyl substituents are shown in Table 1.

In general, the compounds with epoxy rings showed a higher JH activity than the corresponding trienoates. In both of the compounds, compounds with *n*-propyl group at C-7 position were less active than those with methyl or ethyl groups at the same position. A long side chain such as *n*-butyl group at C-11 terminal position enhanced the activity. However, the compound with too long alkyl side chain(*n*-amyl) at C-11 possessed a reduced activity.

MORI et al. (1971) and OHTAKI et al. (1972) reported that the two alkyl groups at C-11 of the *Cecropia* JH were indispensable for the biological activity, and that

Table 2. JH ACTIVITY OF PHENYL-GERANYL-ETHERS WITH DIFFERENT ALKYL SUBSTITUENTS^a

Compound				Dose (μg)						
No.	Side chain			10	1	0.1	0.01	0.001	0.0003	0.0001
	R	R'	R''							
II (m=0)										
14	Pr ⁿ	Me	Me	100	80	0	—	—	—	—
15	⋈	Et	⋈	100	10	0	—	—	—	—
16	⋈	Pr ⁿ	⋈	20	0	—	—	—	—	—
17	Bu ⁿ	Me	⋈	100	100	30	0	—	—	—
18	⋈	Et	⋈	95	5	0	—	—	—	—
19	⋈	Pr ⁿ	⋈	100	0	—	—	—	—	—
20	Pr ⁿ	Me	Et	100	55	10	0	—	—	—
21	⋈	Et	⋈	100	85	0	—	—	—	—
22	⋈	Pr ⁿ	⋈	100	0	—	—	—	—	—
23	Bu ⁿ	Me	⋈	100	30	0	—	—	—	—
24	⋈	Et	⋈	60	0	—	—	—	—	—
25	⋈	Pr ⁿ	⋈	0	—	—	—	—	—	—
II (m=1)										
26	Pr ⁿ	Me	Me	—	—	—	100	100	100	55
27	⋈	Et	⋈	—	100	70	0	—	—	—
28	⋈	Pr ⁿ	⋈	100	0	—	—	—	—	—
29	Bu ⁿ	Me	⋈	—	—	—	100	0	—	—
30	⋈	Et	⋈	100	0	—	—	—	—	—
31	⋈	Pr ⁿ	⋈	100	85	0	—	—	—	—
32	Pr ⁿ	Me	Et	—	—	—	100	100	100	55
33	⋈	Et	⋈	—	—	100	0	—	—	—
34	⋈	Pr ⁿ	⋈	100	45	0	—	—	—	—
35	Bu ⁿ	Me	⋈	100	100	100	0	—	—	—
36	⋈	Et	⋈	30	0	—	—	—	—	—
37	⋈	Pr ⁿ	⋈	35	0	—	—	—	—	—
C18- <i>Cecropia</i> -JH ^b				100	70	0				

^a Percentages of larval molt induction are shown in each column. Ten to twelve individuals were used in each test.

^b cf. OHTAKI et al. (1971).

conversion of methyl to ethyl or to propyl at C-11 made the compound more active. They also showed that the absence of alkyl substituents at C-7 position reduced the activity considerably, although those at C-3 position did not seem to possess a significant effect on the activity. Therefore, we can tentatively summarize the effect of alkyl substituents of the JH molecule on the activity, as follows;

- (1) C-3: hydrogen $\rightleftharpoons$ methyl,
- (2) C-7: methyl $\leq$ ethyl $>$ *n*-propyl,
- (3) C-11: hydrogen $<$ methyl $<$ ethyl $<$ *n*-propyl $<$ *n*-butyl $>$ *n*-amyl.

Among the compounds, the most active one was an epoxy compound with methyl (C-3), ethyl (C-7) and *n*-butyl (C-11) groups (Compound No. 11). The activity was about one thousand times as much as the C18-*Cecropia*-JH.

In the aromatic geranyl ethers, the length of each side chain was also found to be relating to juvenile hormone activity, as shown in Table 2. A long side chain (*n*-propyl) at the middle of the molecule led to a decrease in activity, similar to the terpenoid esters. A long side chain, such as *n*-propyl, at the terminal position showed an extremely high activity, although the ethers with longer one (*n*-butyl) showed reduced activity. Epoxy compounds showed a higher activity than those without epoxy rings in the aromatic terpenoid ethers. Among the ethers tested, compounds No. 26 and No. 32 exhibited the highest activity. Their 50% effective dose was about only 0.0001 μ g per animal, being one thousand times or more active than the C18-*Cecropia*-JH.

Fig. 2. Compounds with high JH activity on the silkworm, *Bombyx mori*. The numbers in the figure show the chain lengths of the molecules.

The chemical structure of the compounds with high activity on the silkworm, *Bombyx mori*, are illustrated in Fig. 2, along with their chain length. They are all epoxides with chain lengths of 17 or 18. They are two or three atoms longer than the C18-*Cecropia*-JH. The importance of chain length in JH active compounds has been discussed by many authors (SCHNEIDERMAN et al. 1965, WIGGLESWORTH 1969, SCHWARZ et al. 1969, WAKABAYASHI et al. 1971). WAKABAYASHI et al. (1971) have pointed out that a species specificity occurs based on the length of the molecule in addition to that based on rigidity and functional groups. According to the authors, the optimum chain length is 13 for *Oncopeltus fasciatus*, but 15 for *Tenebrio molitor*. In the silkworm, *Bombyx mori*, a longer chain (17 or 18) is likely to be optimal, judging from the present study.

ACKNOWLEDGEMENTS

We are grateful to Dr. T. ITO, The Sericultural Experiment Station, and Prof. R. C. KING, Northwestern University, for reading the manuscript.

REFERENCES

- AKAI, H., K. KIGUCHI and K. MORI (1971) Increased accumulation of silk protein accompanying JH-induced prolongation of larval life in *Bombyx mori*. *Appl. Ent. Zool.* **6**: 218-220.
- BOWERS, W. S. (1969) Juvenile hormone: Activity of aromatic terpenoid ethers. *Science* **164**: 323-325.
- CHANG, C-F., S. MURAKOSHI and S. TAMURA (1972) Giant cocoon formation in the silkworm, *Bombyx mori* L., topically treated with methylene-dioxyphenyl derivatives. *Agr. Biol. Chem.* **36**: 692-694.
- ITSUI, T., C. NOBUSAWA, J. FUKAMI, K. MORI and K. FUKUNAGA (1973) Compounds with juvenile hormone activity. I. Inhibitory effects of the synthetic juvenile hormone and its analogues on the metamorphosis of several insects. *Appl. Ent. Zool.* **8**: 27-35.
- MORI, K. (1972) Synthesis of compounds with juvenile hormone activity. Part IX. Analogues of the *Cecropia* juvenile hormone lacking the methyl substituent at C-3. *Agr. Biol. Chem.* **36**: 442-445.
- MORI, K., T. ITSUI, J. FUKAMI and T. OHTAKI (1971) Synthesis of compounds with juvenile hormone activity. Part VII. A convenient non-stereoselective synthesis of the C18-*Cecropia* juvenile hormone and its analogues; Effects of the terminal alkyl substituents on biological activity. *Agr. Biol. Chem.* **35**: 1117-1127.
- MURAKOSHI, S., C-F, CHANG and S. TAMURA (1972) Increase in silk production by the silkworm, *Bombyx mori* L., due to oral administration of a juvenile hormone analog. *Agr. Biol. Chem.* **36**: 695-696.
- NIHMURA, M., S. AOMORI, K. MORI and M. MATSUI (1972) Utilization of synthetic compounds with juvenile hormone activity for the silkworm rearing. *Agr. Biol. Chem.* **38**: 889-892.
- OHTAKI, T., S. TAKEUCHI and K. MORI (1971) Juvenile hormone and synthetic analogues: Effect on larval moult of silkworm, *Bombyx mori*. *Jap. J. Med. Sci. Biol.* **24**: 251-255.
- OHTAKI, T., K. KIGUCHI, H. AKAI and K. MORI (1972) Juvenile hormone and synthetic analogues: II. Novel substances with high juvenile hormone activity. *Appl. Ent. Zool.* **7**: 161-167.
- PALLOS, F. M., J. J. MOENN, P. E. LETCHWORTH and J. B. MIANLLIS (1971) Synthetic mimics of insect juvenile hormone. *Nature* **232**: 486-487.
- REDFERN, R. E., T. P. MCGOVERN, R. SARMIENTO and M. BEROZA (1971) Juvenile hormone activity of mixed ethers containing a phenyl and a terpenoid moiety applied topically to the large milkweed bug and the yellow mealworm. *J. Econ. Ent.* **64**: 374-376.
- SCHNEIDERMAN, H. A., A. KRISHNAKUMARAN, V. KULKARNI, and L. FRIEDMAN (1965) Juvenile hormone activity of structurally unrelated compounds. *J. Insect Physiol.*, **11**: 1641-1649.
- SCHWARZ, M., B. H. BRAUN, M. W. LAW, P. E. SONNET, N. WAKABAYASHI, R. M. WATERS and M. JACOBSON (1969) Compounds related to juvenile hormone. III. *Ann. Entomol. Soc. Amer.*, **62**: 668-669.
- VINSON, J. W., and C. M. WILLIAMS (1967) Lethal effects of synthetic juvenile hormone of the human body louse. *Pro. N. A. S.* **58**: 294-297.
- WAKABAYASHI, N., M. SCHWARZ, P. E. SONNET, P. M. WATERS, R. E. REDFERN and M. JACOBSON (1971) Compounds related to insect juvenile hormones. *Mitt. Schweiz. Ent. Ges. Bull. Soc. ent Suisse* **44**: 131-140.
- WIGGLESWORTH, V. B. (1969) Chemical structure and juvenile hormone activity: comparative tests on *Rhodnius prolixus*. *J. Insect Physiol.* **15**: 73-94.