Biological activity of enantiomerically pure forms of insect juvenile hormone I and III in *Bombyx mori*

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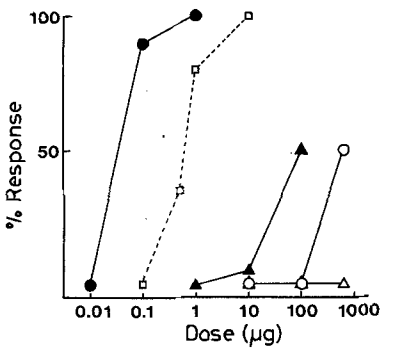
Summary. Biological activity of enantiomerically pure juvenile hormones was assayed by topical application on allatectomized *Bombyx* fourth instar larvae. JHs tested were (10R)-JH I [methyl (2E,6E,10R,11S)-10,11-epoxy-3,11-dimethyl-7-ethyl-2,6-tridecadienoate], (10S)-JH I [methyl (2E, 6E, 10S, 11R)-10,11-epoxy-3,11-dimethyl-7-ethyl-2,6-tridecadienoate], (10R)-JH III [methyl (2E,6E,10R)-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate] and (10S)-JH III [methyl (2E,6E,10S)-10,11-epoxy-3,7,11-trimethyl-2,6-dodecadienoate]. Among these compounds, natural (10R)-JH I was most active and the dose needed to induce 50% larval molting was 0.04 µg/larva; it was approximately 12,000 times more active than unnatural (10S)-JH I. Though natural (10R)-JH III showed slight biological activity, it was only one three-thousandth of that of (10R)-JH I. Unnatural (10S)-JH III exhibited no biological activity at the levels assayed.
Key words. Juvenile hormone; enantiomer; biological activity; *Bombyx mori*; allatectomy.

Since Röller's discovery of insect juvenile hormone I (JH I)[1], five JH homologs have been identified[2], i.e., JH I, II, III, 0 and iso-JH 0. Among these, JH I, II and III are involved in larval growth and metamorphosis. Though the absolute configurations of these JHs have been established as 10R[3-5], the biological activity of the natural JHs is not always known precisely, since for some time only (10R)-JH contaminated with corresponding (10S)-JH was available because of the difficulty of synthesizing the enantiomerically pure forms[6]. For the same reason, it was unclear whether unnatural (10S)-JH possesses biological activity. Both the enantiomers of JH III have now been synthesized[7] in pure form and their biological activity has been determined[8]. However, the biological activity of JH I remained to be determined, though it is of considerable significance in Lepidoptera[2], since JH I is biologically most active in this order[9,10]. Recently, both the enantiomers of JH I were chemically synthesized in 100% purity[6,11]. We therefore determined the activity of these optical isomers using *Bombyx* larvae in which JH I is the main hemolymph JH[12,13].

Eggs of the silkworm, *Bombyx mori* (racial hybrid between Gunpo and Shugyoku), were purchased from Gunze Sericultural Co, Tochigi. Larvae were reared on an artificial diet at 25 °C under a 12-h light-dark photo regime[14]. Fourth instar larvae were allatectomized at 24 h after the third ecdysis and JH, dissolved in 10 µl acetone containing 10% peanut oil, was immediately applied to the dorsal thorax and abdomen with a 100-µl microsyringe. After JH application, groups of 10 larvae were placed in a glass petri dish, fed on the diet used before, and observed until all the larvae had undergone either larval molting, larval-pupal molting or gut purge followed by pupation. The effects were classified into three grades and the percentage response was calculated according to the method described by Ohtaki et al.[15]. The results are shown in the figure, which is redrawn from the data in the table. In the *Bombyx* larval assay,



Dose-response curves for maintaining larval characteristics after the subsequent ecdysis in the allatectomized fourth instar larvae by (10R)-JH I (closed circles), (10S)-JH I (open circles), (10R)-JH III (closed triangles) and (10S)-JH III (open triangles). The curve for racemic JH I (open squares) is depicted from the data as previously reported[15].

Biological activity of JH enantiomers

Compound	Dose (µg)	Larvae molted into*		
		++	+	−
(10R)-JH I	0.01	0	0	10
	0.1	9	0	1
	1	10	0	0
(10S)-JH I	10	0	0	10
	100	0	0	10
	500	4	2	4
(10R)-JH III	1	0	0	10
	10	0	1	9
	100	3	3	3
(10S)-JH III	10	0	0	10
	100	0	0	9
	500	0	0	10

* ++, larvae molted into perfect fifth instar larvae;
+, those developing into larval-pupal intermediates;
−, those developing into precocious pupae[15].
Ten larvae were used for each dose. At the doses of 100 µg (10R)-JH III and 100 µg (10S)-JH III, one larva at each dose died within 2 days, and, therefore, these larvae were excluded from the data.

(10R)-JH I was most potent in maintaining larval characteristics in the allatectomized larvae. The effective dose of (10R)-JH I for 50% larval molting (ED_{50}) was 0.04 µg/larva which was approximately 12,000 times less than that of (10S)-JH I. It should be noted that (10S)-JH I did exhibit biological activity, though ED_{50} was 500 µg/larva.

JH III is a minor component of hemolymph JH in *Bombyx*[12] and the least effective of the three JHs[9,10]. Natural (10R)-JH III exhibited biological activity but the activity was one three-thousandth of that of (10R)-JH I. The unnatural (10S)-JH III was also investigated, but no effect was detected at a dose of 500 µg/larva. The biological activity of both the enantiomers of JH III in *Bombyx* was examined by Imai et al.[16] who found that (10S)-JH III showed weak activity which was about one-fiftieth of that of (10R)-JH III. The present study, however, failed to detect any biological activity of (10S)-JH III at the levels assayed; it is possible that the activity of (10S)-JH III in the previous study originated in the contamination of (10R)-JH III.

In the present bioassay, allatectomized *Bombyx* larvae were used as the test animals, so that they had no internal JH of their own. We can, therefore, exclude the possibility that the samples tested were active because they stimulated the corpora allata, acted as synergists of JH, or inhibited a metabolic pathway that inactivated JH.

It may be of interest to compare the JH concentration in the hemolymph of intact 4th instar larvae with that 24 h after JH application onto the allatectomized larvae. Using the values presented by Rountree and Bollenbacher[17] for the rate of penetration of topically applied JH I, the (10R)-JH I concentration in the hemolymph 24 h after the application of 0.1 µg/larva might be of the order of 10^{-10} M. The minimum hemolymph JH titer is approximately 0.7×10^{-8} M (2.2 ng/ml) JH I (racemic JH I) equivalents in *Bombyx* fourth instar (unpublished). JH titer at any time in the fourth instar is, therefore, much more than enough to maintain larval characteristics after the subsequent ecdysis. In the same way, (10S)-JH I concentration in hemolymph, when 500 µg were applied, may be estimated to be 10^{-8} M. This value is still lower than the concentration (3×10^{-7} M) of racemic JH I required for in vitro induction of larval cuticle in *Manduca* epidermis[18].

It has been suggested that the difference in the activity of the enantiomers results from differences in affinity to JH binding protein (JHBP), since JH in hemolymph may bind to JHBP, which protects JH against general esterase in hemolymph[19]. However, the affinity of (10R)-JH I to JHBP of *Manduca sexta* is only 3.3 times greater than that of (10S)-JH I, and is the same as that of racemic JH I[20]. Consequently, the difference in affinity appears not to explain the difference in biological activity between the enantiomers. A similar consideration is also true for the different biological activity of the two enantiomers of JH III[21]. The determination of the affinity of both the enantiomers to intracellular JH receptor protein might help to explain the difference in the biological activity.

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