Note
Ethyl 4-[2-(6-Methyl-3-pyridyloxy)butyloxy]benzoate, a Novel Anti-juvenile Hormone Agent

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Ethyl 4-[2-(6-methyl-3-pyridyloxy)butyloxy]benzoate (2) was prepared as a novel anti-juvenile hormone (anti-JH) agent. Compound 2 induced precocious metamorphosis in larvae of the silkworm and black pigmentation of the larval cuticle, which are clearly recognized as JH-deficiency symptoms. The 4-ethoxycarbonyl group on the benzene ring was indispensable for activity. The activity of compound 2 could be fully counteracted by methoprene, a JH agonist, but not by the dietary administration of 20-hydroxyecdysone.

Key words: anti-juvenile hormone; 6-methyl-3-pyridyl ethers; precocious metamorphosis; silkworm

Since insect juvenile hormone (JH) influences a wide range of physiological processes such as metamorphosis, reproduction, diapause, and behavior,⁵ compounds with anti-JH activity are useful as biochemical probes to assist in elucidating the role of JH in insect development. Although several anti-JH agents have so far been found, their activities were restricted to some insect species and were not sufficiently high as insect growth regulators.⁶ Ethyl 4-[2-(t-butylcarbonyloxy)butyloxy]benzoate (ETB) is known to show anti-JH activity as well as JH activity for the tobacco hornworm, Manduca sexta⁷ and the silkworm, Bombyx mori,⁸ depending on the dose applied; low doses of ETB induced precocious metamorphosis, a clear JH-deficiency symptom, but at higher doses JH-like activity was observed. No other anti-JH agents with such action have been found to date and the exact mode of action of ETB is still unknown.

By modifying the structure of ETB, we have recently found that 2-(3-chlorophenoxy)ethyl 6-methyl-3-pyridyl ether (I) induces precocious metamorphosis in the 3rd instar larvae of B. mori at both low and high doses.⁹ However, the activity of compound 1 was very weak and there was quite a difference in critical times between ETB and compound 1 treatment for induction of precocious metamorphosis; ETB was active only when applied to newly molted 3rd instar larvae, while compound 1 induced precocious metamorphosis when applied to larvae from 24 hr-old 3rd instar to 24 hr-old 4th instar. In our continuing studies on ETB and compound 1 analogs, we have discovered ethyl 4-[2-(6-methyl-3-pyridyloxy)butyloxy]benzoate (2) as a novel anti-JH agent. In this paper we report the activity of compound 2 and briefly discuss the structure-activity relationships.

Compound 2⁶ and related compounds were prepared in a similar manner to that reported previously.⁹ Bombyx mori (Shunrei × Shougetsu strain) larvae were reared on an artificial diet as previously described.⁹ Test compounds in acetone solution (1~4 µl/larva) were applied topically to 3rd or 4th instar larvae. Twenty larvae were used for each dose. The activity was evaluated by induction of precocious metamorphosis; puation or formation of larval-pupal intermediates from the 4th instar (penultimate) larval stage.

Table 1 shows precocious-metamorphosis-inducing activity of ETB, compounds 1 and 2 against
newly molted 3rd instar larvae. ETB at 10 µg induced precocious pupation, but no activity was observed at a high dose of 100 µg, similar to that reported previously.\(^4,5\) Compound 1 had no precocious-metamorphosis-inducing activity against newly molted 3rd instar larvae. In contrast to ETB, the activity of compound 2 to induce precocious metamorphosis correlated with the applied dose. When newly molted 3rd instar larvae were treated with compound 2, precocious metamorphosis always occurred in the 4th larval stage. None of the treated 3rd instar larvae metamorphosed into precocious pupae in the same larval stage. None of the treated 3rd instar larvae induced precocious metamorphosis in B. mori larvae by causing a temporary deficiency of ecdysteroid titers in the larval hemolymph, because their activity was completely reversible by the dietary administration of 20-hydroxyecdysone. Therefore, we examined the effects of methoprene, a JH agonist, and 20-hydroxyecdysone on precocious metamorphosis induced by compound 2 (Table 2). The activity of compound 2 to induce precocious metamorphosis was completely blocked by simultaneous application of methoprene to newly molted 3rd instar larvae or methoprene applied immediately after 3rd ecdisis. In contrast to the results found in 1,5-disubstituted imidazoles and 3-pyridine derivatives, precocious pupation induced by compound 2 was not counteracted by the dietary administration of 20-hydroxyecdysone. The results of the rescue experiments indicate that compound 2 induces precocious metamorphosis in B. mori larvae by causing a deficiency of JH titers in the larval hemolymph.

Ohashi et al. have reported that JH regulates larval coloration in the silkworm and allatectomy causes black pigmentation of the larval cuticle.\(^10\) When compound 2 was applied to 3rd instar larvae, the prominent blackening of the cuticle was observed after the 3rd ecdisis. Simultaneous application of compound 2 and methoprene changed the larval body color to yellowish brown. The black coloration of the cuticle has not been observed in the larvae treated with 1,5-disubstituted imidazoles and 3-pyridine derivatives. Although the mode of action of compound 2 remains to be examined, compound 2 would be a structurally novel class of leads for the development of anti-JH agents.

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### References

4) Kiguchi, K., Mori, T., and Akai, H., Effects of anti-juvenile hormone “ETB” on the development and metamorphosis of the silkworm, *Bombyx mori*. *J.

6) Compound 2: $^1$H-NMR (400 MHz, CDCl$_3$, TMS) $\delta$: 1.06 (3H, t, $J = 7.3$ Hz), 1.37 (3H, t, $J = 7.2$ Hz), 1.84–1.88 (2H, m), 2.50 (3H, s), 4.12–4.21 (2H, m), 4.34 (2H, q, $J = 7.2$ Hz), 4.50–4.53 (1H, m), 6.90 (2H, d, $J = 8.5$ Hz), 7.07 (1H, d, $J = 8.4$ Hz), 7.20 (1H, d, $J = 2.9$, 8.4 Hz), 7.98 (2H, d, $J = 8.5$ Hz), 8.26 (1H, d, $J = 2.9$ Hz). Anal. Found: C, 69.28; H, 7.10; N; 4.33%. Calcd. for C$_{19}$H$_{23}$NO$_4$: C, 69.30; H, 6.99; N; 4.26%.


