Synthesis and Anti-Juvenile Hormone Activity of Ethyl 4-(2-Aryloxyhexyloxy)benzoates

Kenjiro Furuta, Hiromitsu Shirahashi, Haruna Yamashita, Kiyo Ashibe, and Eiichi Kuwano

Laboratory of Pesticide Chemistry, Department of Applied Genetics and Pest Management, Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan

Received October 5, 2005; Accepted November 7, 2005

A series of ethyl 4-(2-aryloxyhexyloxy)benzoates was prepared and tested for their activity to induce precocious metamorphosis in larvae of the silkworm. Phenyl analog 5 showed activity comparable to that of the 6-methyl-3-pyridyl analog reported as a novel anti-JH agent. The activity of 5 could be fully counteracted by methoprene, a JH agonist. The ethoxycarbonyl group of 5 was essential for its activity.

Key words: anti-juvenile hormone; precocious metamorphosis; silkworm

The juvenile hormone (JH) is involved in a wide range of physiological processes in both developing and mature insects. JH is critical for the regulation of metamorphosis and is required in the adult for such reproductive functions as pheromone biosynthesis, ovarian development, and maturation of eggs in females. Therefore, an anti-JH agent, which chemically blocks the functioning of the JH control system, could be an effective tool for studies on insect physiology as well as a potential insect growth regulator (IGR). Although some anti-JH agents have so far been found, including precocenes, fluorenonevalonate, ethyl 4-[2-(tert-butylocarbonyloxy)butyloxy]benzoate (ETB) and 1,5-disubstituted imidazoles, their degree of activity is not sufficiently high for use as IGRs.

ETB is known to show both JH activity and anti-JH activity toward the tobacco hornworm, Manduca sexta, and the silkworm, Bombyx mori, depending on the dose applied: a low dose of ETB induced precocious metamorphosis, a clear sign of JH-deficiency, but at higher doses, the precocious metamorphosis-inducing activity disappeared and instead, JH-like activity was observed. Riddiford et al. have reported that ETB acted as a partial JH antagonist in the target tissue of the larval epidermis. By modifying the structure of ETB, we have recently found that ethyl 4-[2-(6-methyl-3-pyridyloxy)butyloxy]benzoate (1) induced precocious metamorphosis in 3rd instar larvae of B. mori at higher doses. We have further synthesized analogs in which the ethyl side chain of 1 was modified, and found that butyl-substituted analog 2 was more potent than 1. In contrast to ETB, the activity of compounds 1 and 2 to induce precocious metamorphosis was correlated with the applied dose to some extent. In our continuing studies on this series of compounds, the butyl side chain was fixed in the molecule, and a modification was made by replacing the 6-methyl-3-pyridyl moiety with other aromatic rings. In the present paper, we report the activity of a novel series of ethyl 4-(2-aryloxyhexyloxy)benzoates as anti-JH agents causing precocious metamorphosis in B. mori larvae.

Ethyl 4-(2-aryloxyhexyloxy)benzoates were prepared in a similar manner to that reported previously. All compounds showed a single spot by thin-layer chromatography and an appropriate 1H-NMR spectrum. B. mori (Shunrei × Shougetsu strain) larvae were reared on an artificial diet as previously reported. Test compounds in an acetone solution (1–4 μl/larva) were topically applied to the dorsal abdomen of 24-hr-old 3rd instar and newly molted 4th instar larvae. Twenty larvae were used for each dose. The activity of the compounds was evaluated by the induction of precocious metamorphosis: spinning a cocoon and subsequent pupation from the 4th instar (penultimate) larval period.

Table 1 shows the precocious metamorphosis-inducing activity of the ethyl 4-(2-aryloxyhexyloxy)benzoates against 3rd instar larvae of B. mori. As previously reported, compound 2 induced precocious metamorphosis in a dose range of 1–40 μg. 3-Pyridyl analog 3 showed some activity, while 4-pyridyl analog 4 had no activity at 1 μg and 10 μg. Phenyl analog 5 as well as 2 induced precocious metamorphosis at each of the treated doses, indicating that the 6-methyl-3-pyridyl moiety was
not essential for activity. The introduction of a methyl substituent on the benzene ring (6–8) decreased the degree of the activity in comparison with that of 5. 3-Chlorophenyl analog 10 showed activity comparable to that of 5, whereas the 2-chlorophenyl (9) and the 4-chlorophenyl (11) analogs had lower activity. When 24-hr-old 3rd instar larvae were treated with each of these compounds, precocious metamorphosis always occurred in the 4th larval stage. None of the compounds induced precocious pupation when applied to newly molted 4th instar larvae (data not shown).

We have previously reported that the precocious metamorphosis-inducing activity of 1 was blocked by the simultaneous application of methoprene, a JH agonist.6) We examined the effect of methoprene on the precocious metamorphosis induced by 5 as well. The activity of 5 to induce precocious pupation was completely counteracted by the simultaneous application of methoprene (10 µg) to 24-hr-old 3rd instar larvae or of methoprene applied immediately after the 3rd ecdysis, indicating that 5 as well as 1 induced precocious metamorphosis in B. mori larvae by causing a deficiency in JH titers of the larval hemolymph.

Since ethyl 4-substituted benzoates can be hydrolyzed to the corresponding 4-substituted benzoic acids in the larval hemolymph, we examined the activity of 4-(2-phenoxycarboxyloxy)benzoic acid (5-acid) by dietary administration (Table 2). Compound 5 induced precocious metamorphosis by dietary administration as well as by topical application when applied to 3rd instar larvae, while 5-acid showed no activity at concentrations of 50 and 200 ppm. This result indicates that the ethoxycarbonyl group itself is responsible for the activity.7)

In conclusion, we found ethyl 4-(2-aryloxyhexyloxy)benzoates as a new class of potent anti-JH agents. Further studies on the structure-activity relationship of this series of compounds are in progress.

Acknowledgment

This work was supported by a grant-in-aid to E.K. for scientific research (no. 17208007) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References and Notes

6) Ishiguro, H., Fujita, N., Kim, I.-H., Shiotsuki, T., and Kuwano, E., Ethyl 4-[2-(6-methyl-3-pyridyloxy)butyl-


9) Compound 5: $^1$H-NMR (400 MHz, CDCl$_3$, TMS) δ: 0.92 (3H, t, $J = 7.1$ Hz), 1.38 (3H, t, $J = 7.2$ Hz), 1.39–1.55 (4H, m), 1.80–1.84 (2H, m), 4.09–4.20 (2H, m), 4.33 (2H, q, $J = 7.2$ Hz), 4.56–4.61 (1H, m), 6.89 (2H, d, $J = 8.7$ Hz), 6.91–6.97 (2H, m), 7.24–7.31 (3H, m), 7.97 (2H, d, $J = 8.7$ Hz). *Anal. Found:* C, 73.60; H, 7.62%. Calcd. for C$_{21}$H$_{26}$O$_4$: C, 73.66; H, 7.65%.