早熟変態誘導活性を有する光学活性なエチル4-（2-ベンジルアルキルオキシ）ベンゾエート類の幼若ホルモン活性

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Juvenile hormone activity of optically active ethyl 4-(2-benzylalkyloxy)-benzoates inducing precocious metamorphosis

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A series of ethyl 4-(2-benzylalkyloxy)benzoates possessing precocous metamorphosis-inducing activity showed juvenile hormone (JH) activity when topically applied to allatectomized 4th instar larvae of Bombyx mori. Hexyl (KF-13) and heptyl analogs, which induced precocuous metamorphosis at low doses, had relatively high JH activity. In both compounds, (S)-enantiomers were more active than (R)-enantiomers. A correlation was observed between JH activity and anti-JH activity in the ethyl 4-(2-benzylalkyloxy)benzoate series. Replacement of the 4-ethoxycarbonyl group with a 4-ethyl or 3,4-methylenedioxy group in KF-13 eliminated both JH and anti-JH activity. © Pesticide Science Society of Japan

Keywords: juvenile hormone, anti-juvenile hormone, precocious metamorphosis, silkworm.

Introduction

We have recently reported ethyl 4-(2-benzylhexyloxy)benzoate (KF-13) as a novel anti-juvenile hormone (JH) agent.† This compound induced precocious metamorphosis in larvae of the silkworm, Bombyx mori, a clear sign of JH deficiency, and its activity could be completely counteracted by the simultaneous application of methoprene, a JH agonist. KF-13 was designed by modifying the structure of ethyl 4-[2-(tert-butylcarbonyloxy)butyloxy]benzoate (ETB), which is the only compound that is reported to act as a partial JH antagonist in the larval epidermis of Manduca sexta in vitro.2) KF-13 and its analogs (1–3) showed stronger precocious metamorphosis-inducing activity than ETB (Fig. 1).1) ETB is known to show JH activity as well as anti-JH activity for M. sexta2b) and B. mori,6) depending on the dose applied; low doses of ETB induced precocious metamorphosis, but at higher doses precocious metamorphosis-inducing activity disappeared and instead, JH activity was observed. ETB as a JH agonist counteracted the effect of allatectomy, i.e., induction of precocious metamorphosis, in a dose-dependent manner.4) In our previous study, KF-13 as well as methoprene prolonged the duration of the instar and delayed the onset of cocoon spinning when applied to 24-hr-old 5th instar larvae, suggesting that KF-13 as well as ETB has JH-like activity.5) It has been reported that the JH and anti-JH activity of ETB in M. sexta larvae is entirely due to the (S)-enantiomer, the (R)-isomer being completely inactive.5) In the alkyl (2E,4E)-3,7,11-trimethyl-2,4-dodecadienoate series with JH activity, (S)-enantiomers have shown considerably higher activity on several insect species than (R)-enantiomers;6) therefore, we examined the JH activity of optically active KF-13 and its analogs by bioassay using allatectomized 4th instar larvae, which proved to be sensitive and satisfactory for the evaluation of JH activity.5) Moreover, the effect of the 4-ethoxycarbonyl group of KF-13 on JH activity was investigated.

Materials and Methods

1. Instrumental analysis

The 1H NMR spectra were determined with a JEOL EX-400 (400 MHz) spectrometer, using tetramethylsilane as an internal standard, and all samples were prepared in deuteriochloroform. Optical rotation values were measured with a Union Giken PM-101 polarimeter. HPLC analysis was carried out with a Shimadzu LC-10A equipped with a Shimadzu UV-VIS diode array.

2. Chemicals

Methoprene (93.4%) was kindly supplied by Earth Biochemical Co. Racemic 1–3, KF-13,† and ETB6) were synthesized according to the procedure reported previously. Enantiomers of KF-13 and the heptyl analog 2 were prepared using a chiral auxiliary oxazolidinone according to the process already described.1)

2.1. Ethyl 4-[(S)-2-benzylpentyloxy]benzoate (IS)

This compound was prepared in the same manner as ethyl 4-[(S)-2-benzylhexyloxy]benzoate (KF-13S) using n-pentanoyl chloride instead of n-hexanoyl chloride as a starting material. [α]D8) +43° (c 1, ethanol). Enantiomeric purity was 99% ee by HPLC analysis under the following conditions: column, CHIRALPAC OD-H (4.6×250 mm, Daicel Chemical Industry Co.); mobile phase, hexane-2-propanol (99:1); detection, UV 260 nm; flow rate, 1 ml/min.

Ethyl 4-[(R)-2-benzylpentyloxy]benzoate (1R) was prepared in the same manner as IS using (S)-4-benzyl-2-oxazolidinone in-
stead of (R)-isomer.

Compound 1R: \([\alpha]_D^{20} + 36^\circ\) (c 1, ethanol); enantiomeric purity, 98% ee. The \(^1\text{H}\) NMR spectra of 1S and 1R were fully consistent with that already reported for racemic mixture 1.

2.2 Ethyl 4-(S)-2-benzylhexyloxy]benzoate (3S) and its enantiomer (3R)

3S and 3R were prepared in the same manner as KF-13S and KF-13R, respectively, using n-octanoyl chloride instead of n-hexanoyl chloride.

Compound 3S: \([\alpha]_D^{20} + 35^\circ\) (c 1, ethanol), 90% ee.

Compound 3R: \([\alpha]_D^{20} - 48^\circ\) (c 1, ethanol), 95% ee. The \(^1\text{H}\) NMR spectra of 3S and 3R were completely consistent with that of 3.

2.3 2-Benzyl-1-(4-ethylphenoxy)hexane (4)

This compound was prepared in the same manner as KF-13 using 4-ethylphenol instead of ethyl 4-hydroxybenzoate as the starting material. \(^1\text{H}\) NMR \(\delta: 0.88\) (3H, t, \(J=7.3\) Hz, \(CH_3\)) 1.21 (3H, t, \(J=7.8\) Hz, \(CH_3\)), 1.22-1.54 (6H, m, \(3CH_2\)), 2.02-2.06 (1H, m, \(CH\)), 2.58 (2H, q, \(J=7.8\) Hz, \(CH_2\)), 2.68-2.81 (2H, m, \(CH_2\)), 3.73-3.79 (2H, m, \(CH_2\)), 6.80 (2H, d, \(J=8.3\) Hz, phenyl), 7.09 (2H, d, \(J=8.3\) Hz, phenyl), 7.15-7.20 (3H, m, phenyl), 7.24-7.29 (2H, m, phenyl).

2.4 5-(2-Benzilhexyloxy)1,3-benzodioxole (5)

This was similarly prepared from 3,4-methylenedioxybenzole. \(^1\text{H}\) NMR \(\delta: 0.89\) (3H, t, \(J=7.3\) Hz, \(CH_3\)), 1.29-1.48 (6H, m, \(3CH_2\)), 2.01-2.04 (1H, m, \(CH\)), 2.68-2.79 (2H, m, \(CH_2\)), 3.68-3.73 (2H, m, \(CH_2\)), 5.90 (2H, s), 6.27 (1H, dd, \(J=2.4\) and 8.8 Hz, phenyl), 6.47 (1H, d, \(J=2.4\) Hz), 6.68 (1H, d, \(J=8.8\) Hz), 7.15-7.20 (3H, m, phenyl), 7.25-7.28 (2H, m, phenyl).

3. Biological Evaluation

B. mori (Shunrei×Shougetsu) larvae were reared on an artificial diet as previously reported.\(^9\) Twenty-four hours after the 3rd molt, the corpora allata were extirpated with fine forceps under a binocular microscope, as described by Ohtaki et al.\(^7\) Test compounds in acetone solution (1–4 \(\mu\)l/ler) were each topically applied to the dorsal abdomen of the larvae within 1 hr after the allatectomy. Ten larvae were used for each dose. JH activity was evaluated by molting into normal 5th instar larvae. Anti-JH activity was determined against B. mori larvae as previously described.\(^5\)

\[
\text{ETB : } R^1 = \begin{array}{c} O \\ - \end{array} ; R^2 = H \\
1: R^1 = \begin{array}{c} \end{array} ; R^2 = CH_3 \\
KF-13: R^1 = \begin{array}{c} \\ \end{array} ; R^2 = C_2H_5 \\
2: R^1 = \begin{array}{c} \\ \end{array} ; R^2 = n-C_3H_7 \\
3: R^1 = \begin{array}{c} \end{array} ; R^2 = n-C_4H_9 
\]

Fig. 1. Structures of ETB and ethyl 4-(2-benzylalkyloxy)benzoates.

Results and Discussion

As previously reported,\(^1\) in our bioassay using 24-h-old 3rd instar larvae of B. mori, KF-13 and 2 showed stronger precocious metamorphosis-inducing activity than pentyl (1) and octyl (3) analogs. For both KF-13 and 2, (S)-enantiomer was more active than (R)-isomer at low doses of 0.1 and 1 \(\mu\)g, but at higher doses their activity was reversed.

Table 1 summarizes the JH activity of ETB, methoprene, KF-13, 1, 2 and 3 when topically applied to allatectomized 4th instar larvae. All of the allatectomized and acetone-treated control larvae underwent precocious metamorphosis. ETB at 1 \(\mu\)g prevented precocious metamorphosis so that all treated larvae molted into 5th instar larvae; at a dose of 0.1 \(\mu\)g it showed lower activity. These results were almost the same as those reported by Kiguchi et al.\(^6\) Methoprene showed the same level of activity as ETB.

Racemic 1 showed JH activity at a high dose of 40 \(\mu\)g. Compound 1S at 40 \(\mu\)g had obvious JH activity, while 1R was inactive at this dose, indicating that the activity of racemic 1 is apparently due to the (S)-enantiomer. KF-13 had high JH activity in comparison with 1, but not as high as ETB. The JH activity of KF-13S was higher than that of KF-13R, indicating that the JH activity of racemic KF-13 is also essentially due to the (S)-enantiomer. Racemic 2 showed almost the same activity as KF-13. The (R)-enantiomer 2R showed increased activity in comparison with that observed for KF-13R. 2S was somewhat more active than 2R. Racemic 3 showed lower activity than racemic 2 but higher activity than racemic 1. No remarkable difference in JH activity between 3S and 3R was observed, probably due to the similar size of the benzyl and n-hexyl substituents at the chiral carbon atom. Thus, KF-13 and 2, which induced precocious metamorphosis at low doses, had relatively high JH activity. This result suggests that the decrease of precocious metamorphosis-inducing activity resulting from treatment with KF-13 and 2 at high doses is due to the counteraction caused by these compounds as JH agonists.

From detailed structure-JH activity relationship studies for aryl geranyl ethers,\(^10\) the placement of appropriate substituents on the benzene ring, such as a 4-ethyl or a 3,4-methylenedioxy group, has been shown to lead to comparatively high JH activity; there-
Table 1. Effects of methoprene, ETB, KF-13, 1, 2 and 3 on the development of allatectomized 4th instar larvae of *B. mori*

<table>
<thead>
<tr>
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<tr>
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<td></td>
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<td>1</td>
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*Number of larvae tested: 10.*
Therefore, we examined the activity of KF-13 analogs with a 4-ethyl (4) and a 3,4-methylenedioxy (5) group on the benzene ring (Fig. 2). These compounds had neither anti-JH activity in a dose range of 1–40 µg nor JH activity at 40 µg (Table 1), indicating that the 4-ethoxycarbonyl group of KF-13 is necessary for both activities.

Although numerous compounds, such as aryl geranyl ethers, alkyl (2E,4E)-3,7,11-trimethyl-2,4-dodecadienoates and 4-phenoxypyrenoxo derivatives, have been described to exhibit JH activity, there has been no report on JH activity of branched compounds like ethyl 4-(2-alkylalkyloxy)benzoates. ETB was the first compound showing both JH and anti-JH activity. This study indicates that ethyl 4-(2-alkylalkyloxy)benzoates, which have stronger anti-JH activity than ETB, show lower JH activity than ETB against B. mori larvae. In a series of ethyl 4-(2-alkylalkyloxy)benzoates, a correlation was observed between JH activity and anti-JH activity. In order to develop a genuine anti-JH agent showing no JH activity, further studies on the structure-activity relationships of these kinds of compounds are under investigation.

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References