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Synthesis and Structure–Activity Relationship of a New Series of Anti–Juvenile Hormone Agents: Alkyl 4–(2–Benzylhexyloxy)benzoates and Ethyl 4–Substituted Benzoates

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A series of alkyl 4–(2–benzylhexyloxy)benzoates and related compounds were synthesized and investigated for their ability to induce precocious metamorphosis in larvae of *Bombyx mori*, a clear sign of JH deficiency in the hemolymph of early larval stages. In the alkyl 4–(2–benzylhexyloxy)benzoate series, the methyl and ethyl (KF–13) esters induced precocious metamorphosis at relatively low doses. Replacement of the ester group with an amide, alcohol, oxime, ethyl or 3,4–methylenedioxy group eliminated the activity, indicating that the ester group on the benzene ring is essential for activity. A modification was made between the two benzene rings in KF–13. None of the compounds showed higher activity than KF–13 at low doses. The JH activity of synthesized compounds was assayed using allatectomized 4th instar larvae of *B. mori*. The aniline analog, which showed stronger precocious metamorphosis–inducing activity than KF–13 at higher doses, had obvious JH activity. The *n*–propyl ester possessing moderate precocious metamorphosis–inducing activity did not show any JH activity. There was some correlation between the ability of compounds to cause precocious metamorphosis and their JH activity.

INTRODUCTION

Juvenile hormone (JH) is involved in a wide range of physiological events in both developing and mature insects (Riddiford, 1994). It is critical for the regulation of molting and metamorphosis. Precocious metamorphosis results from surgical removal of the corpora allata from early larval stages of the commercial silkworm, *Bombyx mori* (Fukuda, 1944). Similar effects have been elicited by administration of chemical compounds which act as anti–JH agents at a critical time point. Although some anti–JH agents have so far been found (Staal, 1986), their degree of activity is not sufficiently high for use as insect growth regulators (IGRs). Our studies have been concentrated on compounds inducing precocious metamorphosis, anti–JH agents (Kuwano *et al.*, 2008), which would be expected as practical IGRs. We have recently reported that ethyl 4–(2–benzylhexyloxy)benzoate (KF–13) induced precocious metamorphosis in larvae of *B. mori* at relatively low doses (Furuta *et al.*, 2007). Its activity was completely counteracted by the simultaneous application of methoprene, a JH agonist, showing that KF–13 caused a clear deficiency of JH titers in the larval hemolymph. From a preliminary study of the structure–activity relationship, the hexyl (KF–13) or heptyl group as an alkyl side chain was found to be optimal for high activity. The (*S*)–enantiomer of KF–13

was more active than the (*R*)–enantiomer at low doses, but at high doses the activity was reversed. In our continuing studies on this series of compounds, the hexyl group was fixed as an alkyl chain in the molecule, and modifications were made by replacing 4–ethoxycarbonyl group on the benzene ring with a variety of substituents, and by introducing some functional groups between the two benzene rings. In the present paper, we report the structure–activity relationships of a new series of alkyl 4–(2–benzylhexyloxy)benzoates and related compounds derived from KF–13.

MATERIALS AND METHODS

Chemicals

The ¹H–NMR spectra were determined with JEOL EX–400 (400 MHz) spectrometer. All melting points (mp) are uncorrected.

Compound **1** and **13** were prepared according to the procedure reported previously (Furuta *et al.*, 2007 and Furuta *et al.*, 2006). Compounds **11** and **12** were prepared according to the method described by Fujita *et al.* (2008). The preparation of 4–(2–benzylhexyloxy)benzoic acid derivatives and related compounds (**1–4** and **6–10**) is outlined in Fig. 1. 4–(2–Benzylhexyloxy)benzoic acid (**III**) was prepared by basic hydrolysis of **1** or **2**. Compounds **2**, **5** and **19** were prepared in the same manner as **1** but with use of methyl 4–hydroxybenzoate, ethyl 3–hydroxybenzoate and methyl 3–hydroxy–5–isoxazolecarboxylate, respectively, instead of ethyl 4–hydroxybenzoate.

Methyl 4–(2–benzylhexyloxy)benzoate (2)

¹H–NMR (CDCl₃) δ: 0.89 (3H, t, *J*=7.3 Hz), 1.24–1.52

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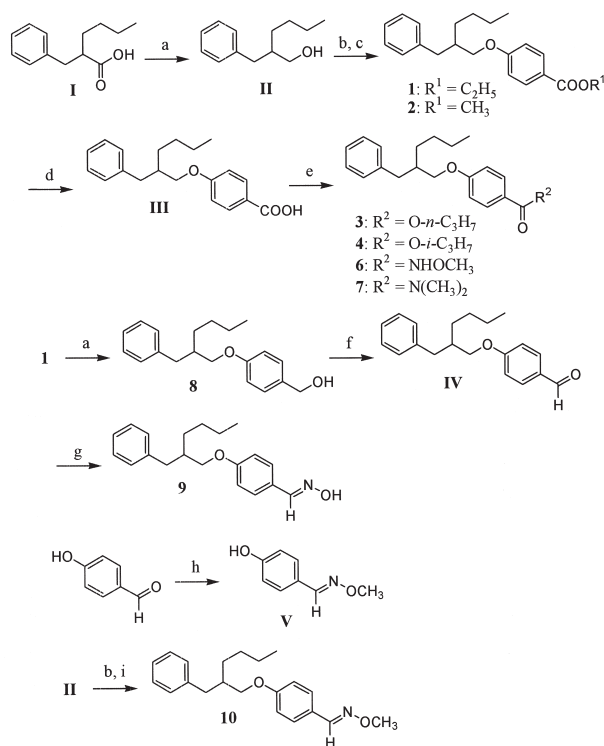


Fig. 1. Synthetic scheme for preparation of 4-(2-benzylhexyloxy)benzoic acid derivatives and related compounds (**1–4** and **6–10**).

(a) LiAlH₄, THF; (b) *p*-toluenesulfonyl chloride, N(C₂H₅)₃, THF; (c) ethyl 4-hydroxybenzoate or methyl 4-hydroxybenzoate, K₂CO₃, DMF; (d) NaOH, EtOH and H₂O; (e) NH₂OCH₃·HCl, NH(CH₃)₂·HCl, 1-propanol or 2-propanol, N(C₂H₅)₃, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide·HCl, DMAP, CH₂Cl₂; (f) (COCl)₂, DMSO, N(C₂H₅)₃, CH₂Cl₂; (g) NH₂OCH₃·HCl, pyridine, EtOH; (h) NH₂OCH₃·HCl, pyridine, EtOH; (i) compound **V**, K₂CO₃, DMF.

(6H, m), 2.07–2.11 (1H, m), 2.75 (2H, d, *J*=6.8 Hz), 3.83 (2H, d, *J*=5.4 Hz), 3.88 (3H, s), 6.87 (2H, d, *J*=8.8 Hz), 7.14–7.20 (3H, m), 7.24–7.29 (2H, m), 7.96 (2H, d, *J*=8.8 Hz).

Ethyl 3-(2-benzylhexyloxy)benzoate (5)

¹H-NMR (CDCl₃) δ: 0.90 (3H, t, *J*=6.8 Hz), 1.24–1.53 (6H, m), 1.39 (3H (overlapped), t, *J*=7.3 Hz), 2.04–2.09 (1H, m), 2.71–2.82 (2H, m), 3.83 (2H, d, *J*=5.4 Hz), 4.35 (2H, q, *J*=7.3 Hz), 7.06 (1H, dd, *J*=2.0 and 7.8 Hz), 7.15–7.24 (3H, m), 7.28–7.34 (3H, m), 7.52–7.53 (1H, m), 7.62 (1H, d, *J*=7.8 Hz).

Methyl 3-(2-benzylhexyloxy)-5-isoxazolecarboxylate (19)

¹H-NMR (CDCl₃) δ: 0.88 (3H, t, *J*=6.8 Hz), 1.26–1.45 (6H, m), 2.08–2.11 (1H, m), 2.70–2.73 (1H, m), 3.95 (3H, s), 4.15 (2H, dd, *J*=2.0 and 5.4 Hz), 6.54 (1H, s), 7.14–7.21 (3H, m), 7.25–7.29 (2H, m).

***N*-Methoxy-4-(2-benzylhexyloxy)benzamide (6)**

A mixture of *O*-methylhydroxylamine hydrochloride (0.13 g, 1.6 mmol) and triethylamine (0.26 g, 2.6 mmol) in 10 ml of dichloromethane was stirred for 1 hr at room temperature. To the mixture was added compound **III** (0.4 g, 1.3 mmol), 4-(*N,N*-dimethylamino)pyridine (DMAP, 0.02 g, 0.16 mmol) and 1-[3-(dimethylamino)

propyl]-3-ethylcarbodiimide hydrochloride (0.35 g, 1.8 mmol) at 0 °C. After stirring overnight at room temperature, a 2 M aqueous solution of HCl (5 ml) was added to the mixture and the product was extracted with ethyl acetate. The ethyl acetate solution was washed with brine, dried over Na₂SO₄, and concentrated. The residue was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (1:1) to afford 0.34 g (78%) of **6** as a colorless oil. ¹H-NMR (CDCl₃) δ: 0.89 (3H, t, *J*=6.8 Hz), 1.24–1.52 (6H, m), 2.06–2.09 (1H, m), 2.75 (2H, d, *J*=6.8 Hz), 3.82 (2H, d, *J*=5.4 Hz), 3.87 (3H, s), 6.87 (2H, d, *J*=8.8 Hz), 7.14–7.20 (3H, m), 7.24–7.28 (2H, m), 7.68 (2H, d, *J*=8.8 Hz), 8.58 (1H, br s).

Compound **7** was prepared in the same manner as compound **6** with use of dimethylamine hydrochloride instead of *O*-methylhydroxylamine hydrochloride.

***N,N*-Dimethyl-4-(2-benzylhexyloxy)benzamide (7)**

¹H-NMR (CDCl₃) δ: 0.89 (3H, t, *J*=7.3 Hz), 1.24–1.53 (6H, m), 2.04–2.10 (1H, m), 2.72–2.78 (2H, m), 3.06 (6H, s), 3.80 (2H, d, *J*=4.9 Hz), 6.85 (2H, d, *J*=8.3 Hz), 7.15–7.20 (3H, m), 7.25–7.36 (2H, m), 7.37 (2H, d, *J*=8.3 Hz).

In a similar manner, compounds **3** and **4** were prepared by reacting **III** with 1-propanol and 2-propanol, respectively.

***n*-Propyl 4-(2-benzylhexyloxy)benzoate (3)**

¹H-NMR (CDCl₃) δ: 0.89 (3H, t, *J*=7.3 Hz), 1.02 (3H, t, *J*=7.3 Hz), 1.20–1.55 (6H, m), 1.70–1.85 (2H, m), 2.02–2.12 (1H, m), 2.75 (2H, d, *J*=7.3 Hz), 3.83 (2H, d, *J*=5.3 Hz), 4.24 (2H, t, *J*=6.8 Hz), 6.87 (2H, d, *J*=8.8 Hz), 7.10–7.30 (5H, m), 7.96 (2H, d, *J*=8.8 Hz).

Isopropyl 4-(2-benzylhexyloxy)benzoate (4)

¹H-NMR (CDCl₃) δ: 0.89 (3H, t, *J*=7.3 Hz), 1.35 (6H, d, *J*=6.3 Hz), 1.30–1.60 (6H, m), 2.04–2.10 (1H, m), 2.75 (2H, d, *J*=7.3 Hz), 3.83 (2H, d, *J*=6.4 Hz), 5.19–5.23 (1H, m), 6.86 (2H, d, *J*=8.8 Hz), 7.14–7.27 (5H, m), 7.96 (2H, d, *J*=8.8 Hz).

4-(2-Benzylhexyloxy)benzyl alcohol (8)

A mixture of compound **1** (1.0 g, 2.9 mmol) and lithium aluminum hydride (0.11 g, 2.9 mmol) in 15 ml of tetrahydrofuran (THF) was stirred for 2 hr at room temperature. The reaction mixture was quenched with saturated NH₄Cl solution at 0 °C. After removal of the solvent under reduced pressure, the product was extracted with ethyl acetate. The ethyl acetate solution was washed with brine and dried over Na₂SO₄. After concentration under reduced pressure, the residue was chromatographed on silica gel by elution with hexane and ethyl acetate (5:1) to afford 0.85 g (97%) of **8** as a colorless oil. ¹H-NMR (CDCl₃) δ: 0.89 (3H, t, *J*=6.8 Hz), 1.26–1.53 (6H, m), 2.04–2.08 (1H, m), 3.78 (2H, d, *J*=5.6 Hz), 4.61 (2H, d, *J*=5.9 Hz), 6.85 (2H, d, *J*=8.3 Hz), 7.15–7.17 (3H, m), 7.19–7.24 (2H, m), 7.27 (2H, d, *J*=8.3 Hz).

4-(2-Benzylhexyloxy)benzaldehyde (IV)

To a solution of oxalyl chloride (0.43 g, 3.4 mmol) in 10 ml of dichloromethane at -78 °C was added dimethylsulfoxide (0.39 g, 5.0 mmol) and the solution was stirred for 1 hr. To the mixture was added a solution of compound **5** (0.5 g, 1.7 mmol) in 5 ml of dichloromethane. After stirring for 1.5 hr at -78 °C, triethylamine (1.2 g,

11.8 mmol) was added to the mixture. The mixture was stirred for 0.5 hr for -78°C and then for 0.5 hr at 0°C . The mixture was quenched by adding saturated NH_4Cl solution at 0°C . The product was extracted with ethyl acetate. The ethyl acetate solution was washed with brine, dried over Na_2SO_4 , and concentrated. The residue was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (5:1) to afford 0.56 g (94%) of **IV** as a colorless oil. $^1\text{H-NMR}$ (CDCl_3) δ : 0.89 (3H, t, $J=6.8$ Hz), 1.24–1.55 (6H, m), 2.04–2.13 (1H, m), 2.75 (2H, d, $J=6.8$ Hz), 3.86 (2H, d, $J=5.4$ Hz), 7.95 (2H, d, $J=8.3$ Hz), 7.14–7.21 (3H, m), 7.24–7.27 (2H, m), 7.81 (2H, d, $J=8.3$ Hz), 9.88 (1H, s).

4-(2-Benzylhexyloxy)benzaldehyde oxime (**9**)

To a solution of compound **IV** (0.5 g, 1.7 mmol) in pyridine and ethanol was added hydroxylamine hydrochloride (0.13 g, 1.9 mmol). After stirring for 12 hr at room temperature, the mixture was concentrated under reduced pressure. To the residue was added 2 M HCl solution and the product was extracted with ethyl acetate. The ethyl acetate solution was washed with brine, dried over Na_2SO_4 , and concentrated. The residue was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (4:1) to afford 0.33 g (63%) of **9** as a colorless oil. $^1\text{H-NMR}$ (CDCl_3) δ : 0.89 (3H, t, $J=6.8$ Hz), 1.24–1.53 (6H, m), 2.06–2.10 (1H, m), 2.74–2.76 (2H, m), 3.81 (2H, d, $J=5.4$ Hz), 6.86 (2H, d, $J=8.8$ Hz), 7.15–7.20 (3H, m), 7.24–7.28 (2H, m), 7.41 (2H, d, $J=8.8$ Hz), 8.08 (1H, s).

4-Hydroxybenzaldehyde *O*-methyloxime (**V**)

A mixture of 4-hydroxybenzaldehyde (0.5 g, 4.1 mmol) and *O*-methylhydroxylamine hydrochloride (0.38 g, 4.6 mmol) in pyridine (5 ml) and ethanol (5 ml) was stirred for 12 hr at room temperature. After removal of the solvent, the product was extracted with ethyl acetate. The organic layer was washed with water and brine, dried over Na_2SO_4 , and concentrated. The residue was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (2:1) to afford 0.59 g (95%) of **V** as a colorless oil. $^1\text{H-NMR}$ (CDCl_3) δ : 3.95 (3H, s), 5.01 (1H, br s), 6.83 (2H, dd, $J=2.0$ and 6.4 Hz), 7.48 (2H, dd, $J=2.0$ and 6.4 Hz), 8.01 (1H, s).

4-(2-Benzylhexyloxy)benzaldehyde *O*-methyloxime (**10**)

A solution of compound **II** (0.5 g, 2.6 mmol), triethylamine (0.29 g, 2.9 mmol) and *p*-toluenesulfonyl chloride (0.55 g, 2.9 mmol) in 15 ml of dichloromethane was stirred for 36 hr at room temperature. After removal of the solvent, the product was extracted with ethyl acetate. The organic layer was washed with brine, and dried over Na_2SO_4 . Concentration of the solvent gave crude 2-benzylhexyl *p*-toluenesulfonate. A mixture of the above *p*-toluenesulfonate, compound **V** (0.43 g, 2.8 mmol) and potassium carbonate (0.43 g, 3.1 mmol) in 25 ml of dimethylformamide (DMF) was heated at 90 – 100°C for 6 hr. To the mixture was added 30 ml of water and the product was extracted with ethyl acetate. The ethyl acetate solution was washed with 2 M NaOH solution, water, and brine, dried over Na_2SO_4 , and concentrated. The residue was purified by column chromatography on silica

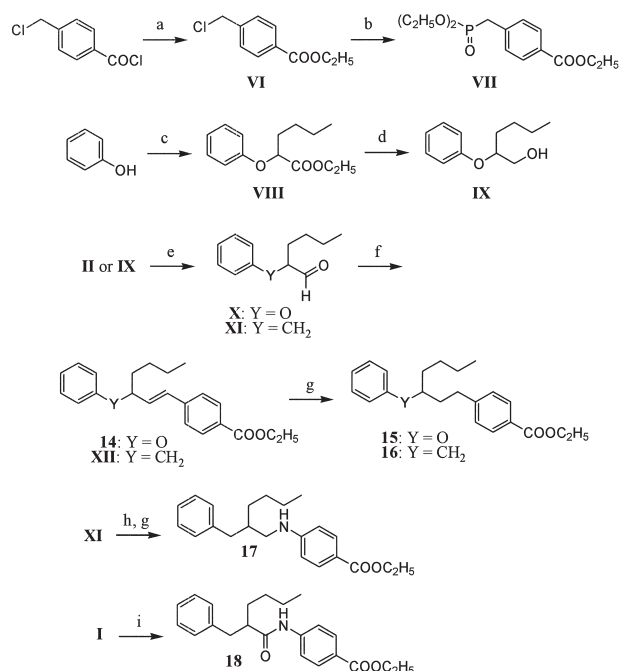


Fig. 2. Synthetic scheme for preparation of ethyl 4-substituted benzoates **14**–**18**.

(a) 4-chloromethylbenzoyl chloride, $\text{N}(\text{C}_2\text{H}_5)_3$, EtOH; (b) triethyl phosphite; (c) ethyl 2-bromohexanoate, K_2CO_3 , DMF; (d) LiAlH_4 , THF; (e) $(\text{COCl})_2$, DMSO, $\text{N}(\text{C}_2\text{H}_5)_3$, CH_2Cl_2 ; (f) compound **VII**, NaH, THF; (g) Pd/C , H_2 , MeOH; (h) ethyl 4-aminobenzoate, MgSO_4 , EtOH; (i) ethyl 4-aminobenzoate, DMAP, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide-HCl, CH_2Cl_2 .

gel eluting with hexane and ethyl acetate (15:1) to afford 0.59 g (69%) of **10** as a colorless oil. $^1\text{H-NMR}$ (CDCl_3) δ : 0.89 (3H, t, $J=6.8$ Hz), 1.26–1.50 (6H, m), 2.05–2.08 (1H, m), 2.73–2.76 (2H, m), 3.80 (2H, d, $J=4.9$ Hz), 3.95 (3H, s), 6.85 (2H, d, $J=8.8$ Hz), 7.14–7.20 (3H, m), 7.26–7.28 (2H, m), 7.49 (2H, d, $J=8.8$ Hz), 8.01 (1H, s).

Ethyl 4-substituted benzoates (**14**–**18**) were synthesized as shown in Fig. 2.

Ethyl 4-chloromethylbenzoate (**VI**)

To a solution of 4-chloromethylbenzoyl chloride (0.6 g, 3.2 mmol) in 30 ml of ethanol was added triethylamine (0.32 g, 3.2 mmol) at 0°C and the mixture was stirred for 12 hr at room temperature. After removal of the solvent, the product was extracted with ethyl acetate. The ethyl acetate solution was washed with water and brine, dried over Na_2SO_4 , and concentrated. The residue was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (5:1) to afford 0.62 g (98%) of **VI** as a colorless oil. $^1\text{H-NMR}$ (CDCl_3) δ : 1.40 (3H, t, $J=7.3$ Hz), 4.38 (2H, q, $J=7.3$ Hz), 4.62 (2H, s), 7.46 (2H, d, $J=8.3$ Hz), 8.04 (2H, d, $J=8.3$ Hz).

Diethyl 4-(ethoxycarbonyl)benzylphosphonate (**VII**)

A mixture of compound **VI** (0.46 g, 2.8 mmol) and triethyl phosphite (0.5 g, 2.5 mmol) was heated for 6 hr. The crude product was purified by silica gel column chromatography eluting with hexane and ethyl acetate (5:1) to give 0.38 g (50%) of **VII** as a yellow oil. $^1\text{H-NMR}$

(CDCl₃) δ : 1.25 (6H, t, $J=7.3$ Hz), 1.39 (3H, t, $J=7.3$ Hz), 3.20 (2H, d, $J=22$ Hz), 4.00–4.04 (4H, m), 4.37 (2H, q, $J=7.3$ Hz), 7.37 (2H, d, $J=8.3$ Hz), 7.99 (2H, d, $J=8.3$ Hz).

Ethyl 2-phenoxyhexanoate (VIII)

A mixture of phenol (0.5 g, 5.3 mmol) and potassium carbonate (0.88 g, 6.4 mmol) in 15 ml of DMF was stirred for 0.5 hr at room temperature. To the mixture was added ethyl 2-bromohexanoate (0.81 g, 3.6 mmol). After stirring for 12 hr at room temperature, the product was poured into water and the product was extracted with ethyl acetate. The ethyl acetate solution was washed with brine, dried over Na₂SO₄, and concentrated. The residue was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (10:1) to afford 0.6 g (48%) of **VIII** as a yellow oil. ¹H-NMR (CDCl₃) δ : 0.93 (3H, t, $J=7.3$ Hz), 1.24 (3H, t, $J=7.3$ Hz), 1.34–1.57 (4H, m), 1.90–1.98 (2H, m), 4.21 (2H, q, $J=7.3$ Hz), 4.59 (1H, dd, $J=5.4$ and 7.8 Hz), 6.87–6.89 (2H, m), 6.95–6.98 (1H, m), 7.24–7.29 (2H, m).

2-Phenoxy-1-hexanol (IX)

Compound **VIII** was reduced to **IX** with LiAlH₄ in the same manner as that described in **5**. Yield 100%. ¹H-NMR (CDCl₃) δ : 0.89 (3H, t, $J=6.8$ Hz), 1.32–1.43 (4H, m), 1.58–1.67 (2H, m), 1.70–1.77 (1H, m), 3.70–3.75 (1H, m), 3.80–3.84 (1H, m), 4.32–4.37 (1H, m), 6.93–6.98 (3H, m), 7.26–7.30 (2H, m).

2-Phenoxy-1-hexanal (X)

Compound **IX** was oxidized to **X** using oxalyl chloride and DMSO in the same manner as that described in **IV**. After normal workup, chromatography of the crude product on silica gel using hexane and ethyl acetate (10:1) afforded the desired compound **X**. Yield 85%. ¹H-NMR (CDCl₃) δ : 0.92 (3H, t, $J=7.3$ Hz), 1.24–1.43 (2H, m), 1.45–1.55 (2H, m), 1.82–1.90 (2H, m), 4.49 (1H, dd, $J=2.0$ and 5.3 Hz), 6.86–6.88 (2H, m), 6.98–7.01 (1H, m), 7.26–7.31 (2H, m), 9.69 (1H, d, $J=2.4$ Hz).

Ethyl 4-(3-phenoxy-1-heptenyl)benzoate (14)

To a suspension of NaH (60% in oil, 0.33 g, 14 mmol) in 20 ml of THF at 0 °C was added compound **VII** (4.18 g, 14 mmol) and the mixture was stirred for 0.5 hr at room temperature. To the mixture was added compound **X** (2.23 g, 12 mmol). After stirring for 12 hr at room temperature, 30 ml of water was added to the mixture, and the product was extracted with ethyl acetate. The ethyl acetate solution was washed with brine, dried over Na₂SO₄, and concentrated. The residue was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (10:1) to give 2.42 g (51%) of **14** which was a mixture of *E* and *Z* isomer. ¹H-NMR (CDCl₃) δ : 0.92 (3H, t, $J=7.3$ Hz), 1.39 (3H, t, $J=6.8$ Hz), 1.42–1.53 (4H, m), 1.74–1.80 (1H, m), 1.85–1.90 (1H, m), 4.36 (2H, q, $J=6.8$ Hz), 4.75–4.80 (1H, m), 6.36 (1H, dd, $J=6.4$ and 16.1 Hz), 6.62 (1H, d, $J=16.1$ Hz), 6.93–6.95 (3H, m), 7.24–7.29 (3H, m), 7.41 (2H, d, $J=8.3$ Hz), 7.97 (2H, d, $J=8.3$ Hz).

Ethyl 4-(3-phenoxyheptyl)benzoate (15)

Compound **14** (0.9 g, 2.7 mmol) was dissolved in 10 ml of methanol and to this solution was added a catalytic amount of palladium on carbon. The mixture was stirred for 15 hr at room temperature in a hydrogen

atmosphere. The mixture was filtered through a pad of Celite, and then concentrated. The resulting residue was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (10:1) to give **15** (0.86 g, 95%) as a colorless oil. ¹H-NMR (CDCl₃) δ : 0.88 (3H, t, $J=7.3$ Hz), 1.26–1.35 (4H, m), 1.39 (3H, t, $J=7.3$ Hz), 1.60–1.73 (2H, m), 1.93–2.02 (2H, m), 2.70–2.87 (2H, m), 4.21–4.24 (1H, m), 4.36 (2H, q, $J=7.3$ Hz), 6.85–6.87 (2H, m), 6.91–6.95 (1H, m), 4.21 (2H, d, $J=8.3$ Hz), 7.24–7.29 (2H, m), 7.94 (2H, d, $J=8.3$ Hz).

2-Benzyl-1-hexanal (XI)

Compound **II** was oxidized to **XI** using oxalyl chloride and DMSO in the same manner as that described in **IV**. After normal workup, chromatography of the crude product on silica gel using hexane and ethyl acetate (8:1) gave the desired compound **XI** as a yellow oil. Yield 85%. ¹H-NMR (CDCl₃) δ : 0.87 (3H, t, $J=6.8$ Hz), 1.23–1.38 (4H, m), 1.44–1.53 (1H, m), 1.60–1.67 (1H, m), 2.57–2.65 (1H, m), 2.70–2.75 (1H, m), 2.96–3.01 (1H, m), 7.15–7.22 (3H, m), 7.26–7.30 (2H, m), 9.66 (1H, d, $J=2.9$ Hz).

Ethyl 4-(3-benzyl-1-heptenyl)benzoate (XII)

This compound was prepared in the same manner as compound **9** with use of compound **XI** instead of compound **X**. Compound **XII** was obtained as a mixture of *E* and *Z* isomer in 71% yield. ¹H-NMR (CDCl₃) δ : 0.86 (3H, t, $J=5.9$ Hz), 1.24–1.40 (5H, m), 1.39 (3H, t, $J=7.3$ Hz, overlapped), 1.51–1.56 (1H, m), 2.45–2.68 (1H, m), 2.70–2.76 (2H, m), 4.36 (2H, q, $J=7.3$ Hz), 6.13–6.27 (2H, m), 7.14–7.19 (2H, m), 7.23–7.27 (3H, m), 7.34 (2H, d, $J=8.3$ Hz), 7.95 (2H, d, $J=8.3$ Hz).

Ethyl 4-(3-benzylheptyl)benzoate (16)

Compound **XII** was reduced to **16** using palladium on carbon and hydrogen gas in the same manner as that described in **15**. Yield 93%. ¹H-NMR (CDCl₃) δ : 0.88 (3H, t, $J=7.3$ Hz), 1.24–1.32 (6H, m), 1.38 (3H, t, $J=7.3$ Hz), 1.57–1.61 (2H, m), 1.67 (1H, m), 2.58–2.70 (4H, m), 4.34 (2H, q, $J=7.3$ Hz), 7.12–7.20 (3H, m), 7.17 (2H, d, $J=8.3$ Hz), 7.25–7.28 (2H, m), 7.93 (2H, d, $J=8.3$ Hz).

Ethyl 4-(2-benzylhexyl)aminobenzoate (17)

A mixture of compound **XI** (0.59 g, 3.1 mmol), ethyl 4-aminobenzoate (0.56 g, 3.4 mmol) and MgSO₄ (2 g) in 10 ml of ethanol was refluxed for 3 hr. After cooling, the mixture was filtered through a pad of Celite, and then concentrated. The product was extracted with ethyl acetate. The ethyl acetate solution was washed with brine, dried over Na₂SO₄, and concentrated. The obtained crude imine was reduced to **17** using palladium on carbon and hydrogen gas in the same manner as that described in **15**. After normal workup, chromatography of the crude product on silica gel using hexane and ethyl acetate (10:1) afforded the desired compound **17** as a pale yellow oil in 48% yield. ¹H-NMR (CDCl₃) δ : 0.89 (3H, t, $J=6.8$ Hz), 1.24–1.39 (6H, m), 1.38 (3H, t, $J=6.8$, overlapped), 1.93–2.04 (1H, m), 2.57 (1H, dd, $J=7.8$ and 13.7 Hz), 2.74 (1H, dd, $J=5.9$ and 13.7 Hz), 3.05–3.08 (2H, m), 4.29 (1H, br s), 4.30 (2H, q, $J=6.8$ Hz), 6.39 (2H, d, $J=8.8$ Hz), 7.14–7.21 (3H, m), 7.26–7.31 (2H, m), 7.81 (2H, d, $J=8.8$ Hz).

Ethyl 4-(2-benzylhexanamido)benzoate (18)

To a mixture of compound **1** (0.5 g, 2.4 mmol), ethyl 4-aminobenzoate (0.44 g, 2.7 mmol) and DMAP (0.03 g, 0.25 mmol) in 10 ml of dichloromethane was added 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (0.52 g, 2.7 mmol) at 0 °C. After stirring for 14 hr at room temperature, a 2 M aqueous solution of HCl (5 ml) was added to the mixture and the product was extracted with ethyl acetate. The ethyl acetate solution was washed with water and brine, dried over Na₂SO₄, and concentrated. The residue was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (3:1) to afford 0.29 g (40%) of **18** as a white solid, mp 104.5–106.5 °C. ¹H-NMR (CDCl₃) δ: 0.89 (3H, t, *J*=7.3 Hz), 1.32–1.35 (4H, m), 1.38 (3H, t, *J*=7.3 Hz), 1.59–1.60 (1H, m), 1.82–1.87 (1H, m), 2.39–3.43 (1H, m), 2.81–2.86 (1H, m), 2.94–3.00 (1H, m), 4.34 (2H, q, *J*=7.3 Hz), 6.82–6.87 (1H, br s), 7.16–7.20 (3H, m), 7.24–7.27 (2H, m), 7.39 (2H, d, *J*=8.3 Hz), 7.95 (2H, d, *J*=8.3 Hz).

Biological evaluation*Anti-JH activity (precocious metamorphosis-inducing activity)*

B. mori (Shunrei × Shougetsu) larvae were reared on artificial diet as previously reported (Yoshida *et al.*, 2000). Test compounds in 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 compounds was evaluated by the induction of precocious metamorphosis: spinning a cocoon and subsequent pupation or formation of larval-pupal intermediates from the 4th instar (penultimate) larval period.

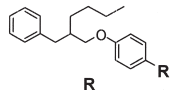
JH activity

Twenty-four hours after 3rd molt, the corpora allata were extirpated with fine forceps under a binocular microscope as described by Ohtaki *et al.* (1972). Test compounds in an acetone solution (4 μl/larva) were each applied topically to the dorsal abdomen of the larvae within 1 hour after the allatectomy. JH activity was evaluated by the molting into normal 5th instar larvae.

RESULTS AND DISCUSSION

Table 1 shows the activity of alkyl 4-(2-benzylhexyloxy)benzoates to induce precocious metamorphosis in

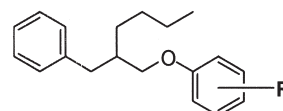
Table 1. Precocious metamorphosis-inducing activity of alkyl 4-(2-benzylhexyloxy)benzoates against 3rd instar larvae of *B. mori*

No		Precocious metamorphosis (%)			
		1	10	40	(μg/larva)
1	4-COOC ₂ H ₅ (KF-13)	90	34	12	
2	4-COOCH ₃	60	80	69	
3	4-COO- <i>n</i> -C ₃ H ₇	0	45	85	
4	4-COO- <i>i</i> -C ₃ H ₇	0	0	0	

the 4th larval stage of *B. mori* when applied to 24-hr-old 3rd instar larvae. As previously reported (Furuta *et al.*, 2007), treatment with KF-13 at 1 μg induced precocious pupation in 90%, but the activity decreased at higher doses. The activity of the methyl ester **2** at 1 μg decreased in comparison with that of KF-13, but at higher doses of 10 and 40 μg **2** was more active than KF-13. In contrast to KF-13, the activity of the *n*-propyl ester **3** correlated with the applied dose. Although **3** had no activity at 1 μg, it showed the highest activity at 40 μg among the ester series tested. A dramatic decrease in activity was observed in changing from ethyl to isopropyl ester **4**. The activity was found to disappear by replacing 4-ethoxycarbonyl group with 3-ethoxycarbonyl group on the benzene ring (**5**), indicating the *para*-substituted benzoate is essential for activity. To see whether the ester group was necessary for precocious metamorphosis-inducing activity, the amide (**6** and **7**), alcohol (**8**), oxime (**9** and **10**), ethyl (**11**) and 3,4-methylenedioxy (**12**) analogs were assayed for their activity (Fig. 3). None of them induced precocious metamorphosis in a dose range of 1–40 μg. We have already reported (Furuta *et al.*, 2007) that conversion of the ethyl ester in KF-13 to the corresponding benzoic acid eliminated the activity. These results indicate that the ester group on the benzene ring plays an important role for activity.

Since KF-13 showed the highest activity among the compounds tested, the 4-ethoxycarbonylphenyl moiety was fixed in the molecule, and further modification was made by introducing some functional groups between the two benzene rings. Precocious metamorphosis-inducing activity of ethyl 4-substituted benzoates and related compounds is shown in Table 2. As previously reported (Furuta *et al.*, 2006), the phenoxy analog **13** at 1 μg showed lower activity than KF-13, but its activity correlated with the applied dose to some extent. The introduction of a double bond between the two benzene rings (**14**) decreased the degree of the activity in comparison with that of **13**. The 2-phenoxyheptylbenzoate **15** and the 2-benzylheptylbenzoate **16** had almost the same level of activity as **13**.

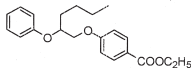
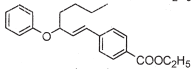
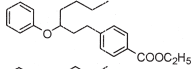
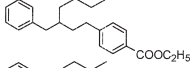
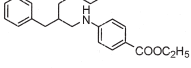
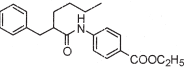
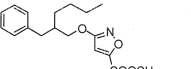
The aniline analog **17**, in which the oxygen atom in KF-13 was replaced with a nitrogen atom, showed much higher activity at 10 μg compared with that observed for KF-13, while the activity at 1 μg was somewhat less than



- 5** : R = 3-COOC₂H₅ **9** : R = 4-CH=NOH
6 : R = 4-CONHOCH₃ **10** : R = 4-CH=NOCH₃
7 : R = 4-CON(CH₃)₂ **11** : R = 4-C₂H₅
8 : R = 4-CH₂OH **12** : R = 3,4-OCH₂O-

Fig. 3. Structures of inactive compounds.

Table 2. Precocious metamorphosis-inducing activity of ethyl 4-substituted benzoates and related compounds against 3rd instar larvae of *B. mori*

No	Compound	Precocious metamorphosis (%)		
		1	10	40
		($\mu\text{g/larva}$)		
13		32	66	70
14		0	10	40
15		45	65	79
16		50	55	65
17		67	94	NT
18		0	0	NT
19		0	0	NT

NT: not tested

that of KF-13. Thus, the optimal structure for higher activity was represented by the 4-(2-benzylalkoxy) benzoate as KF-13. The placement of an amide function between the two benzene rings (**18**) completely eliminated the activity. To see whether the presence of a benzene moiety was essential for activity, the isoxazolecarboxylate analog **19** was evaluated. This compound did not induce precocious metamorphosis, suggesting that the six-membered ring is favorable for activity.

On the other hand, we have recently found that KF-13 showed clear JH activity when topically applied to allatectomized 4th instar larvae of *B. mori* (Fujita *et al.*, 2008). Preliminary structure-JH activity relationship studies for ethyl 4-(2-benzylalkoxy)benzoates exhibited that there was an apparent correlation between the ability of these compounds to cause precocious metamorphosis and their JH activity against allatectomized

Table 3. Effects of compounds **3**, **4**, **6**, **11**, **12** and **17** on the development in allatectomized 4th instar larvae of *B. mori*

Treatment	Number of larvae transformed into	
	Precocious pupa	5th instar larva
Allatectomized control	10	0
+ 3	10	0
+ 4	10	0
+ 6	10	0
+ 11	10	0
+ 12	10	0
+ 17	0	10

Forty micrograms of each compound in acetone solution ($4\mu\text{l}$) was topically applied to allatectomized 4th instar larvae. Number of larvae tested: 10

4th instar larvae; KF-13, which induced precocious metamorphosis at low doses, showed much stronger JH activity than other alkyloxy analogs. We therefore examined further structure-JH activity relationship of a new series of KF-13 analogs described in this article. Table 3 shows the JH activity of a number of compounds when topically applied to allatectomized 4th instar larvae. All of the allatectomized and acetone-treated control larvae underwent precocious metamorphosis. Compounds **4**, **6**, **11** and **12**, which did not induce precocious metamorphosis at 1–40 μg , had no JH activity at 40 μg . The aniline analog **17**, which had the highest precocious metamorphosis-inducing activity among the compounds tested in the present study, showed obvious JH activity so that all treated larvae molted into 5th instar larvae. It is noteworthy that the *n*-propyl ester **3**, which induced precocious metamorphosis in a dose-dependant manner, showed no JH activity, indicating that **3** might represent a reasonable lead for the development of a genuine anti-JH agent. Further studies on the structure-activity relationships of this series of compounds are in progress.

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