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Yoshida, Shuhei
Laboratory of Pesticide Chemistry, Division of Plant Protection, Department of Applied Genetics and Pest Management, Faculty of Agriculture, Kyushu University

Furuta, Kenjiro
Laboratory of Pesticide Chemistry, Division of Plant Protection, Department of Applied Genetics and Pest Management, Faculty of Agriculture, Kyushu University

Ashibe, Kiyo
Laboratory of Pesticide Chemistry, Division of Plant Protection, Department of Applied Genetics and Pest Management, Faculty of Agriculture, Kyushu University

Fujita, Norihiro
Laboratory of Pesticide Chemistry, Division of Plant Protection, Department of Applied Genetics and Pest Management, Faculty of Agriculture, Kyushu University

他

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Ethyl 4–[2–(Substituted Benzyl)hexyloxy]benzoates: Anti–Juvenile Hormone Agents with Juvenile Hormone Activity

Shuhei YOSHIDA[1], Kenjiro FURUTA[1], Kiyo ASHIBE[1], Norihiro FUJITA[1], Seiji NISHIKAWA[1], Naotaka YAMADA and Eiichi KUWANO*

Laboratory of Pesticide Chemistry, Division of Plant Protection, Department of Applied Genetics and Pest Management, Faculty of Agriculture, Kyushu University, Fukuoka 812–8581, Japan

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A large number of ethyl [2–(substituted benzyl)hexyloxy]benzoates and related compounds were prepared and their activity to induce precocious metamorphosis was evaluated in larvae of *Bombyx mori*, which was obviously recognized as a juvenile hormone (JH)–deficiency symptom. Introduction of a methyl, chloro or fluoro substituent at the 2–position on the benzene ring increased the activity in comparison with that of ethyl (2–benzylhexyloxy)benzoate (KF–13) in a dose range of 1–40 μg, however, no consistent dose–response relationship was obtained in these compounds as well as KF–13. The 4–methoxybenzyl analog showed strong precocious metamorphosis–inducing activity at both low and high doses, while introduction of other substituents such as a methyl, chloro, fluoro or ethyl group at the 3– and 4–position on the benzene ring decreased the activity at low doses. The JH activity of synthesized compounds was examined by bioassay using allatectomized 4th instar larvae. In the ethyl [2–(substituted benzyl)hexyloxy]benzoate series, a correlation was observed between JH activity and anti–JH activity; Compounds which induced high percentages of precocious metamorphosis at lower doses had obvious JH activity. Compounds possessing weak precocious metamorphosis–inducing activity showed little JH activity.

INTRODUCTION

We have recently discovered ethyl 4–(2–benzylhexyloxy)benzoate (KF–13) as a novel anti–juvenile hormone (anti–JH) agent (Furuta *et al*., 2007). 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. Although KF–13 induced obvious precocious metamorphosis at lower doses, its activity drastically decreased with increasing the applied doses. More recently, we have found that KF–13 showed JH activity as well as anti–JH activity for *B. mori* larvae (Fujita *et al*., 2008). KF–13 as a JH agonist counteracted the effect of allatectomy, *i.e*., induction of precocious metamorphosis, in a dose–dependant manner. Therefore, the activity of KF–13 observed in *B. mori* larvae is a consequence of both JH and anti–JH activity; low precocious metamorphosis–inducing activity of KF–13 at higher doses is due to the counteraction caused by KF–13 itself as a JH agonist. From studies of the structure–activity relationship of KF–13 analogs, the 4–ethoxycarbonylphenoxy moiety and the butyl side chain were apparently essential for both JH and anti–JH activity, and in the ethyl 4–(2–benzylalkyloxy)benzoate series, a correlation was observed between JH activity and anti–JH activity. In order to elucidate the detailed structure–activity relationships of this series of compounds, we further synthesized analogs in which the benzyl moiety of KF–13 was modified, and evaluated their activity to induce precocious metamorphosis and their JH activity against allatectomized 4th instar larvae.

MATERIALS AND METHODS

Synthesis

The ^{1}H–NMR spectra were determined with JEOL EX–400 (400 MHz) spectrometer.

KF–13 was prepared according to the procedure reported previously (Furuta *et al*., 2007). Compounds **3**, **4**, **9**–**13** and ethyl 4–[2–(4–benzyloxy)benzylhexyloxy] benzoate (**III**, R = 4–$OCH_2C_6H_5$) were prepared in the same manner as KF–13 but using the corresponding substituted benzyl bromide or substituted benzyl chloride instead of benzyl bromide (Method A).

Ethyl 4–[2–(3–methylbenzyl)hexyloxy]benzoate (**3**)

^{1}H–NMR ($CDCl_3$) δ: 0.89 (3H, t, J=6.8 Hz), 1.24–1.54 (6H, m), 1.38 (3H, t, J=6.8 Hz), 2.07–2.10 (1H, m), 2.29 (3H, s), 2.71 (2H, d, J=7.3 Hz), 3.82 (2H, d, J=4.9 Hz), 4.34 (2H, q, J=6.8 Hz), 6.86 (2H, d, J=8.8 Hz), 6.88–7.01 (3H, m), 7.16 (1H, t, J=7.8 Hz), 7.98 (2H, d, J=8.8 Hz).

Ethyl 4–[2–(4–methylbenzyl)hexyloxy]benzoate (**4**)

^{1}H–NMR ($CDCl_3$) δ: 0.89 (3H, t, J=6.8Hz), 1.26–1.53 (6H, m), 1.38 (3H, t, J=7.3 Hz), 2.02–2.08 (1H, m), 2.31 (3H, s), 2.71 (2H, d, J=6.8 Hz), 3.82 (2H, d, J=5.4 Hz), 4.34 (2H, q, J=7.3 Hz), 6.87 (2H, d, J=8.8 Hz), 7.02–7.08 (4H, m), 7.97 (2H, d, J=8.8 Hz).

Ethyl 4–[2–(3–chlorobenzyl)hexyloxy]benzoate (**9**)

^{1}H–NMR ($CDCl_3$) δ: 0.89 (3H, t, J=7.3 Hz), 1.30–1.34

[1] Laboratory of Pesticide Chemistry, Division of Plant Protection, Department of Applied Genetics and Pest Management, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University

* Corresponding author (E–mail: ekuwano@agr.kyushu–u.ac.jp)

(6H, m), 1.38 (3H, t, *J*=7.3 Hz), 2.04–2.10 (1H, m), 2.67–2.80 (2H, m), 3.80–3.87 (2H, m), 4.34 (2H, q, *J*=7.3 Hz), 6.87 (2H, d, *J*=8.8 Hz), 7.03 (1H, d, *J*=8.3 Hz), 7.16–7.20 (3H, m), 7.97 (2H, d, *J*=8.8 Hz).

Ethyl 4–[2–(4–chlorobenzyl)hexyloxy]benzoate (**10**)

^{1}H–NMR ($CDCl_3$) δ: 0.89 (3H, t, *J*=7.3 Hz), 1.24–1.49 (6H, m), 1.38 (3H, t, *J*=6.8 Hz), 2.03–2.06 (1H, m), 2.67–2.79 (2H, m), 3.78–3.85 (2H, m), 4.35 (2H, q, *J*=6.8 Hz), 6.87 (2H, d, *J*=8.8 Hz), 7.07 (2H, d, *J*=8.3 Hz), 7.22 (2H, d, *J*=8.3 Hz), 7.98 (2H, d, *J*=8.8 Hz).

Ethyl 4–[2–(2–fluorobenzyl)hexyloxy]benzoate (**11**)

^{1}H–NMR ($CDCl_3$) δ: 0.89 (3H, t, *J*=7.1 Hz), 1.28–1.58 (6H, m), 1.38 (3H, t, *J*=7.1 Hz), 2.09–2.14 (1H, m), 2.74–2.80 (2H, m), 3.82–3.88 (2H, m), 4.34 (2H, q, *J*=7.1 Hz), 6.85 (2H, d, *J*=8.8 Hz), 6.90–7.03 (2H, m), 7.11–7.20 (2H, m), 7.97 (2H, d, *J*=8.8 Hz).

Ethyl 4–[2–(3–fluorobenzyl)hexyloxy]benzoate (**12**)

^{1}H–NMR ($CDCl_3$) δ: 0.89 (3H, t, *J*=7.3 Hz), 1.26–1.35 (6H, m), 1.38 (3H, t, *J*=7.3 Hz), 2.05–2.11 (1H, m), 2.70–2.82 (2H, m), 3.83–3.84 (2H, m), 4.34 (2H, q, *J*=7.3 Hz), 6.87 (2H, d, *J*=8.8 Hz), 6.89–6.93 (3H, m), 7.22 (1H, t, *J*=7.8 Hz), 7.98 (2H, d, *J*=8.8 Hz).

Ethyl 4–[2–(4–fluorobenzyl)hexyloxy]benzoate (**13**)

^{1}H–NMR ($CDCl_3$) δ: 0.89 (3H, t, *J*=7.3 Hz), 1.26–1.50 (6H, m), 1.38 (3H, t, *J*=7.3 Hz), 2.01–2.05 (1H, m), 2.68–2.79 (2H, m), 3.80–3.83 (2H, m), 4.34 (2H, q, *J*=7.3 Hz), 6.87 (2H, d, *J*=8.8 Hz), 6.93 (2H, d, *J*=8.8 Hz), 7.09 (2H, d, *J*=8.8 Hz), 7.98 (2H, d, *J*=8.8 Hz).

Ethyl 4–[2–(4–hydroxybenzyl)hexyloxy]benzoate (**16**)

To a solution of compound **III** (R=4–$OCH_2C_6H_5$, 0.15 g, 0.3 mmol) in 15 ml of methanol was added a catalytic amount of palladium on carbon, and the mixture was stirred at room temperature under a hydrogen atmosphere for 3 days. The mixture was filtrated through a pad of Celite, and the filtrate was concentrated. The residue was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (3:1) to afford 0.1 g (83%) of **16**. ^{1}H–NMR ($CDCl_3$) δ: 0.89 (3H, t, *J*=6.8 Hz), 1.38 (3H, t, *J*=7.3 Hz), 1.30–1.49 (6H, m), 1.99–2.03 (1H, m), 2.68 (2H, d, *J*=7.3 Hz), 3.82 (2H, d, *J*=4.8 Hz), 4.35 (2H, q, *J*=7.3 Hz), 4.87 (1H, s), 6.73 (2H, d, *J*=8.8 Hz), 6.87 (2H, d, *J*=8.8 Hz), 7.01 (2H, d, *J*=8.8 Hz), 7.97 (2H, d, *J*=8.8 Hz).

2–(4–Methoxybenzyl)hexanoic acid (**VII**, R^1=4–OCH_3)

To a suspension of sodium hydride (60% in oil, 1.55 g, 39 mmol) in 30 ml of tetrahydrofuran (THF) at 0–5 °C was added ethyl diethylphosphonoacetate (8.67 g, 39 mmol), and the mixture was stirred for 10 minutes at the same temperature. 4–Methoxybenzaldehyde (4.0 g, 29 mmol) was added to the mixture at 0–5 °C. After stirring for 30 minutes 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, and dried over Na_2SO_4. Concentration of the solvent gave crude ethyl 4–methoxycinnamate (**V**, R^1=4–OCH_3). To a solution of **V** in 30 ml of methanol was added a catalytic amount of palladium on carbon, and the mixture was stirred at room temperature under a hydrogen atmosphere for 3 days. The mixture was filtrated through a pad of Celite, and the filtrate was concentrated. To the residue was added a solution of NaOH (4.0 g, 0.1 mol) dissolved in 15 ml of ethanol and 15 ml of water, and the mixture was stirred for 24 hr at room temperature. After removal of the solvent, the residue was dissolved in water and the aqueous solution was acidified with 2 M HCl solution. The product was extracted with ethyl acetate and the ethyl acetate solution was washed with brine, and dried over Na_2SO_4. Concentration of the ethyl acetate solution afforded 4.52 g (85%) of crude 3–(4–methoxyphenyl)propanoic acid (**VI**, R^1=4–OCH_3). A solution of **VI** (2.1 g, 11 mmol) in 5 ml of THF was added dropwise at −5 °C to a solution of lithium diisopropylamide (LDA, 2 M THF solution, 12.2 ml, 24 mmol) dissolved in 10 ml of THF. To the mixture was added hexamethylphosphoramide (HMPA, 2.38 g, 13 mmol) at the same temperature. The mixture was warmed to 50 °C and stirred for 40 minutes. The mixture was cooled to room temperature, and 1–iodobutane (2.45 g, 13 mmol) was added to the mixture. The mixture was stirred for 7 hr at 50 °C and then for 12 hr at room temperature. After removal of the solvent under reduced pressure, to the residue was added ethyl acetate and water. The aqueous solution was separated, washed with ethyl acetate, and acidified with 2 M HCl solution. The product was extracted with ethyl acetate and 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 1.23 g (47%) of **VII** (R^1=4–OCH_3). ^{1}H–NMR ($CDCl_3$) δ: 0.88 (3H, t, *J*=6.8 Hz), 1.24–1.35 (4H, m), 1.48–1.53 (1H, m), 1.59–1.66 (1H, m), 2.59–2.65 (1H, m), 2.68–2.73 (1H, m), 2.88–2.93 (1H, m), 3.78 (3H, s), 6.82 (2H, d, *J*=8.8 Hz), 7.09 (2H, d, *J*=8.8 Hz).

Ethyl 4–[2–(4–methoxybenzyl)hexyloxy]benzoate (**7**)

This substance was prepared from **VII** (R^1=4–OCH_3) according to the procedure (method A) described for KF–13 (Furuta *et al.*, 2007). ^{1}H–NMR ($CDCl_3$) δ: 0.89 (3H, t, *J*=6.8 Hz), 1.28–1.48 (6H, m), 1.37 (3H, t, *J*=7.3 Hz), 2.00–2.05 (1H, m), 2.69 (2H, d, *J*=6.8 Hz), 3.78 (3H, s), 3.82 (2H, d, *J*=5.3 Hz), 4.34 (2H, q, *J*=7.3 Hz), 6.80 (2H, d, *J*=8.3 Hz), 6.87 (2H, d, *J*=8.8 Hz), 7.06 (2H, d, *J*=8.3 Hz), 7.97 (2H, d, *J*=8.8 Hz).

Compounds **5**, **6**, **14**, **15** and **17**–**25** were prepared in the same manner as compound **7** but using the corresponding aldehyde instead of 4–methoxybenzaldehyde.

Ethyl 4–[2–(2–methoxybenzyl)hexyloxy]benzoate (**5**)

^{1}H–NMR ($CDCl_3$) δ: 0.90 (3H, t, *J*=7.1 Hz), 1.24–1.51 (6H, m), 1.37 (3H, t, *J*=7.3 Hz), 2.08–2.12 (1H, m), 2.66–2.79 (2H, m), 3.73 (3H, s), 3.79–3.91 (2H, m), 4.34 (2H, q, *J*=7.3 Hz), 6.82–6.90 (4H, m), 7.05–7.10 (1H, m), 7.16–7.20 (1H, m), 7.96 (2H, d, *J*=8.8 Hz).

Ethyl 4–[2–(3–methoxybenzyl)hexyloxy]benzoate (**6**)

^{1}H–NMR ($CDCl_3$) δ: 0.90 (3H, t, *J*=7.3 Hz), 1.29–1.46 (6H, m), 1.38 (3H, t, *J*=6.8 Hz), 2.06–2.11 (1H, m), 2.73 (2H, d, *J*=6.8 Hz), 3.72 (3H, s), 3.83 (2H, d, *J*=5.4 Hz), 4.34 (2H, q, *J*=6.8 Hz), 6.69 (1H. s), 6.72–6.75 (2H, m), 6.87 (2H, d, *J*=8.8 Hz), 7.17 (1H, t, *J*=7.8 Hz), 7.97 (2H, d, *J*=8.8 Hz).

Ethyl 4–[2–(4–ethylbenzyl)hexyloxy]benzoate (**14**)

^{1}H–NMR ($CDCl_3$) δ: 0.89 (3H, t, *J*=7.3 Hz), 1.22 (3H,

t, *J*= 7.8 Hz), 1.28–1.52 (6H, m), 1.38 (3H, t, *J*=7.3 Hz), 2.03–2.07 (1H, m), 2.61 (2H, q, *J*=7.8 Hz), 2.71 (2H, d, *J*=7.3 Hz), 3.83 (2H, d, *J*=5.7 Hz), 4.34 (2H, q, *J*=7.3 Hz), 6.87 (2H, d, *J*=8.8 Hz), 7.05–7.11 (4H, m), 7.97 (2H, d, *J*=8.8 Hz).

Ethyl 4–[2–(4–ethoxybenzyl)hexyloxy]benzoate (**15**)

^{1}H–NMR ($CDCl_3$) δ: 0.89 (3H, t, *J*=7.3 Hz), 1.24–1.50 (12H, m), 1.99–2.04 (1H, m), 2.69 (2H, d, *J*=6.8 Hz), 3.82 (2H, d, *J*=5.3 Hz), 4.00 (2H, q, *J*=6.8 Hz), 4.34 (2H, q, *J*=7.3 Hz), 6.79 (2H, d, *J*=8.8 Hz), 6.87 (2H, d, *J*=8.8 Hz), 7.05 (2H, d, *J*=8.8 Hz), 7.97 (2H, d, *J*=8.8 Hz).

Ethyl 4–[2–(3,4–difluorobenzyl)hexyloxy]benzoate (**17**)

^{1}H–NMR ($CDCl_3$) δ: 0.89 (3H, t, *J*=7.3 Hz), 1.28–1.48 (6H, m), 1.38 (3H, t, *J*=7.3 Hz), 2.02–2.05 (1H, m), 2.65–2.70 (1H, m), 2.74–2.79 (1H, m), 3.78–3.86 (2H, m), 4.35 (2H, q, *J*=7.3 Hz), 6.83–6.89 (1H, m), 6.87 (2H, d, *J*=8.8 Hz), 6.93–7.07 (2H, m), 7.99 (2H, d, *J*=8.8 Hz).

Ethyl 4–[2–(3,5–difluorobenzyl)hexyloxy]benzoate (**18**)

^{1}H–NMR ($CDCl_3$) δ: 0.89 (3H, t, *J*=7.3 Hz), 1.26–1.49 (6H, m), 1.38 (3H, t, *J*=7.3 Hz), 2.03–2.09 (1H, m), 2.67–2.72 (1H, m), 2.77–2.83 (1H, m), 3.79–3.88 (2H, m), 4.34 (2H, q, *J*=7.3 Hz), 6.62–6.71 (3H, m), 6.87 (2H, d, *J*=8.8 Hz), 7.98 (2H, d, *J*=8.8 Hz).

Ethyl 4–[2–(3,4–dimethylbenzyl)hexyloxy]benzoate (**19**)

^{1}H–NMR ($CDCl_3$) δ: 0.89 (3H, t, *J*=7.3 Hz), 1.29–1.54 (6H, m), 1.38 (3H, t, *J*=7.3 Hz), 2.02–2.07 (1H, m), 2.21 (6H, d, *J*=8.8 Hz), 2.67 (2H, d, *J*=7.3 Hz), 3.82 (2H, d, *J*=5.3 Hz), 4.34 (2H, q, *J*=7.3 Hz), 6.86–6.91 (4H, m), 7.01 (1H, d, *J*=7.3 Hz), 7.97 (2H, d, *J*=8.3 Hz).

Ethyl 4–[2–(2,4–dimethoxybenzyl)hexyloxy]benzoate (**20**)

^{1}H–NMR ($CDCl_3$) δ: 0.90 (3H, t, *J*=6.8 Hz), 1.29–1.53 (6H, m), 1.38 (3H, t, *J*=7.3 Hz), 2.07–2.08 (1H, m), 2.59–2.72 (2H, m), 3.71 (3H, s), 3.78 (3H, s), 3.79–3.87 (2H, m), 4.34 (2H, q, *J*=7.3 Hz), 6.37 (1H, m), 6.42 (1H, d, *J*=2.4 Hz), 6.85 (2H, d, *J*=8.8 Hz), 6.96 (1H, d, *J*=7.8 Hz), 7.96 (2H, d, *J*=8.8 Hz).

Ethyl 4–[2–(3,4–dimethoxybenzyl)hexyloxy]benzoate (**21**)

^{1}H–NMR ($CDCl_3$) δ: 0.90 (3H, t, *J*=7.3 Hz), 1.24–1.49 (6H, m), 1.38 (3H, t, *J*=7.3 Hz), 2.04–2.07 (1H, m), 2.71 (2H, d, *J*=7.3 Hz), 3.72 (3H, s), 3.81–3.87 (2H, m), 3.85 (3H, s), 4.34 (2H, q, *J*=7.3 Hz), 6.64–6.69 (2H, m), 6.76 (1H, d, *J*=8.3 Hz), 6.88 (2H, d, *J*=8.8 Hz), 7.97 (2H, d, *J*=8.8 Hz).

Ethyl 4–[2–(3,5–dimethoxybenzyl)hexyloxy]benzoate (**22**)

^{1}H–NMR ($CDCl_3$) δ: 0.90 (3H, t, *J*=7.3 Hz), 1.31–1.37 (6H, m), 1.38 (3H, t, *J*=7.3 Hz), 2.02–2.05 (1H, m), 2.70 (2H, d, *J*=7.3 Hz), 3.69 (6H, s), 3.83 (2H, d, *J*=4.9 Hz), 4.34 (2H, q, *J*=7.3 Hz), 6.30 (3H, s), 6.87 (2H, d, *J*=8.8 Hz), 7.97 (2H, d, *J*=8.8 Hz).

Ethyl 4–[2–(3,4–methylenedioxybenzyl)hexyloxy]benzoate (**23**)

^{1}H–NMR ($CDCl_3$) δ: 0.89 (3H, t, *J*=7.3 Hz), 1.30–1.50 (6H, m), 1.38 (3H, t, *J*=7.3 Hz), 2.00–2.05 (1H, m), 2.66–2.68 (2H, m), 3.83 (2H, d, *J*=4.9 Hz), 4.34 (2H, q, *J*=7.3 Hz), 5.91 (2H, s), 6.58–6.71 (3H, m), 6.87 (2H, d, *J*=8.8 Hz), 7.97 (2H, d, *J*=8.8 Hz).

Ethyl 4–[2–(1,4–benzodioxan–6–yl)methylhexyloxy]benzoate (**24**)

^{1}H–NMR ($CDCl_3$) δ: 0.89 (3H, t, *J*=7.1 Hz), 1.26–1.45 (6H, m), 1.37 (3H, t, *J*=7.3 Hz), 1.99–2.04 (1H, m), 2.63 (2H, d, *J*=7.4 Hz), 3.83 (2H, d, *J*=5.3 Hz), 4.23 (4H, s), 4.34 (2H, q, *J*=7.3 Hz), 6.59–6.62 (1H, m), 6.66 (1H, d, *J*=2.0 Hz), 6.74 (1H, d, *J*=8.8 Hz), 6.87 (2H, d, *J*=8.8 Hz), 7.97 (2H, d, *J*=8.8 Hz).

Ethyl 4–[2–(2–naphthylmethyl)hexyloxy]benzoate (**25**)

^{1}H–NMR ($CDCl_3$) δ: 0.89 (3H, t, *J*=6.8 Hz), 1.29–1.55 (6H, m), 1.38 (3H, t, *J*=6.8 Hz), 2.16–2.22 (1H, m), 2.87–2.97 (2H, m), 3.87 (2H, dd, *J*=1.5 and 5.3 Hz), 4.34 (2H, q, *J*=6.8 Hz), 6.88 (2H, d, *J*=8.8 Hz), 7.29–7.32 (1H, m), 7.40–7.46 (2H, m), 7.58 (1H, s), 7.71–7.81 (3H, m), 7.97 (2H, d, *J*=8.8 Hz).

2–(2–Methylbenzyl)–1–hexanol (**X**, R^2=CH_3)

To a solution of ethyl diethylphosphonoacetate (4.05 g, 18 mmol) in 20 ml of dimethylsulfoxide (DMSO) at 0–5 °C was added potassium *tert*–butoxide (*t*–BuOK, 2.2 g, 20 mmol), and the mixture was stirred for 10 minutes at room temperature. To the mixture was added 1–bromobutane (2.69 g, 20 mmol), and then the mixture was stirred for 2 hr at 60 °C. The mixture was cooled to 0–5 °C and the reaction was quenched by the addition of saturated NH4Cl solution. The product was extracted with tert–butyl methyl ether. The ether solution was washed with brine, and dried over Na_2SO_4. Concentration of the solvent afforded crude ethyl 2–(diethylphosphono) hexanoate (**VIII**).

To a suspension of sodium hydride (60% in oil, 0.34 g, 14 mmol) in 20 ml of THF at 0–5 °C was added the above **VIII**, and the mixture was stirred for 10 minutes at the same temperature. To the mixture was added 2–methylbenzaldehyde (1.0 g, 83 mmol). After stirring for 3 hr at 50 °C, the reaction was quenched by the addition of saturated NH_4Cl solution. The product was extracted with ethyl acetate and the ethyl acetate solution was washed with brine, and dried over Na_2SO_4. Concentration of the solvent afforded crude ethyl 2–(2–benzylidene)hexanoate (**IX**, R^2=CH_3).

A mixture of crude **IX** (1.69 g) and lithium aluminum hydride (0.27 g, 71 mmol) in 20 ml of dry THF was stirred for 3 hr at room temperature. The mixture was quenched at 0–5 °C with saturated NH_4Cl solution. After removal of the solvent, 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 with column chromatography on silica gel eluting with hexane–ethyl acetate (10:1) to afford 1.12 g (80%) of 2–(2–methylbenzylidene)–1–hexanol.

To a solution of the above alcohol in 20 ml of methanol was added a catalytic amount of palladium on carbon, and the mixture was stirred at room temperature under a hydrogen atmosphere for 12 hr. The mixture was filtrated through a pad of Celite, and the filtrate was concentrated to give 2–(2–methylbenzyl)–1–hexanol (**X**,

R^2=CH_3).

Ethyl 4–[2–(2–methylbenzyl)hexyloxy]benzoate (**2**)

This was prepared from compound **X** (R^2=CH_3) and ethyl 4–hydroxybenzoate by the method described for KF–13 (Furuta *et al*., 2007). Purification was done by column chromatography (hexane–ethyl acetate, 10:1). ^{1}H–NMR (CDCl3) δ: 0.89 (3H, t, *J*=7.1 Hz), 1.21–1.63 (6H, m), 1.38 (3H, t, *J*=7.1 Hz), 1.99–2.06 (1H, m), 2.30 (3H, s), 2.67–2.81 (2H, m), 3.83–3.86 (2H, m), 4.34 (2H, q, *J*=7.1 Hz), 6.85 (2H, d, *J*=8.8 Hz), 7.07–7.15 (4H, m), 7.96 (2H, d, *J*=8.8 Hz).

Compound **8** was prepared in the same manner as compound **2** but using 2–chlorobenzaldehyde instead of 2–methylbenzaldehyde.

Ethyl 4–[2–(2–chlorobenzyl)hexyloxy]benzoate (**8**)

^{1}H–NMR ($CDCl_3$) δ: 0.89 (3H, t, *J*=7.1 Hz), 1.26–1.60 (6H, m), 1.36 (3H, t, *J*=6.9 Hz), 2.17–2.20 (1H, m), 2.84–2.90 (2H, m), 3.81–3.89 (2H, m), 4.33 (2H, q, *J*=6.9 Hz), 6.85 (2H, d, *J*=8.8 Hz), 7.11–7.17 (3H, m), 7.32–7.35 (1H, m), 7.96 (2H, d, *J*=8.8 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 24hr–old 3rd instar larvae. Eighteen to 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. Ten larvae were used for each treatment.

RESULTS AND DISCUSSION

Synthesis

A large number of ethyl 4–[2–(substituted benzyl) hexyloxy]benzoates were synthesized from 2–(substituted benzyl)–1–hexanols, which were prepared by three different procedures (Fig. 1). According to the procedure previously reported (Furuta *et al*., 2007), some 2–(substituted benzyl)hexanoic acids (**I**) were prepared by reaction of hexanoic acid with substituted benzyl bromides or benzyl chlorides in the presence of LDA (Method A). Compounds (**I**) were reduced to 2–(substituted benzyl)–1–hexanols (**II**) with $LiAlH_4$. Compounds (**II**) were converted to the corresponding toluenesulfonates, which were treated with ethyl 4–hydroxybenzoate in DMF in the presence of K_2CO_3 to afford ethyl 4–[2–(substituted benzyl)hexyloxy]benzoates (**III**).

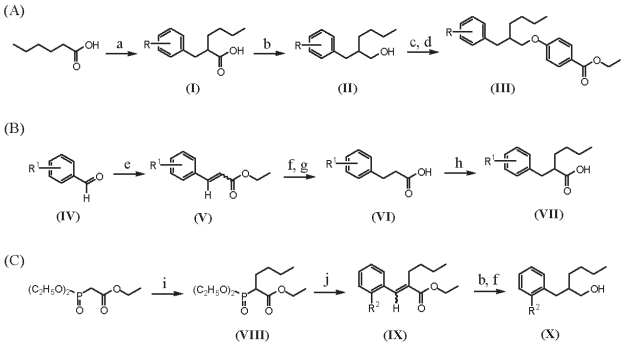


Fig. 1. Synthetic scheme for preparation of (A) 4–[2–(substituted benzyl)hexyloxy]benzoates (**III**), (B) 2–(substituted benzyl)hexanoic acids (**VII**), and (C) 2–(2–substituted benzyl)hexanols (**X**).
(a) substituted benzyl bromides or benzyl chlorides, LDA, THF, HMPA; (b) LiAlH4, THF; (c) *p*–toluenesulfonyl chloride, $N(C_2H_5)_3$, CH_2Cl_2; (d) ethyl 4–hydroxybenzoate, K_2CO_3, DMF; (e) NaH, ethyl diethylphosphonoacetate, THF; (f) H_2, Pd/C, MeOH; (g) NaOH, EtOH and H_2O; (h) n–C_4H_9I, LDA, THF, HMPA; (i) n–C_4H_9Br, t–C_4H_9OK, DMSO; (j) NaH, 2–substituted benzaldehyde, THF.

A variety of commercially available aromatic aldehydes were utilized as starting materials to prepare 2–(substituted benzyl)–1–hexanoic acids (Method B). The Wittig–Horner reaction of substituted benzaldehydes (**IV**) with ethyl diethylphosphonoacetate in THF in the presence of NaH as a base afforded ethyl substituted cinnamates (**V**). Catalytic hydrogenolysis over palladium–carbon of (**V**) followed by alkaline hydrolysis gave the corresponding 3–(substituted phenyl)propanoic acids (**VI**). Standard alkylation of (**VI**) with 1–iodobutane using LDA as a base afforded 2–(substituted benzyl)–1–hexanoic acids, which were reduced to the corresponding 1–hexanols in the same manner as compound (**I**).

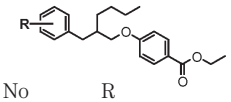
Since alkylation of the α–position of 3–substituted propanoic acids sometimes proceeded in poor yields, an alternative procedure was used for the preparation of 2–(2–substituted benzyl)–1–hexanols (Method C). Alkylation of ethyl diethylphosphonoacetate with 1–bromobutane using *t*–BuOK as a base gave ethyl 2–(diethylphosphono)hexanoate (**VIII**). Treatment of (**VIII**) with 2–substituted benzaldehydes afforded ethyl 2–(2–substituted benzylidene)hexanoates (**IX**). Reduction of (**IX**) with LiAlH4 followed by catalytic hydrogenolysis afforded 2–(2–substiuted benzyl)–1–hexanols in relatively good yields.

Biological activity

Anti–JH activity

The anti–JH activity of KF–13 analogs was determined by precocious metamorphosis of the treated larvae as previously reported (Fujita *et al*., 2005). When 24–hr–old 3rd instar larvae were treated, precocious pupation occurred after the 4th larval stage. None of the treated 3rd instar larvae metamorphosed into precocious miniature pupae after the same larval stage. Bioassay data are presented in Table 1 for a number of ethyl

Table 1. Precocious metamorphosis–inducing activity of KF–13 and its derivatives against 3rd instar larvae of *B. mori*

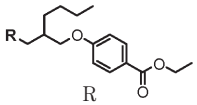


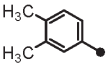
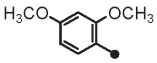
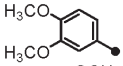
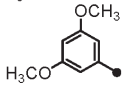
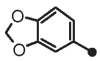
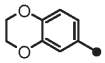
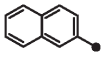
No	R	Precocious metamorphosis (%) 1	10	40	(μg/larva)
1	H (KF–13)	90	34	12	
2	2–CH_3	100	89	43	
3	3–CH_3	52	87	97	
4	4–CH_3	95	85	28	
5	2–OCH_3	67	76	92	
6	3–OCH_3	68	92	100	
7	4–OCH_3	95	94	94	
8	2–Cl	100	80	44	
9	3–Cl	30	69	74	
10	4–Cl	59	89	68	
11	2–F	100	70	39	
12	3–F	58	79	85	
13	4–F	58	85	82	
14	4–C_2H_5	20	45	68	
15	4–OC_2H_5	5	47	80	
16	4–OH	15	55	70	

4–[2–(monosubstituted benzyl)hexyl]benzoates. As previously described (Furuta *et al*., 2007), KF–13 strongly induced precocious metamorphosis at 1 μg and its activity decreased with increasing the applied doses. Introduction of a methyl (**2**), chloro (**8**) or fluoro (**11**) substituent at the 2–position on the benzene ring increased the activity in comparison with that of KF–13 in a dose range of 1–40 μg, however, no consistent dose–response relationship was obtained in these compounds as well as KF–13. The 2–methoxybenzyl analog **5** at 1 μg showed lower activity than KF–13, but it had higher activity at 10 and 40 μg. Although precocious metamorphosis–inducing activity decreased by introducing a substituent at the 3–position on the benzene ring (**3**, **6**, **9** and **12**) at a low dose of 1 μg, in contrast to KF–13, they showed activity in a dose–response manner to some extent. The 4–methylbenzyl (**4**) and 4–methoxybenzyl (**7**) analogs at 1 μg had almost the same level of activity as KF–13. It is noteworthy that introduction of a methoxy substituent on the benzene ring irrespective of its position (**5**, **6** and **7**) increased activity at a high dose of 40 μg, and especially **7** induced high percentages of precocious metamorphosis in a dose range of 1–40 μg. Introduction of a chloro (**10**), fluoro (**13**), ethyl (**14**), ethoxy (**15**) or hydroxy (**16**) substituent at the 4–position decreased the activity at 1 μg, however, these compounds showed moderate activity at 40 μg.

Since precocious metamorphosis–inducing activity was significantly affected by the substituent on the benzene ring, we also examined the activity of disubstituted benzyl analogs and related compounds (Table 2). Additional introduction of a fluoro substituent (**17** and **18**) decreased the degree of the activity at 1 μg in comparison with those of monofluoro–substituted analogs (**12** and **13**). The 3,4–dimethylbenzyl analog **19** did not induce precocious metamorphosis at 1 μg. The 3,5–dimethoxybenzyl analog **22** had no activity at 1–40 μg, while the 2,4–dimethoxybenzyl analog 20 showed considerable activity. It is interesting to note

Table 2. Precocious metamorphosis–inducing activity of KF–13 derivatives and related compounds against 3rd instar larvae of *B. mori*



No	R	Precocious metamorphosis (%) 1	10	40	(μg/larva)
17		22	60	97	
18		10	32	55	
19		0	25	70	
20		37	95	85	
21		0	0	5	
22		0	0	0	
23		45	95	100	
24		0	26	29	
25		0	10	10	

that 3,4–dimethoxybenzyl analog **21** had little activity, whereas 3,4–methylendioxybenzyl analog **23** showed moderate activity in a dose–response manner. Benzodioxan (**24**) and naphthalene (**25**) analogs had low activity, indicating that 5–membered ring is favorable for activity. Thus, compounds **2**, **8** and **11** were the most active of the analogs tested at a low dose of 1 μg.

JH activity

As previously described (Fujita *et al*., 2008), KF–13 showed JH activity as well as anti–JH activity for *B. mori* larvae. When topically applied to allatectomized 4th instar larvae of *B. mori*, KF–13 as a JH agonist counteracted the precocious metamorphosis induced by allatectomy in a dose–dependant manner. In the ethyl 4–(2–benzylalkyloxy)benzoate series, a correlation was observed between JH activity and anti–JH activity; KF–13, which clearly induced precocious metamorphosis against 3rd instar larvae at low doses, had comparatively high JH activity against allatectomized 4th instar larvae. We therefore examined whether or not a new series of KF–13 analogs had JH activity when topically applied to allatectomized 4th instar larvae (Table 3). All of the allatectomized and acetone–treated control larvae underwent precocious metamorphosis. Compounds with a methyl substituent on the benzene ring (**2**, **3** and **4**) showed JH activity so that all treated larvae molted into 5th instar larvae. The 2–methoxybenzyl analog **5**, which had the same level of precocious metamorphosis–inducing activity as **3**, showed somewhat low JH activity, while the 3–methoxybenzyl (**6**) and 4–methoxybenzyl (**7**) ana-

Table 3. JH activity of KF–13 derivatives against allatectomized 4th instar larvae of *B. mori*

Treatment	Number of larvae transformed into		
	Precocious pupa	Larval–pupal intermediate	5th instar larva
Allatectomized control	10	0	0
+2	0	0	10
+3	0	0	10
+4	0	0	10
+5	2	3	7
+6	0	1	9
+7	0	0	10
+8	0	0	10
+9	7	3	0
+10	1	0	9
+11	0	0	10
+13	0	0	10
+14	1	1	8
+18	10	0	0
+21	9	0	1
+22	8	1	1

Forty micrograms of each compound in acetone solution (4 μl) was topically applied to allatectomized 4th instar larvae. Number of larvae tested: 10

logs had obvious JH activity. The 3–chlorobenzyl analog **9** showing low precocious metamorphosis–inducing activity at 1 μg exhibited relatively weak JH activity. Compounds **8** and **11**, which were the most effective in inducing precocious metamorphosis at low doses, completely counteracted precocious metamorphosis caused by allatectomy. Compounds **18**, **21** and **22** possessing weak precocious metamorphosis–inducing activity showed little JH activity. Thus, in a series of ethyl 4–[2–(substituted benzyl)hexyloxy]benzoates and related compounds, there was a correlation between JH activity and anti–JH activity as well. In order to develop a genuine anti–JH agent showing no JH activity further modification of structure is under investigation.

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