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Synthesis and Anti–Juvenile Hormone Activity of Alkyl 4–(2–Phenoxyalkyloxy)benzoates and Related Compounds

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A number of alkyl 4–(2–phenoxyhexyloxy)benzoates and related compounds were synthesized and evaluated their activity to induce precocious metamorphosis in larvae of the silkworm, which was clearly recognized as a juvenile hormone–deficiency symptom. In the alkyl 4–(2–phenoxyhexyloxy)benzoate series, only the methyl and ethyl esters showed precocious metamorphosis–inducing activity. Replacement of the ester group with an ethylcarbamoyl, butanoyl, nitro or a phenoxy group dramatically decreased or eliminated the activity. Both enantiomers of ethyl 4–(4–methyl–2–phenoxypentyloxy)benzoate (**13**) were prepared by starting with L– and D–leucine. There was no significant difference in precocious metamorphosis–inducing activity between **13R**(+)– and **13S**(–)–enantiomers. Conversion of the 4–ethoxycarbonyl group of **13** to the corresponding carboxylic acid eliminated the activity, indicating that the methyl or ethyl ester group is indispensable for the activity.

INTRODUCTION

Since juvenile hormone (JH) is involved in a wide range of physiological processes in both developing and mature insects (Riddiford, 1994), compounds that possess anti–JH activity are potentially useful not only as biochemical probes to assist in elucidating the role of JH in insect development and reproduction, but also as insect growth regulators (Staal, 1986). Several compounds including precocenes (Bowers *et al*., 1976), fluoromevalonate (Quistad *et al*., 1981), ethyl 4–[2–(*tert*–butylcarbonyloxy)butyloxy]benzoate (ETB) (Staal, 1982), dichloroallyl hexanoate (Quistad *et al*., 1985), 1,5–disubstituted imidazoles (Pratt *et al*., 1990) and brevioxime (Moya *et al*., 1997) have so far been found to have anti–JH activity. However, none of the compounds has been developed for practical use in pest control as yet. Among them, ETB is known to be a unique anti–JH agent. It shows anti–JH activity as well as JH activity for the tobacco hornworm, *Manduca sexta* (Staal, 1982) and the silkworm, *Bombyx mori* (Kiguchi *et al*., 1984), depending on the dose applied; low doses of ETB induced precocious metamorphosis, a clear JH–deficiency symptom, but at higher doses only JH–like activity was observed. Riddiford *et al*. (1983) have reported that ETB acts as a partial JH antagonist at the target tissue of the larval epidermis. No other anti–JH agents with such action have been found to date.

In our previous studies (Ishiguro *et al*., 2003), we found that ethyl 4–[2–(6–methyl–3–pyridyloxy)butyloxy] benzoate (**1**), structurally derived from ETB (Fig. 1), showed stronger activity than ETB. Modifications of the ethyl side chain of **1** revealed that the butyl (**2**) and isobutyl (**3**) groups were optimal for high activity (Fujita *et al*., 2005). Further structure activity relationship studies on this series of compounds indicated that the phenyl analog **4** showed activity comparable to that of **2**, suggesting that the 6–methyl–3–pyridyl moiety is not significant for activity (Furuta *et al*., 2006). Conversion of the 4–ethoxycarbonyl group of **2** and **4** to the corresponding carboxylic acids eliminated the activity, indicating that the ester moiety is responsible for the activity. We therefore synthesized additional analogs in which the ethoxycarbonyl group of **4** was modified, and evaluated their activity to induce precocious metamorphosis in *B. mori* larvae. We also examined the precocious metamorphosis–inducing activity of both optical isomers of ethyl 4–(4–methyl–2–phenoxypentyloxy) benzoate. We report here the structure–activity relationships of new phenyl ether analogs derived from compound **4**.

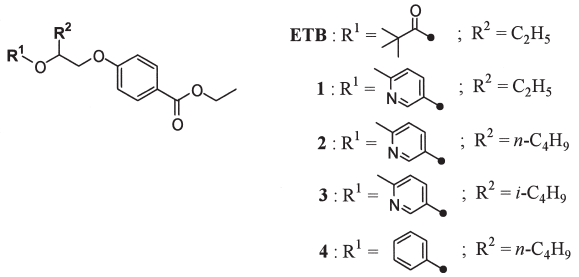

Fig. 1. Structures of ETB and ethyl 4–substituted benzoates.

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MATERIALS AND METHODS

Synthesis

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

Methyl (*S*)–2–hydroxy–4–methylpentanoate and its enantiomer (*R*) were prepared from L–leucine and D–leucine respectively according to the method described by Valls *et al*. (2002). The preparation of a series of phenyl ether analogs (**4–12**) and optically active **13R** is outlined in Fig. 2 (A) and (B), respectively.

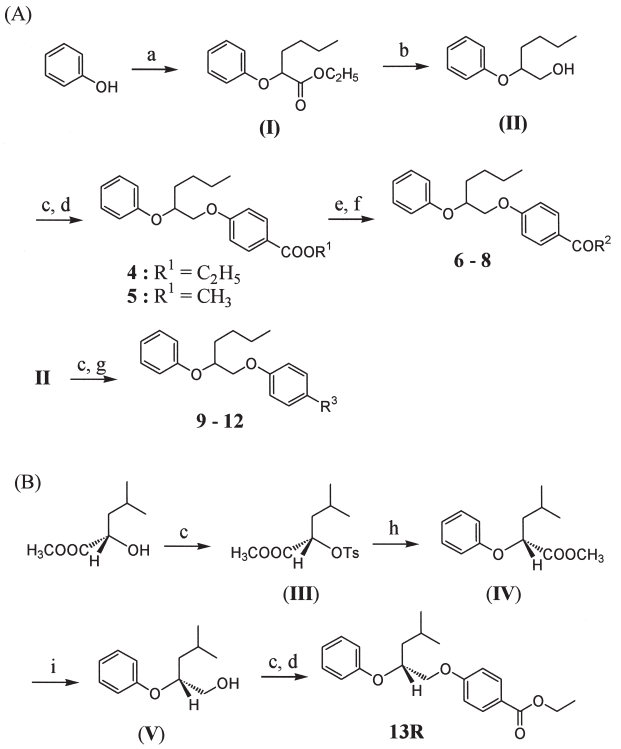


Fig. 2. Synthetic scheme for preparation of (A) ethyl 4–(2–phenoxyhexyloxy)benzoates and related compounds, and (B) ethyl 4–[(*R*)–4–methyl–2–phenoxypentyloxy]benzoate (**13R**). (a) ethyl 2–bromohexanoate, K_2CO_3, DMF; (b) $LiAlH_4$, THF; (c) *p*–toluenesulfonyl chloride, triethylamine, THF; (d) ethyl 4–hydroxybenzoate or methyl 4–hydroxybenzoate, K_2CO_3, DMF; (e) NaOH, EtOH and H_2O; (f) corresponding alcohol or amine, 4–(*N*,*N*–dimethylamino) pyridine, 1–[3–(dimethylamino)propyl]–3–ethylcarbodiimide hydrochloride, CH_2Cl_2; (g) 4–substituted phenol, K_2CO_3, DMF; (h) phenol, K_2CO_3, DMF; (i) NaBH4, ethanol.

Ethyl 2–phenoxyhexanoate (**I**)

A mixture of ethyl 2–bromohexanoate (2.7 g, 12 mmol), phenol (1.0 g, 11 mmol) and potassium carbonate (1.7 g, 12 mmol) in 20 ml of dimethylformamide (DMF) was stirred for 5 hr at room temperature. The mixture was poured into 30 ml of water and the product was extracted with 50 ml of 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 2.4 g (93%) of compound (**I**). ^{1}H–NMR ($CDCl_3$) δ : 0.93 (3H, t, J= 7.3 Hz, CH_3), 1.24 (3H, t, J=6.8 Hz, CH_3), 1.35–1.43 (2H, m, CH_2), 1.46–1.54 (2H, m, CH_2), 1.89–1.98 (2H, m, CH_2), 4.30 (2H, q, J=6.8 Hz, CH_2), 4.54–4.60 (1H, m, CH), 6.88 (2H, d, J=8.3 Hz, phenyl), 6.96 (1H, t, J= 7.3 Hz, phenyl), 7.25–7.29 (2H, m, phenyl).

2–Phenoxy–1–hexanol (**II**)

A mixture of **I** (2.36 g, 10 mmol) and lithium aluminum hydride (0.38 g, 10 mmol) in 30 ml of tetrahydrofuran (THF) was stirred for 2.5 hr at room temperature. The reaction mixture was quenched with saturated NH_4Cl solution. After removal of the solvent under reduced pressure, 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 organic layer gave 1.87 g (96%) of **II**. ^{1}H–NMR ($CDCl_3$) δ : 0.89 (3H, t, J= 7.3 Hz, CH_3), 1.24–1.38 (4H, m, CH_2CH_2), 3.71–3.83 (2H, m, CH_2), 4.32–4.38 (1H, m, CH), 6.90–6.98 (3H, m, phenyl), 7.25–7.32 (2H, m, phenyl).

Ethyl 4–(2–phenoxyhexyloxy)benzoate (**4**)

To a solution of **II** (1.9 g, 9.8 mmol) in 30 ml of dichloromethane was added triethylamine (1.46 g, 14 mmol) and *p*–toluenesulfonyl chloride (2.24 g, 12 mmol), and the mixture was stirried for 48 hr at room temperature. After removal of the solvent under reduced pressure, the product was extracted with *tert*–butyl methyl ether. The ether solution was washed with brine, dried over Na_2SO_4. Concentration of the solvent gave crude 2–phenoxyhexyl *p*–toluenesulfonate. A mixture of this *p*–toluenesulfonate (3.17 g, 9.5 mmol), ethyl 4–hydroxybenzoate (1.9 g, 11 mmol) and potassium carbonate (1.61 g, 12 mmol) in 30 ml of DMF was heated at 90–100 ˚C for 8 hr. To the mixture was added 30 ml of water and the product was extracted with *tert*–butyl methyl ether. The ether solution was washed with 1N NaOH solution and brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel eluting with hexane–ethyl acetate (5:1) to afford 2.73g (84%) of **4** as an oil. ^{1}H–NMR ($CDCl_3$) δ : 0.92 (3H, t, J=7.1 Hz, CH_3), 1.38 (3H, t, J= 7.2 Hz, CH_3), 1.39–1.55 (4H, m, CH_2), 1.80–1.84 (2H, m, CH_2), 4.09–4.20 (2H, m, CH_2), 4.33 (2H, q, J=7.2 Hz, CH_2), 4.56–4.61 (1H, m, CH), 6.89 (2H, d, J=8.7 Hz, phenyl), 6.91–6.97 (2H, m, phenyl), 7.24–7.31 (3H, m, phenyl), 7.97 (2H, d, J= 8.7 Hz, phenyl).

Compound **5** was prepared in the same manner as compound **4** with use of methyl 4–hydroxybenzoate instead of ethyl 4–hydroxybenzoate.

Methyl 4–(2–phenoxyhexyloxy)benzoate (**5**)

^{1}H–NMR ($CDCl_3$) δ : 0.89 (3H, t, J=7.1 Hz, CH_3), 1.24–1.52 (6H, m, $3CH_2$), 2.05–2.11 (1H, m, CH), 2.75 (2H, d, J=6.9 Hz, CH_2), 3.83 (2H, d, J=5.4 Hz, CH_2), 3.88 (3H, s, CH_3), 6.87 (2H, d, J= 8.8 Hz, phenyl), 7.16–7.28 (5H, m, phenyl), 7.96 (2H, d, J= 8.8 Hz, phenyl).

4–(2–Phenoxyhexyloxy)benzoic acid (**4–acid**)

A mixture of **4** (0.48 g, 1.4 mmol) and NaOH (0.24 g, 6 mmol) in 15 ml of ethanol and 15 ml of water was refluxed for 6 hr. After removal of the solvent, the product was extracted with *tert*–butyl methyl ether. The ether solution was washed with 1N HCl solution 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 (1:1) to afford 0.40 g (93%) of **4–acid** as a solid, mp 56.5–58 °C. ^{1}H–NMR ($CDCl_3$) δ : 0.92 (3H, t, J= 7.3 Hz, CH_3), 1.32–1.56 (4H, m, $2CH_2$), 1.81–1.87 (2H, m, CH_2), 4.11–4.23 (2H, m, CH_2), 4.59–4.64 (1H, m, CH), 6.93–6.98 (5H, m, phenyl), 7.26–7.31 (2H, m, phenyl), 8.05 (2H, d, J=9.3 Hz, phenyl).

Propyl 4–(2–phenoxyhexyloxy)benzoate (**6**)

To a mixture of **4–acid** (0.50 g, 1.6 mmol), 4–(*N,N*–dimethylamino)pyridine (0.05 g, 0.41 mmol) and 1–propanol (0.19 g, 3.2 mmol) in 30 ml of dichloromethane was added 1–[3–(dimethylamino)propyl]–3–ethylcarbodiimide hydrochloride (0.35 g, 1.8 mmol) at 0 °C. After stirring for 30 hr at room temperature, the product was extracted with *tert*–butyl methyl ether. The ether solution was washed with 1N NaOH solution 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 0.56 g (99%) of **6** as an oil. ^{1}H–NMR ($CDCl_3$) δ : 0.92 (3H, t, J=7.3 Hz, CH_3), 1.02 (3H, t, J=7.3 Hz, CH_3), 1.35–1.43 (4H, m, $2CH_2$), 1.75–1.86 (4H, m, $2CH_2$), 4.09–4.21 (2H, m, CH_2), 4.25 (2H, t, J=6.4 Hz, CH_2), 4.58–4.61 (1H, m, CH), 6.89–6.98 (5H, m, phenyl), 7.27–7.30 (2H, m, phenyl), 7.98 (2H, d, J=8.8 Hz, phenyl).

Compound **7** was similarly synthesized by reaction of **4–acid** with 1–butanol.

n–Butyl 4–(2–phenoxyhexyloxy)benzoate (**7**)

Yield 99%; oil; 1H–NMR ($CDCl_3$) δ : 0.91 (3H, t, J= 7.3 Hz, CH_3), 0.98 (3H, t, J=7.3 Hz, CH_3), 1.33–1.53 (6H, m, $3CH_2$), 1.70–1.77 (2H, m, CH_2), 1.81–1.85 (2H, m, CH_2), 4.09–4.13 (1H, m, CH), 4.17–4.21 (1H, m, CH), 4.29 (2H, t, J=6.4 Hz, CH_2), 4.57–4.60 (1H, m, CH), 6.88–6.98 (5H, m, phenyl), 7.26–7.31 (2H, m, phenyl), 7.98 (2H, d, J=8.8 Hz, phenyl).

N–Ethyl–4–(2–phenoxyhexyloxy)benzamide (**8**)

A mixture of ethylamine hydrochloride (0.14 g, 1.7 mmol) and triethylamine (0.32 g, 3.2 mmol) in 10 ml of dichloromethane was stirred for 1 hr at room temperature. To the mixture was added **4–acid** (0.52 g, 1.5 mmol), 4–(*N,N*–dimethylamino)pyridine (0.03 g, 0.25 mmol) and 1–[3–(dimethylamino)propyl]–3–ethylcarbodiimide hydrochloride (0.37 g, 1.9 mmol). The mixture was stirred for 2 hr at 0 °C and then for 20 hr at room temperature. After normal workup, the crude product was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (1:1) to afford 0.56 g (100%) of **8** as an oil. ^{1}H–NMR ($CDCl_3$) δ : 0.92 (3H, t, J=6.8 Hz, CH_3), 1.24 (3H, t, J=6.8 Hz, CH_3), 1.33–1.55 (4H, m, $2CH_2$), 1.80–1.86 (2H, m, CH_2), 3.47 (2H, q, J= 6.8 Hz, CH_2), 4.08–4.11 (1H, m, CH), 4.15–4.19 (1H, m, CH), 4.56–4.62 (1H, m, CH), 5.99 (1H, s, NH), 6.90 (2H, d, J=8.3 Hz, phenyl), 6.92–6.98 (3H, m, phenyl), 7.27 (2H, d, J=8.3 Hz, phenyl), 7.70 (2H, d, J=8.3 Hz, phenyl).

Compounds **9–12** were prepared in the same manner as compound **4** with use of the corresponding 4–substituted phenol instead of ethyl 4–hydroxybenzoate. The yields were calculated as based on 4–substituted phenol.

1–[4–(2–Phenoxyhexyloxy)phenyl]–1–butanone (**9**)

Yield 34%; oil; ^{1}H–NMR ($CDCl_3$) δ : 0.92 (3H, t, J= 7.3 Hz, CH_3), 1.00 (3H, t, J=7.3 Hz, CH_3), 1.35–1.56 (4H, m, $2CH_2$), 1.70–1.86 (4H, m, $2CH_2$), 2.89 (2H, t, J= 7.3 Hz, CH_2), 4.10–4.14 (1H, m, CH), 4.18–4.26 (1H, m, CH), 4.57–4.63 (1H, m, CH), 6.91–6.98 (4H, m, phenyl), 7.27–7.31 (3H, m, phenyl), 7.92 (2H, d, J= 8.3 Hz, phenyl).

4–Nitrophenyl 2–phenoxyhexyl ether (**10**)

Yield 80%; oil; ^{1}H–NMR ($CDCl_3$) δ : 0.93 (3H, t, J= 7.3 Hz, CH_3), 1.34–1.54 (4H, m, $2CH_2$), 1.81–1.86 (2H, m, CH_2), 4.15–4.25 (2H, m, CH_2), 4.59–4.65 (1H, m, CH), 6.91–6.99 (5H, m, phenyl), 7.27–7.31 (2H, m, phenyl), 8.19 (2H, d, J=9.3 Hz, phenyl).

4–(Imidazol–1–yl)phenyl 2–phenoxyhexyl ether (**11**)

Yield 69%; oil; ^{1}H–NMR ($CDCl_3$) δ : 0.93 (3H, t, J= 7.3 Hz, CH_3), 1.36–1.53 (4H, m, $2CH_2$), 1.81–1.86 (2H, m, CH_2), 4.08–4.19 (2H, m, CH_2), 4.59–4.62 (1H, m, CH), 6.95–6.99 (5H, m, phenyl), 7.18–7.31 (6H, m, phenyl, imidazolyl), 7.75 (1H, s, imidazolyl).

4–Phenoxyphenyl 2–phenoxyhexyl ether (**12**)

Yield 82%; oil; ^{1}H–NMR ($CDCl_3$) δ : 0.92 (3H, t, J= 7.3 Hz, CH_3), 1.35–1.46 (4H, m, $2CH_2$), 1.80–1.86 (2H, m, CH_2), 4.02–4.06 (1H, m, CH), 4.11–4.15 (1H, m, CH), 4.55–4.60 (1H, m, CH), 6.85–7.06 (9H, m, phenyl), 7.26–7.31 (5H, m, phenyl).

Methyl (S)–4–methyl–2–p–toluenesulfonyloxypentanoate (**III**)

To a solution of methyl (*S*)–2–hydroxy–4–methylpentanoate (1.0 g, 6.8 mmol) and triethylamine (0.83 g, 8.2 mmol) in 15 ml of dichloromethane was added *p*–toluenesulfonyl chloride (1.6 g, 8.2 mmol). After stirring for 12 hr at room temperature, 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 by eluting with hexane–ethyl acetate (3:1) to afford 1.07 g (52%) of **III** as an oil. ^{1}H–NMR ($CDCl_3$) δ : 0.80 (3H, d, J=6.8 Hz, CH_3), 0.90 (3H, d, J= 6.8 Hz, CH_3), 1.52–1.59 (1H, m, CH), 1.65–1.72 (1H, m, CH), 1.75–1.82 (1H, m, CH), 2.45 (3H, s, CH_3), 3.64 (3H, s, CH_3), 4.86 (1H. d,d, J=3.9, 9.3 Hz, CH), 7.33–7.35 (2H, d, J=8.3 Hz, phenyl), 7.81–7.83 (2H, d, J=8.3 Hz, phenyl).

Methyl (R)–4–methyl–2–phenoxypentanoate (**IV**)

A suspension of phenol (0.37 g, 3.9 mmol) and potassium carbonate (0.54 g, 3.9 mmol) in 15 ml of DMSO was stirred for 30 min at room temperature. To the mixture was added **III** (1.07 g, 3.6 mmol), and the mixture was stirred for 36 hr at room temperature. The product was extracted with ethyl acetate and the ethyl

acetate solution was washed with 1N NaOH solution 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 (3:2) to afford 0.55 g (69%) of **IV** as an oil. $[\alpha]^{19}_D$; +27 (*c*1, ethanol). ^{1}H–NMR ($CDCl_3$) δ: 0.94 (3H, t, *J*=6.8 Hz, CH_3), 1.00 (3H, d, *J*= 6.8 Hz, CH_3), 1.68–1.72 (1H, m, CH), 1.90–1.99 (2H, m, CH_2), 3.75 (3H, s, CH_3), 4.67 (1H. d,d, *J*= 3.9, 9.3 Hz, CH), 6.86–6.89 (2H, d, *J*= 7.8 Hz, phenyl), 6.96–7.00 (3H, m, phenyl), 7.26–7.30 (2H, m, phenyl).

(R)–4–Methyl–2–phenoxy–1–pentanol (**V**)

To a solution of **IV** (1.2 g, 5.4 mmol) in 15 ml of ethanol was added sodium borohydride (0.25 g, 6.6 mmol) and the mixture was stirred for 36 hr at room temperature. After normal workup, the crude product was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (3:1) to afford 0.66 g (63%) of **V** as an oil. ^{1}H–NMR ($CDCl_3$) δ: 0.90 (3H, t, *J*=6.8 Hz, CH_3), 0.97 (3H, d, *J*=6.8 Hz, CH_3), 1.40–1.47 (1H, m, CH), 1.65–1.81 (2H, m, CH_2), 3.67–3.72 (1H, m, CH), 3.82–3.85 (1H, m, CH), 4.44–4.47 (1H, m, CH), 6.94–6.98 (3H, m, phenyl), 7.26–7.31 (2H, m, phenyl).

Ethyl 4–[(R)–4–methyl–2–phenoxypentyloxy]benzoate (**13R**)

To a solution of **V** (0.65 g, 3.4 mmol) in 15 ml of dichloromethane was added triethylamine (0.41 g, 4.1 mmol) and *p*–toluenesulfonyl chloride (0.77 g, 4.1 mmol). The mixture was stirred for 36 hr at room temperature. The product was extracted with ethyl acetate and the organic layer was washed with water and brine, dried over Na_2SO_4. Concentration of the solvent afforded 1.17 g of crude (*R*)–4–methyl–2–phenoxypentyl *p*–toluenesulfonate. A suspension of ethyl 4–hydroxybenzoate (0.34 g, 2.1 mmol) and potassium carbonate (0.29 g, 2.1 mmol) in 15 ml of DMF was stirred for 30 min at room temperature. To the mixture was added above (*R*)–4–methyl–2–phenoxypentyl *p*–toluenesulfonate (0.60 g, 1.7 mmol), and the mixture was heated at 90–100 ˚C for 7 hr. After normal workup, the crude product was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (4:1) to afford 0.49 g (80%) of **13R** as an oil. $[\alpha]^{19}_D$; +27 (*c*1, ethanol). ^{1}H–NMR ($CDCl_3$) δ: 0.94 (3H, t, *J*= 6.8 Hz, CH_3), 1.00 (3H, d, *J*=6.8 Hz, CH_3), 1.37 (3H, t, *J*=7.3 Hz, CH_3), 1.57–1.65 (1H, m, CH), 1.78–1.92 (2H, m, CH_2), 4.07–4.11 (1H, m, CH), 4.14–4.18 (1H, m, CH), 4.34 (2H, q, *J*=7.3 Hz, CH_2), 4.68–4.70 (1H, m, CH), 6.89 (2H, d, *J*=7.3 Hz, phenyl), 6.90 (2H, d, *J*=8.8 Hz, phenyl), 7.27–7.31 (3H, m, phenyl), 7.97 (2H, d, *J*= 8.8 Hz, phenyl). The optical purity was 63% ee by HPLC analysis under 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.

Compound **13S** was prepared in the same manner as **13R** with use of methyl (*R*)–2–hydroxy–4–methylpentanoate instead of (*S*)–isomer. Compound **13S**: oil, $[\alpha]^{19}_D$; –27 (*c*1, ethanol), Optical purity; 59% ee. The ^{1}H–NMR spectrum of **13S** was completely consistent with that of **13R**.

Methyl 4–(4–methyl–2–phenoxypentyloxy)benzoate (**14**) was prepared in the same manner as compound **4** with use of ethyl 2–bromo–4–methylpentanoate and methyl 4–hydroxybenzoate instead of ethyl 2–bromohexanoate and ethyl 4–hydroxybenzoate, respectively. ^{1}H–NMR ($CDCl_3$) δ: 0.94 (3H, d, *J*=6.4 Hz, CH_3), 1.00 (3H, d, *J*= 6.8 Hz, CH_3), 1.58–1.83 (1H, m, CH), 1.85–1.91 (2H, m, CH_2), 3.88 (3H, s, CH_3), 4.07–4.11 (1H, m, CH), 4.15–4.18 (1H, m, CH), 4.67–4.70 (1H, m, CH), 6.89 (2H, d, *J*=8.8 Hz, phenyl), 6.94–6.99 (3H, m, phenyl), 7.26–7.30 (2H, m, phenyl), 7.97 (2H, d, *J*= 8.8 Hz, phenyl).

4–(4–Methyl–2–phenoxypentyloxy)benzoic acid (**14–acid**) was prepared by the alkaline hydrolysis of **14** in the same way as described in **4–acid**. Mp 96–98 ˚C. 1H–NMR ($CDCl_3$) δ: 0.95 (3H, d, *J*=6.4 Hz, CH_3), 1.01 (3H, d, *J*= 6.8 Hz, CH_3), 1.58–1.65 (1H, m, CH), 1.81–1.93(2H, m, CH_2), 4.09–4.20 (2H, m, CH_2), 4.66–4.72 (1H, m, CH), 6.91–6.99 (5H, m, phenyl), 7.25–7.31 (2H, m, phenyl), 8.03–8.05 (2H, m, phenyl).

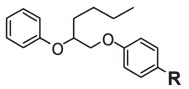
Biological evaluation

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. Compounds **14** and **14–acid** were mixed with the artificial diet at concentrations of 50 and 200 ppm according to the procedure reported (Asano *et al.*, 1984). The diet containing test compound was administered for the first 48 hr to newly molted 3rd and 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.

RESULTS AND DISCISSION

Table 1 shows precocious metmorphosis–inducing activity of alkyl 4–(2–phenoxyhexyloxy)benzoates and related compounds against 3rd and 4th instar larvae of *B. mori*. In contrast to ETB (Kiguchi *et al.*, 1984), the activity of the ethyl ester **4** to induce precocious metamorphosis correlated with the applied dose when applied to 3rd instar larvae. The methyl ester **5** showed somewhat higher activity than **4**. In these cases, precocious metamorphosis was always caused in the 4th larval stage. None of the treated 3rd instar larvae metamorphosed into precocious pupae in the same larval stage by a single topical application of these compounds. A dramatic decrease in activity was observed in changing from ethyl to *n*–propyl (**6**) and *n*–butyl (**7**) ester, indicating that the size of the alkyl group in the ester moiety is significant for activity. Replacement of the ethoxycarbonyl group with the ethylcarbamoyl substituent (**8**) led

Table 1. Precocious metamorphosis–inducing activity of alkyl 4–(2–phenoxyhexyloxy)benzoates and related compounds against 3rd and 4th instar larvae of *B. mori*



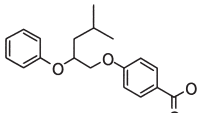
No	**R**	Precocious metamorphosis (%) 3rd instar (μg/larva) 1	10	40	4th instar 1	10
4	$COOC_2H_5$	32	66	70	0	0
5	$COOCH_3$	70	97	95	NT	0
6	COO–*n*–C_3H_7	0	0	NT	0	0
7	COO–*n*–C_4H_9	0	0	NT	0	0
8	$CONHC_2H_5$	0	0	15	0	0
9	COC_3H_7	0	0	NT	0	0
10	NO_2	0	0	NT	0	0
11		5	5	NT	0	0
12		0	0	NT	0	0

NT: not tested

to a drastic decrease in precocious metamorphosis–inducing activity. The butanone analog **9** did not show any activity at 1 and 10 μg. Introduction of a stronger electron–withdrawing nitro group on the benzene ring (**10**) eliminated the activity at 10 μg. The imidazolyl analog **11** slightly induced precocious pupation. The phenoxy analog **12** had little activity. These results indicate that in this series of compounds the ethyl and methyl ester groups play an important role for activity. None of the compounds tested showed precocious–metamorphosis inducing activity when applied to newly molted 4th instar larvae.

As previously reported (Fujita *et al.*, 2005), in the ethyl 4–[2–(6–methyl–3–pyridyl)alkyloxy]benzoate series, the isobutyl analog **3** showed somewhat higher activity than the butyl analog **2** against 3rd instar larvae. Additionally, in contrast to compound **2**, compound **3** induced precocious pupation when applied to 4th instar larvae, though very low activity. We therefore synthesized both enantiomers of ethyl 4–(4–methyl–2–phenoxypentyloxy)benzoate (**13**) by starting with L– and D–leucine, and examined their activity to induce precocious metamorphosis (Table 2). Although the enantiomeric purities of **13R**(+) (63% ee) and **13S**(−) (59% ee) were not so high, these compounds were assumed to be satisfactory for testing as anti–JH agents to determine their potency relative to the racemic compound. There was no significant difference in precocious metamorphosis–inducing activity between **13R**(+) and **13S**(−)–enantiomers. The activity of both enantiomers against 3rd instar larvae correlated with the applied dose. Similar results have been obtained previously in the optical isomers of the isobutyl analog **3** (Fujita *et al.*, 2005). Thus, it was found that the stereochemistry of the isobutyl analogs **3** and **13** did not influence the precocious metamorphosis–inducing activity. It is noteworthy that compound **13** as well as compound **3** slightly induced precocious metamorphosis when applied to 4th instar larvae. The methyl ester analog **14** had almost the same activity as that of the ethyl ester **13**.

Table 2. Precocious metamorphosis–inducing activity of ethyl or methyl 4–(4–methyl–2–phenoxypentyl)benzoate against 3rd and 4th instar larvae of *B. mori*

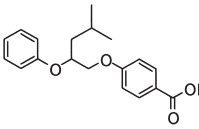


No	**R**		Precocious metamorphosis (%) 3rd instar (μg/larva) 1	10	40	4th instar 1	10
		racemi	0	50	95	5	5
13	C_2H_5	R(+)	7	45	79	15	20
		S(−)	20	60	85	15	0
14	CH_3	racemi	25	70	80	0	15

We have already reported (Fujita *et al.*, 2005) that the ethyl ester of the butyl analog **2** was indispensable for activity, because the corresponding benzoic acid, which might be produced by hydrolysis of **2** in the larval hemolymph, showed no activity. To see whether the ester portion of **13** or **14** was necessary for activity, we tested the activity of the corresponding acid (**14–acid**) as well. Since compound **14–acid** did not show any activity by topical application, presumably due to the low permeability of **14–acid** through the larval cuticle, we compared the activity of the methyl ester **14** and **14–acid** by dietary administration (Table 3). Compound **14** induced precocious metamorphosis by dietary administration as well as topical application when applied to 3rd instar larvae, while **14–acid** did not show activity at 50 and 200 ppm. This result indicates that the ester group of the compounds **13** and **14** is responsible for the activity, similar to the results obtained for compound **2**. Neither compound **14** nor **14–acid** induce precocious pupation by dietary administration against newly molted 4th instar larvae.

In conclusion, we have found that in the alkyl 4–(2–phenoxyhexyloxy)benzoate series only methyl and

Table 3. Precocious metamorphosis–inducing activity of compounds **14** and **14–acid** by dietary administration against 3rd and 4th instar larvae of *B. mori*

Compound	Time of treatment (larval instar)	Precocious metamorphosis (%) 50	200	Concentration (ppm)
14	3rd (0–48 hr)	67	75	
	4th (0–48 hr)	0	0	
 **14–acid**	3rd (0–48 hr)	0	0	
	4th (0–48 hr)	0	0	

The diet containing compounds was administered for the first 48 hr to newly molted 3rd and 4th instar larvae.

ethyl esters showed precocious metamorphosis–inducing activity against 3rd instar larvae of *B. mori*. The isobutyl analogs **13** and **14** as well as compound **2** induced precocious pupation when applied to newly molted 4th instar. Based on these results, further investigations on the structure–activity relationships of this series of compounds are in progress.

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