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Synthesis and Insect Growth Regulating Activity of Ethyl *Meta*-Substituted Benzoates

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Twenty-one ethyl *meta*-substituted benzoates were synthesized and examined their effects on larval growth and molting of the silkworm, *Bombyx mori*. Of the compounds tested, only 2-(3-ethoxycarbonylphenoxy)ethyl 2-methyl-5-pyridyl ether (**21**) induced precocious metamorphosis when applied to 24-hr old 3rd-instar larvae. In contrast to ethyl 4-[2-(*t*-butylcarbonyloxy)butoxy]benzoate (ETB), a juvenile hormone antagonist, compound **21** showed precocious metamorphosis-inducing activity at a high dose of 100 µg. Compound **21** as well as ETB significantly prolonged the duration of the larval period. Preliminary structure-activity relationship studies indicated that the presence of a methyl substituent on the 6-position of 3-pyridine ring was important for precocious metamorphosis-inducing activity.

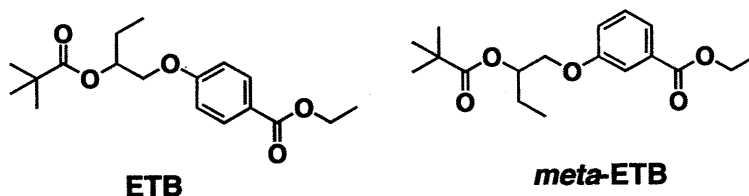
INTRODUCTION

It is well known that precocious metamorphosis is induced by allatectomy or juvenile hormone (JH) deficiency caused by anti-JH agents such as precocenes, fluoromevalonate (FMev), ethyl (*E*)-3-methyl-2-dodecenoate (EMD), and ethyl 4-[2-(*t*-butylcarbonyloxy)butoxy]benzoate (ETB) (Staal, 1986). Among them, ETB has been found to show JH-like activity as well as anti-JH activity for *Manduca sexta* (Staal, 1982) and *Bombyx mori* (Kiguchi *et al.*, 1984), depending on the dose applied; low doses of ETB induced anti-JH activity, but as the dose increasing, JH-like activity was observed. Edwards *et al.* (1983) have reported that application of ETB resulted in a true reduction of endogenous JH titers in *Manduca sexta* larvae, probably due to the inhibition of JH biosynthesis either directly or indirectly. They have proposed the existence of negative-feedback control of JH biosynthesis. However, the exact mode of action of ETB is still unknown.

We have focused on ETB as a lead compound for the development of a new anti-JH agent, and found that ethyl 3-[2-(*t*-butylcarbonyloxy)butoxy]benzoate (*meta*-ETB) induced precocious metamorphosis in the 3rd instar larvae of *Bombyx mori* at both low and high doses (Kuwano *et al.*, 1988). To discover more effective anti-JH agents we synthesized additional ethyl *meta*-substituted benzoates and evaluated their effects on larval growth and molting in *B. mori*. In this paper we report the syntheses and struc-

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ture-activity relationships of a new series of *meta*-ETB analogs.

MATERIALS AND METHODS

Synthesis

All melting points (mp) are uncorrected. $^1\text{H-NMR}$ spectra were recorded on JNM-EX400 spectrometer with tetramethylsilane as an internal standard and all samples were prepared in deuteriochloroform.

Ethyl 3-(4-phenoxybenzyloxy)benzoate (1)

To a solution of 4-phenoxybenzyl alcohol (1.50 g, 7.5 mmol) in dichloromethane was added thionyl chloride (0.65 ml, 9.0 mmol) at room temperature. After stirring for 2 hr, to the reaction mixture was added water (20 ml). The product was extracted with ether, dried over Na_2SO_4 , and concentrated to afford crude 4-phenoxybenzyl chloride (1.31 g, 80%). To a suspension of sodium hydride (0.29 g, 7.2 mmol) in dimethylformamide (DMF, 10 ml) was added ethyl 3-hydroxybenzoate (1.0 g, 6.0 mmol) and the mixture was stirred for 30 minutes at room temperature. To the mixture was added the above chloride. After stirring for 5 hr, to the reaction mixture was added water (10 ml) and the product was extracted with ether. The ether solution was washed with brine, dried over Na_2SO_4 , and concentrated to dryness. The residue was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (5:1) to afford **1** (1.25 g, 60%) as an oil. $^1\text{H-NMR}$ δ : 1.39 (3H, t, $J=7.3$ Hz), 4.37 (2H, q, $J=7.3$ Hz), 5.06 (2H, s), 7.13–7.66 (13H, m).

Compounds **2** and **3** were prepared in the same manner as **1** by using the corresponding chloride instead of 4-phenoxybenzyl chloride.

Ethyl 3-(4-phenylbenzyloxy)benzoate (2). mp 93–94°C. $^1\text{H-NMR}$ δ : 1.40 (3H, t, $J=7.3$ Hz), 4.38 (2H, q, $J=7.3$ Hz), 5.15 (2H, s), 7.23–7.64 (13H, m).

Ethyl 3-(4-benzyloxybenzyloxy)benzoate (3). mp 60°C. $^1\text{H-NMR}$ δ : 1.39 (3H, t, $J=7.3$ Hz), 4.37 (2H, q, $J=6.8$ Hz), 5.03 (2H, s), 5.08 (2H, s), 7.00 (2H, d, 8.8 Hz), 7.14–7.45 (9H, m), 7.64–7.69 (2H, m).

Ethyl 3-octyloxybenzoate (8)

Ethyl 3-hydroxybenzoate (1.0 g, 6.0 mmol) was added to a stirring suspension of NaH (0.29 g, 7.2 mmol) in DMF at room temperature. After stirring for 30 min, to the reaction mixture was added 1-iodooctane (1.16 g, 6.0 mmol). Then 10 ml of water was added to the reaction after 5 hr and the product was extracted with ether. The ether 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 (3:1) to afford **8**

(1.0 g, 60%) as an oil. $^1\text{H-NMR}$ δ : 0.87 (3H, t, $J=6.8\text{ Hz}$), 1.29–1.57 (13H, m), 1.72–1.77 (2H, m), 3.98 (2H, t, $J=6.8\text{ Hz}$), 4.35 (2H, q, $J=7.3\text{ Hz}$), 7.07 (1H, dd, $J=7.8$ and 1.5 Hz), 7.24 (1H, t, $J=7.8\text{ Hz}$), 7.53 (1H, d, $J=1.5\text{ Hz}$), 7.60 (1H, d, $J=7.8\text{ Hz}$).

Compounds **4–7** and **9–10** were prepared in the same manner as **8** by using the corresponding bromide or iodide.

Ethyl 3-[2-(4-phenoxyphenoxy)ethoxy]benzoate (4). mp 75°C . $^1\text{H-NMR}$ δ : 1.39 (3H, t, $J=7.3\text{ Hz}$), 4.33–4.37 (6H, m), 6.94–7.64 (13H, m).

Ethyl 3-[6-(4-phenoxyphenoxy)hexyloxy]benzoate (5). mp $85\text{--}86^\circ\text{C}$. $^1\text{H-NMR}$ δ : 1.39 (3H, t, $J=7.3\text{ Hz}$), 1.55–1.59 (4H, m), 1.81–1.84 (4H, m), 3.96 (2H, t, $J=6.4\text{ Hz}$), 4.02 (2H, t, $J=6.4\text{ Hz}$), 4.37 (2H, q, $J=7.3\text{ Hz}$), 6.86–7.35 (11H, m), 7.56 (1H, d, $J=1.5\text{ Hz}$), 7.63 (1H, d, $J=6.3\text{ Hz}$).

Ethyl 3-[2-(4-ethylphenoxy)ethoxy]benzoate (6). mp $50\text{--}53^\circ\text{C}$. $^1\text{H-NMR}$ δ : 1.21 (3H, t, $J=7.3\text{ Hz}$), 1.39 (3H, t, $J=7.3\text{ Hz}$), 2.59 (2H, q, $J=7.3\text{ Hz}$), 4.28–4.43 (6H, m), 6.88 (2H, d, $J=7.8\text{ Hz}$), 7.07–7.19 (3H, m), 7.33 (1H, t, $J=7.3\text{ Hz}$), 7.58–7.70 (2H, m).

Ethyl 3-[6-(4-ethylphenoxy)hexyloxy]benzoate (7). $^1\text{H-NMR}$ δ : 1.20 (3H, t, $J=7.3\text{ Hz}$), 1.39 (3H, t, $J=7.3\text{ Hz}$), 1.48–1.64 (4H, m), 1.79–1.91 (4H, m), 2.54–2.61 (2H, m), 3.95 (2H, t, $J=6.3\text{ Hz}$), 4.01 (2H, t, $J=6.3\text{ Hz}$), 4.37 (2H, q, $J=7.3\text{ Hz}$), 6.82 (2H, m), 7.05–7.13 (3H, m), 7.32 (1H, t, $J=7.8\text{ Hz}$), 7.56 (1H, d, $J=1.5\text{ Hz}$), 7.62 (1H, d, $J=6.4\text{ Hz}$).

Ethyl 3-hexyloxybenzoate (9). $^1\text{H-NMR}$ δ : 0.91 (3H, t, $J=6.8\text{ Hz}$), 1.34–1.49 (9H, m), 1.76–1.84 (2H, m), 4.00 (2H, t, $J=6.8\text{ Hz}$), 4.37 (2H, q, $J=7.3\text{ Hz}$), 7.08 (1H, dd, $J=7.8$ and 1.5 Hz), 7.32 (1H, t, $J=7.8\text{ Hz}$), 7.55 (1H, d, $J=1.5\text{ Hz}$), 7.61 (1H, d, $J=7.8\text{ Hz}$).

*Ethyl 3-(4-*t*-butylbenzyloxy)benzoate (10)*. $^1\text{H-NMR}$ δ : 1.29–1.42 (12H, m), 4.37 (2H, q, $J=7.3\text{ Hz}$), 5.06 (2H, s), 7.17 (1H, dd, $J=7.8$ and 1.5 Hz), 7.32–7.43 (5H, m), 7.64–7.66 (2H, m).

2-(3-Ethoxycarbonylphenoxy)ethyl 2-methyl-5-pyridyl ether (21)

A mixture of ethyl 3-hydroxybenzoate (1.50 g, 9 mmol), K_2CO_3 (3.74 g, 27 mmol), and 1,2-dibromoethane (8.48 g, 51 mmol) in DMF was stirred for 10 hr at room temperature. Then water was added to the reaction mixture, and the product was extracted with ether. The ether solution was washed with brine, dried over Na_2SO_4 and concentrated to dryness. The residue was purified by column chromatography on silica gel eluting with hexane and ethyl acetate (3:1) to afford ethyl 3-(2-bromoethoxy)benzoate (1.23 g, 50%). To a suspension of sodium hydride (0.14 g, 3.5 mmol) in DMF (10 ml) was added 2-methyl-5-hydroxypyridine (0.32 g, 2.9 mmol) and the mixture was stirred for 30 minutes at room temperature. To the mixture was added the above bromide (0.80 g, 2.9 mmol). After stirring for 5 hr, to the reaction mixture was added water (10 ml) and the product was extracted with ether. The ether solution was washed with brine and the product was extracted with 20 ml of 1 N H_2SO_4 solution from the organic layer. After neutralizing the acidic aqueous solution with K_2CO_3 , the product was extracted with ether again. The ether solution was washed with brine, dried over Na_2SO_4 , and concentrated to afford **21** (0.44 g, 57%) as a white solid, mp 135°C . $^1\text{H-NMR}$ δ : 1.40 (3H, t, $J=7.3\text{ Hz}$), 2.51 (3H, s), 4.35–4.41 (6H, m), 7.08 (1H, d, $J=8.8\text{ Hz}$), 7.14–7.20 (2H, m), 7.36 (1H, t, $J=7.8\text{ Hz}$), 7.62 (1H, d, $J=2.0\text{ Hz}$), 7.67–7.69 (1H, d, $J=7.8\text{ Hz}$), 8.25 (1H, d, $J=2.9\text{ Hz}$).

Compounds **12–20** were prepared in the same manner as that used for compound **21** from ethyl 3-(2-bromoethoxy)benzoate or 3-(3-bromopropoxy)benzoate.

2-(3-Ethoxycarbonylphenoxy)ethyl 2-pyridyl ether (12). mp $48\text{--}49^\circ\text{C}$. $^1\text{H-NMR}$ δ :

1.39 (3H, t, $J=7.3$ Hz), 4.31–4.41 (6H, m), 6.18 (1H, t, $J=6.8$ Hz), 6.58 (1H, d, $J=9.3$ Hz), 7.05 (1H, dd, $J=8.8$ and 2.4 Hz), 7.25–7.46 (3H, m), 7.52 (1H, s), 7.64 (1H, d, $J=7.8$ Hz).

2-(3-Ethoxycarbonylphenoxy)ethyl 3-pyridyl ether (13). mp 50–51 °C. $^1\text{H-NMR}$ δ : 1.40 (3H, t, $J=7.3$ Hz), 4.34–4.43 (6H, m), 7.15 (1H, dd, $J=7.8$ and 1.5 Hz), 7.22–7.29 (2H, m), 7.36 (1H, t, $J=7.8$ Hz), 7.62 (1H, d, $J=1.5$ Hz), 7.68 (1H, d, $J=6.8$ Hz), 8.25 (1H, d, $J=4.4$ Hz), 8.37 (1H, d, $J=2.5$ Hz).

2-(3-Ethoxycarbonylphenoxy)ethyl 4-pyridyl ether (14). mp 50–55 °C. $^1\text{H-NMR}$ δ : 1.40 (3H, t, $J=7.3$ Hz), 4.34–4.44 (6H, m), 6.89 (2H, d, $J=5.4$ Hz), 7.14 (1H, dd, $J=8.8$ and 2.4 Hz), 7.37 (1H, t, $J=7.8$ Hz), 7.62 (1H, s), 7.68 (1H, d, $J=6.8$ Hz), 8.47 (2H, brs)

3-(3-Ethoxycarbonylphenoxy)propyl 2-pyridyl ether (15). $^1\text{H-NMR}$ δ : 1.40 (3H, t, $J=7.3$ Hz), 2.29 (2H, quint, $J=5.9$ Hz), 4.03 (2H, t, $J=5.9$ Hz), 4.16 (2H, t, $J=7.3$ Hz), 4.37 (2H, q, $J=7.3$ Hz), 6.12 (1H, t, $J=6.8$ Hz), 6.58 (1H, d, $J=9.3$ Hz), 7.08 (1H, d, $J=5.9$ Hz), 7.25–7.37 (3H, m), 7.55 (1H, d, $J=1.5$ Hz), 7.65 (1H, d, $J=6.3$ Hz).

3-(3-Ethoxycarbonylphenoxy)propyl 3-pyridyl ether (16). $^1\text{H-NMR}$ δ : 1.39 (3H, t, $J=7.3$ Hz), 2.30 (2H, quint, $J=5.9$ Hz), 4.16–4.27 (4H, m), 4.37 (2H, q, $J=7.3$ Hz), 7.10 (1H, dd, $J=7.8$ and 2.0 Hz), 7.20–7.28 (2H, m), 7.34 (1H, t, $J=7.8$ Hz), 7.58 (1H, d, $J=2.0$ Hz), 7.64 (1H, d, $J=7.8$ Hz), 8.21 (1H, d, $J=5.9$ Hz), 8.32 (1H, d, $J=2.0$ Hz).

3-(3-Ethoxycarbonylphenoxy)propyl 4-pyridyl ether (17). $^1\text{H-NMR}$ δ : 1.40 (3H, t, $J=7.3$ Hz), 2.34 (2H, quint, $J=5.9$ Hz), 4.20–4.40 (6H, m), 6.80 (2H, d, $J=5.4$ Hz), 7.07 (1H, dd, $J=8.8$ and 2.4 Hz), 7.37 (1H, t, $J=7.8$ Hz), 7.62 (1H, s), 7.68 (1H, d, $J=6.8$ Hz), 8.41 (2H, brs).

1-[2-(3-Ethoxycarbonylphenoxy)ethyl]-2-(4-pyridyl)imidazole (18). $^1\text{H-NMR}$ δ : 1.39 (3H, t, $J=7.3$ Hz), 4.26–4.52 (6H, m), 7.00–7.09 (3H, m), 7.34 (1H, td, $J=6.4$ and 2.0 Hz), 7.63 (1H, dd, $J=6.4$ and 1.5 Hz), 7.67 (1H, d, $J=7.3$ Hz), 7.81 (1H, dd, $J=2.9$ and 1.5 Hz), 8.59 (1H, d, $J=4.9$ Hz), 8.72 (1H, d, $J=4.9$ Hz).

2-(3-Ethoxycarbonylphenoxy)ethyl 6-methyl-2-pyridyl ether (19). mp 83 °C. $^1\text{H-NMR}$ δ : 1.39 (3H, t, $J=7.3$ Hz), 2.54 (3H, s), 4.32–4.46 (6H, m), 6.04 (1H, d, $J=6.8$ Hz), 6.45 (1H, d, $J=8.8$ Hz), 7.04 (1H, dd, $J=8.8$ and 2.4 Hz), 7.21–7.32 (2H, m), 7.51 (1H, d, $J=1.5$ Hz), 7.63 (1H, d, $J=7.8$ Hz).

5-Chloro-3-pyridyl 2-(3-ethoxycarbonylphenoxy)ethyl ether (20). $^1\text{H-NMR}$ δ : 1.40 (3H, t, $J=7.3$ Hz), 4.36–4.43 (6H, m), 7.14 (1H, dd, $J=8.8$ and 2.4 Hz), 7.29 (1H, d, $J=2.4$ Hz), 7.37 (1H, t, $J=7.8$ Hz), 7.61 (1H, s), 7.69 (1H, d, $J=7.8$ Hz), 8.23 (1H, d, $J=1.5$ Hz), 8.27 (1H, d, $J=2.4$ Hz)

Ethyl 3-[2-(*t*-butylcarbonyloxy)-2-phenylethoxy]benzoate (11)

To a suspension of sodium hydride (0.48 g, 12 mmol) in DMF (10 ml) was added ethyl 3-hydroxybenzoate (1.67 g, 11 mmol) and the mixture was stirred for 20 minutes at room temperature. To the mixture was added a solution of 2-bromoacetophenone (2.00 g, 11 mmol) in 5 ml of DMF. After stirring for 10 hr, water was added to the reaction mixture and the product was extracted with ether. The ether 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 (3:1) to afford phenylcarbonylmethyl 3-ethoxycarbonylphenyl ether (2.29 g, 80%). To this ketone compound (2.29 g, 8.1 mmol) in ethanol (20 ml) was added NaBH_4 (0.91 g, 2.4 mmol) at 0 °C. After stirring for 3 hr, water was added to the reaction mixture and the alcohol product was extracted with ether. The ether solution was washed with brine, and dried over Na_2SO_4 .

Concentration of the solvent gave crude ethyl 3-(2-hydroxy-2-phenylethoxy)benzoate (2.30 g, 100%). To a solution of the alcohol (0.10 g, 0.42 mmol), 4-dimethylaminopyridine (0.051 g, 0.42 mmol; DMAP) and triethylamine (0.042 g, 0.42 mmol) in dichloromethane was added pivaloyl chloride (0.050 g, 0.42 mmol) with stirring at room temperature. After stirring for 10 hr, the product (**11**) was obtained by same purification manner to that described above in 40% yield (0.062 g) as a yellowish oil. $^1\text{H-NMR}$ δ : 1.22 (9H, s), 1.39 (3H, t, $J=7.3$ Hz), 4.21–4.28 (2H, m), 4.37 (2H, q, $J=7.3$), 6.13–6.17 (1H, m), 7.06–7.09 (1H, m), 7.31–7.41 (6H, m), 7.53 (1H, d, $J=2.9$ Hz), 7.65 (1H, d, $J=7.3$ Hz)

*Ethyl 4-[2-(*t*-butylcarbonyloxy)butoxy]benzoate (ETB)*

A mixture of ethyl 4-hydroxybenzoate (2.00 g, 12 mmol), K_2CO_3 (1.83 g, 13 mmol), and 1,2-butylene oxide (1.74 g, 24 mmol) in acetonitrile was stirred at 60 °C for 24 hr. After cooling the reaction mixture to room temperature, the product was extracted with ether. The ether solution was washed with 2N NaOH solution and brine, and dried over Na_2SO_4 . Concentration of the solvent afforded ethyl 4-(2-hydroxybutoxy)benzoate (1.33 g, 46%) as an oil. To a reaction mixture of the above alcohol (1.33 g, 6.0 mmol), DMAP (0.68 g, 6.0 mmol) and triethylamine (0.57 g, 6.0 mmol) in dichloromethane was added pivaloyl chloride (0.74 g, 6.1 mmol) with stirring at room temperature. After stirring for 4 hr at room temperature, the product was extracted with ether, and the organic layer 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 ETB (1.62 g, 95%) as an oil. $^1\text{H-NMR}$ δ : 0.97 (3H, t, $J=6.8$ Hz), 1.20 (9H, s), 1.38 (3H, t, $J=6.8$ Hz), 1.75–1.77 (2H, m), 4.06 (2H, d, $J=5.9$ Hz), 4.34 (2H, q, $J=6.8$ Hz), 5.13 (1H, m), 6.90 (2H, d, $J=8.3$ Hz), 7.98 (2H, d, $J=8.3$ Hz).

Biological evaluations

B. mori (Shunrei \times Shougetsu strain) larvae were reared on artificial diets as previously described (Yoshida *et al.*, 2000). Test compounds in acetone solution (1–4 μl per larva) were applied topically to 24 h-old 3rd-instar larvae. The time required for ecdysis to 4th and 5th instar was recorded. The precocious metamorphosis-inducing activity was evaluated by spinning a cocoon and subsequent pupation or formation of larval–pupal intermediates from the 4th-instar (penultimate) larval period.

RESULTS AND DISCUSSION

Synthesis

Fig. 1 shows the structures of synthesized compounds. Compounds **1–10** were prepared by reaction of the corresponding alkyl halide with ethyl 3-hydroxybenzoate as shown in Fig. 2 (A). Ethyl 3-[2-(*t*-butylcarbonyloxy)-2-phenylethoxy]benzoate (**11**) was synthesized by the procedure outlined in Fig. 2 (B). Alkylation of ethyl 3-hydroxybenzoate with 2-bromoacetophenone, followed by reduction with sodium borohydride gave alcohol, which was esterified with pivaloyl chloride using 4-dimethylaminopyridine (DMAP) as a base to give **11**. 3-Ethoxycarbonylphenyl pyridyl ethers **12–17** and **19–21** were prepared as illustrated in Fig. 2 (C). Reaction of ethyl 3-hydroxybenzoate with dibromoalkanes gave phenoxyalkyl bromides, which were treated with an appropriate hydroxypyridine in the presence of sodium hydride as a base in dimethylformamide to

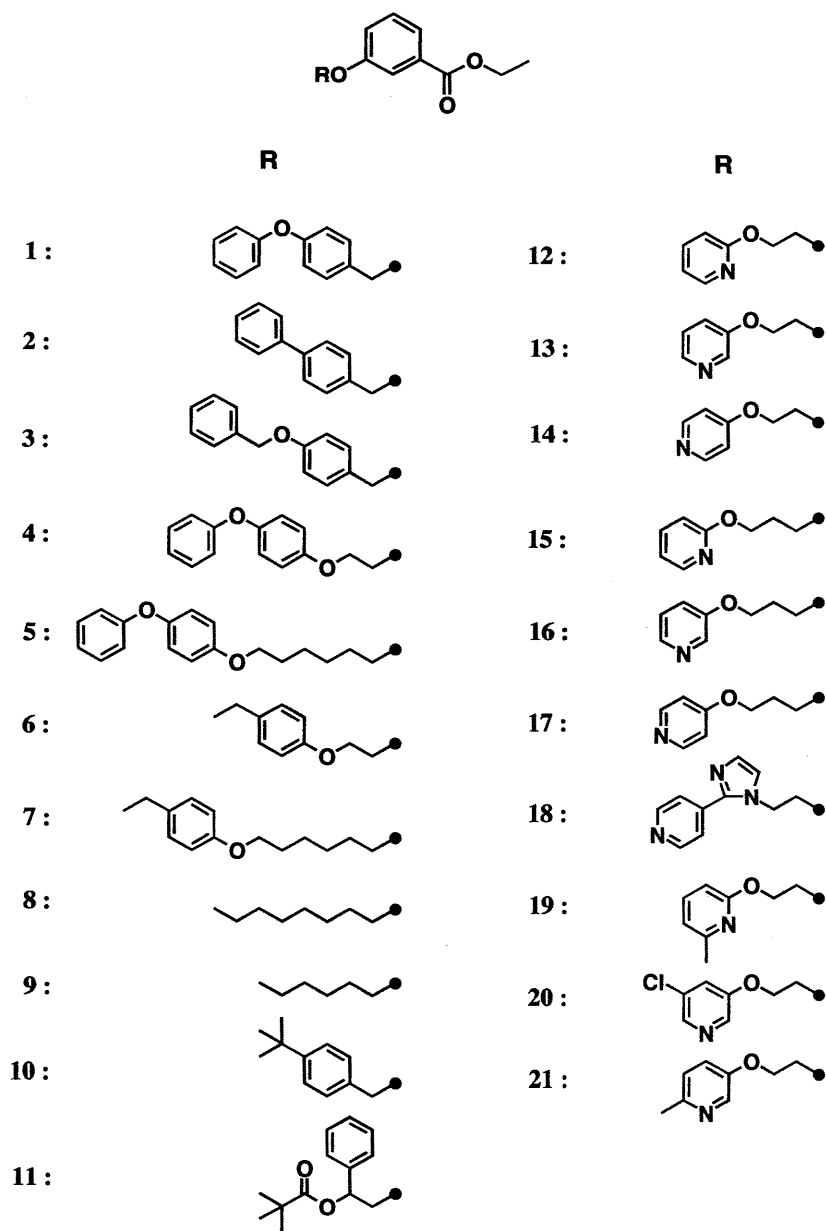


Fig. 1. Structures of synthesized ethyl *meta*-substituted benzoates

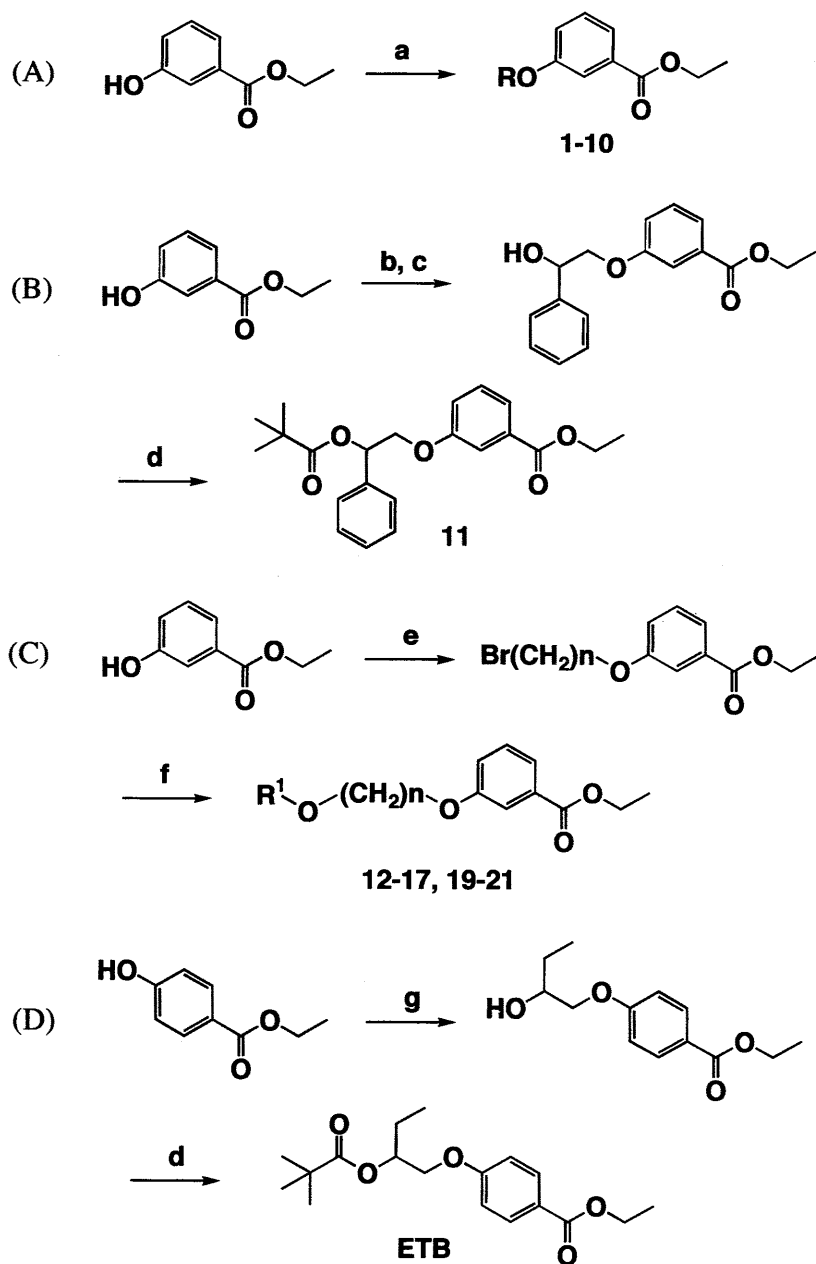


Fig. 2. Synthesis of ethyl *meta*-substituted benzoates
 Reagents: (a) alkyl halide, NaH, DMF, (b) 2-bromoacetophenone, NaH, DMF, (c) NaBH₄, EtOH, (d) pivaloyl chloride, DMAP, triethylamine, CH₂Cl₂, (e) Br(CH₂)_nBr, K₂CO₃, DMF, (f) hydroxypyridine, NaH, DMF, (g) 1,2-butylenedioxy, K₂CO₃, CH₃CN.

afford pyridyl ethers. Imidazole analog **18** was similarly prepared from 2-(3-ethoxycarbonylphenoxy)ethyl bromide and 2-(4-pyridyl)imidazole (Baldwin *et al.*, 1977). The synthesis of ETB was accomplished by the procedure outlined in Fig. 2 (D). Ethyl 4-hydroxybenzoate was treated with 1,2-butylene oxide in the presence of potassium carbonate as a base in acetonitrile to afford ethyl 4-(2-hydroxybutoxy)benzoate, which was esterified with pivaloyl chloride in the same manner as compound **11** to provide ETB.

Biological activity

ETB has been known to induce precocious metamorphosis in the silkworm when applied to 3rd-instar larvae. In this case precocious metamorphosis always occurred in the 4th (penultimate) larval stage. When ETB was applied to 4th-instar larvae, none of the treated larvae showed any sign of precocious metamorphosis, and all of the treated larvae molted into the normal 5th-instar (Kiguchi *et al.*, 1984 and Kuwano *et al.*, 1988). Since 24-hr old 3rd-instar larvae was more susceptible to ETB than newly molted larvae (Kuwano *et al.*, 1988), we examined the effects of ethyl *meta*-substituted benzoates on larval growth, molting and metamorphosis against 24-hr old 3rd-instar larvae.

Although the strain of the silkworm used in this experiment was not susceptible to ETB, clear induction of precocious metamorphosis was observed by ETB treatment (Table 1). As previously reported (Kuwano *et al.*, 1988), the activity of ETB to induce precocious metamorphosis did not correlate with the applied dose; ETB at 10 μ g exhibited some activity, while at a high dose of 100 μ g none of the treated larvae metamorphosed into precocious pupae. Among the compounds shown in Fig. 1, only 2-(3-ethoxycarbonylphenoxy)-ethyl 2-methyl-5-pyridyl ether (**21**) induced precocious metamorphosis against 24-hr old 3rd-instar larvae, though very low activity. In contrast to ETB, compound **21** had activity at a high dose of 100 μ g, but no activity at 10 μ g.

Precocious metamorphosis induced by ETB and compound **21** was always accompanied by the prolongation of the larval period, similar to that induced by treatment with 1,5-substituted imidazoles (Kadono-Okuda *et al.*, 1987) and 3-pyridine derivatives (Yoshida *et al.*, 2000). Therefore, we observed the effects of ethyl *meta*-substituted benzoates on growth and 4th ecdysis in the larvae, in which precocious metamorphosis could not be induced by the compound treatment.

A topical application of ETB at 10 μ g slightly but significantly prolonged the duration of both 3rd and subsequent 4th larval instar (Table 2). In control, the 3rd and 4th larval period was 3.0 and 4.0 days on average, respectively, while ETB treatment resulted in 3.3 ± 0.4 and 4.4 ± 0.5 days, respectively. ETB at 100 μ g clearly delayed the 3rd and 4th ecdysis by 1 to 1.5 days, respectively, compared with the control period. However, it is

Table 1. Precocious metamorphosis-inducing activity of ETB and compound **21** against 24 h-old 3rd-instar larvae

Compound	Dose (μ g/larva)	Activity (%)	
		100	10
ETB		0	10
21		5	0

Table 2. Larval periods of the silkworm treated with ETB and ethyl meta-substituted benzoates

Compound	Dose ($\mu\text{g}/\text{larva}$)	Larval period (Days \pm S.D.)	
		3rd instar	4th instar
ETB	10	3.3 ± 0.4^a	4.4 ± 0.5^a
	100	4.0 ± 0.2^a	5.6 ± 0.5^a
5	10	3.1 ± 0.3	4.3 ± 0.4^a
	100	3.0 ± 0.0	4.3 ± 0.4^a
6	10	3.1 ± 0.2	4.1 ± 0.3
	100	3.2 ± 0.4	4.7 ± 0.6^a
7	10	3.0 ± 0.0	4.2 ± 0.4
	100	3.1 ± 0.2	4.4 ± 0.5^a
Control		3.0 ± 0.0	4.0 ± 0.0

^a Significant difference from the control value ($p < 0.05$).**Table 3.** Larval periods of the silkworm treated with (3-ethoxy-carbonylphenoxy)alkyl substituted pyridyl ethers

Compound	Dose ($\mu\text{g}/\text{larva}$)	Larval period (Days \pm S.D.)	
		3rd instar	4th instar
19	10	3.1 ± 0.3	4.6 ± 0.9
	100	3.4 ± 0.5	4.5 ± 0.6
20	10	3.1 ± 0.3	4.2 ± 0.6
	100	3.5 ± 0.5	4.6 ± 0.9
21	10	4.1 ± 0.3^a	5.1 ± 0.6^a
	100	3.7 ± 0.5^a	4.8 ± 0.5^a
Control		3.2 ± 0.4	4.3 ± 0.5

^a Significant difference from the control value ($p < 0.05$).

not clear whether or not the prolongation of 3rd and 4th periods is due to the anti-JH action of ETB. Compounds **1–4** which have a partial structure of JH mimics such as a phenoxyphenoxy or benzyloxyphenoxy group did not show any effect on larval growth or molting, resulting in molting into the 5th instar similar to that of the control (data not shown). Compound **5** with a phenoxyphenoxy group at $10\mu\text{g}$ did not delay the 3rd ecdysis, but prolonged the duration of the 4th larval period. Compounds **6** and **7** at $100\mu\text{g}$ delayed the 4th ecdysis by an approximately half day compared with that of control, while at a low dose of $10\mu\text{g}$, they had no influence on larval growth.

Pyridyl ethers **12–17** irrespective of the position of the nitrogen atom did not cause any delay of larval growth (data not shown). 2-Methyl-5-pyridyl ether **21**, which showed precocious metamorphosis-inducing activity, significantly prolonged the duration of the instar from 3rd to 4th, being comparable to that of ETB (Table 3). 6-Methyl-2-pyridyl ether **19** and 5-chloro-2-pyridyl ether **20** was inactive, suggesting that the presence of a

methyl substituent on the 6-position of 3-pyridine ring is essential for activity. Although the precocious metamorphosis-inducing activity of compound **21** was weak, a series of 2-methyl-5-pyridyl ether is worthy of further investigation for new insect growth regulators.

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