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Effects of Anti Juvenile Hormones and Related Compounds on Development in the Larvae of *Bombyx mori*

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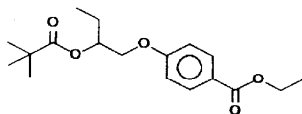
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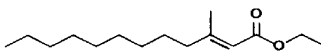
A number of structural analogs of ETB (ethyl 4-[2-(*tert*-butylcarbonyloxy)butoxy]benzoate), EMD (ethyl (*E*)-3-methyl-2-dodecenoate), and J2710 (5-methoxy-6-[1-(4-methoxyphenyl) ethyl] -1, 3-benzodioxole) were synthesized and bioassayed on the silkworm, *Bombyx mori*, in order to assess anti juvenile hormone activity. ETB and related compounds induced precocious pupation in the 3rd instar larvae of *B. mori*. However, attempts to produce analogs with high anti juvenile hormone activity have failed. EMD, J2710 and their analogs demonstrated acute toxicity in *B. mori*, yet failed to induce precocious metamorphosis.

INTRODUCTION

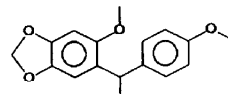
Compounds with anti juvenile hormone (anti JH) activity are expected to be potential insect growth regulators as well as an effective tool in studies of insect physiology. A number of anti JH agents have so far been found such as precocenes (Bowers et al., 1976), fluoromevalonate (FMev) (Quistad et al., 1981), ETB, EMD (Staal et al., 1982), compactin (Monger et al., 1982), benzyl-1, 3-benzodioxoles (Van Mellaert et al., 1983), imidazoles (Kuwano et al., 1983) and dichloroallyl hexanoate (Quistad et al., 1985). These compounds showed anti JH activity on a restricted number of insect species. Among them, ETB has been found to show JH agonist and antagonist activity for *Manduca sexta* and *Bombyx mori* (Kiguchi et al., 1984), depending on the dose applied. EMD has been demonstrated to have anti JH activity on *Heliothis virescens* and *M. sexta* at a high dose. Although the mode of action of EMD is not well understood, the direct action on the JH receptors has been suggested by both in *vitro* and *in vivo* studies (Kramer et al., 1981). No other anti JH agents with such mode of action have been found so far and little information has been reported on detailed structure-activity relationships of ETB and EMD analogs. A benzyl-1,3-benzodioxole derivative, J2710, which was first described as a fly chemosterilant by Jurd (1979), has been reported to inhibit JH III action by means of the *Galleria* wax test, but there is no evidence of anti JH effects on larvae of any insect species.



ETB



EMD



J2710

It is well known that the surgical extirpation of corpora allata in the 3rd or 4th instar larvae of *B. mori* induces precocious metamorphosis. As we have previously reported (Kuwano *et al.*, 1985), a new imidazole compound, 1-benzyl-5-[(*E*)-2,6-dimethyl-1,5-heptadienyl]imidazole (KK-42), induced unequivocal precocious pupation in the 3rd and 4th instar larvae of *B. mori* by topical application and the effects of KK-42 was counteracted by simultaneous application of JH I or methoprene. It appears that *B. mori* is a useful test species for evaluation of anti JH effects. Therefore, we have synthesized a large number of analogs of ETB, EMD and benzyl-1,3-benzodioxoles, and tested these compounds for an anti JH action on *B. mori*.

EXPERIMENTAL

Syntheses

¹H NMR spectra were determined on a JEOL FX-100 spectrometer using Me₄Si as an internal standard. Column chromatography was performed with Merck silica gel 60, 70-230 and 230-400 mesh.

Ethyl 4-(2-(tert-butylcarbonyloxy)butoxy)benzoate (ETB) and Ethyl 4-(2-(tert-butylcarbonyloxy)-1-ethylethoxy)benzoate (4)

To a solution of commercial 1-bromo-2-butanol (3.1 g, 0.02 mol containing ca. 20% of 2-bromo-1-butanol) in CH₂Cl₂ were added dihydropyran (2.5 g, 0.03 mol) and pyridinium *p*-toluenesulfonate (PPTS, 0.5 g, 0.002 mol). The mixture was stirred for 12 hr and concentrated under reduced pressure. The residue was chromatographed on silica gel by elution with hexane-ethyl acetate (18 : 1). The solvent was removed to give 4.2 g (89%) of tetrahydropyranyl ether as an oil.

A mixture of tetrahydropyranyl ether (4.2 g, 0.018 mol) and sodium hydride (0.7 g, 0.018 mol) in 20 ml of DMF was stirred for 30 min at room temperature. To the mixture was added ethyl *p*-hydroxybenzoate (3.1 g, 0.018 mol) in small portion during 10 min, and stirring was continued for 15 hr at room temperature. To the reaction mixture was added 100 ml of H₂O and the product was extracted with ether. The ether solution was washed with brine, and dried over Na₂SO₄. After removal of the solvent, the residue was chromatographed on silica gel and eluted with hexane-ethyl acetate (20 : 1). Removal of the solvent gave *p*-substituted benzoates **1**. A mixture of compound **1** and PPTS in ethanol was refluxed for 3 hr. After concentration of the mixture under reduced pressure, to the residue was added H₂O and ether. The ether layer was washed with brine, dried over Na₂SO₄, and then concentrated. Flash chromatography of the residue on silica gel using hexane-ethyl acetate (30 : 1), (20 : 1) and (15 : 1) afforded 0.36 g (8%) of compound **2** and 0.05 g (1%) of compound **3**.

To a mixture of compound **2** (0.36 g, 1.5 mmol) and 4-dimethylaminopyridine (DMAP) (0.18 g, 1.5 mmol) in CH₂Cl₂ was added dropwise a solution of pivaloyl chloride (0.18 g, 1.5 mmol) in CH₂Cl₂, and the reaction mixture was stirred for 12 hr at room temperature. After concentration of the mixture under reduced pressure, to the residue was added H₂O and ether. The ether layer was washed with 5% NaHCO₃ solution, brine, and dried over Na₂SO₄. After removal of the solvent, the residue was chromatographed on silica gel by elution with hexane-ethyl acetate (20 : 1). The solvent was removed to give 0.24 g (49%) of ETB. NMR (CDCl₃) δ : 0.99 (3H, t), 1.20 (9H, s), 1.39 (3H, t), 1.78 (2H, m), 4.06 (2H, d, J = 6 Hz), 4.34 (2H, q), 5.10 (1H, m), 6.88

(2H, d, J=8 Hz), 7.96 (2H, d, J=8 Hz).

Compound 4 was similarly synthesized from compound 3. Yield 41%. NMR (CDCl₃) δ : 1.00 (3H, t), 1.12 (9H, s), 1.37 (3H, t), 1.70 (2H, m), 4.17-4.36 (4H, m), 4.46 (1H, m), 6.92 (2H, d, J=8 Hz), 7.96 (2H, d, J=8 Hz).

Separation of 1-bromo-2-butanol

To a solution of commercial 1-bromo-2-butanol (5 g, 0.033 mol, containing ca. 20% of 2-bromo-1-butanol) in CH₂Cl₂, pivaloyl chloride (1.6 g, 0.013 mol) and DMAP (1.6 g, 0.013 mol) were added slowly. The reaction mixture was stirred for 72 hr at room temperature and then refluxed for 72 hr. After concentration of the mixture under reduced pressure, to the residue was added H₂O and ether. The ether layer was washed with 5% NaHCO₃ solution, brine, and dried over Na₂SO₄. After removal of the solvent, the residue was chromatographed on silica gel by elution with hexane-ethyl acetate (20 : 1). The solvent was removed to give 1-bromo-2-butanol the purity of which was 98.6% by GLC analysis.

2-Bromo-1-butanol

Bromine (17.6 g, 0.11 mol) was added to n-butyric acid (8.8 g, 0.1 mol) and to the mixture was added 2.3 ml of phosphorus trichloride. The mixture was heated with stirring at 80°C for 8 hr and then at 100°C for 2 hr until the dark red color of bromine disappeared. After cooling of the mixture, 2-bromobutyric acid was distilled at 99-103°C (10 mmHg) in 44% yield. To a cooled solution (ice bath) of borane dimethyl sulfide complex (1.5 g, 0.02 mol) in THF, a solution of 2-bromobutyric acid (3.3 g, 0.02 mol) in THF was added dropwise during a period of 10 min at 0°C. The mixture was stirred for 1.5 hr at the same temperature. After evaporation of the solvent, the product was extracted with ether and the ether solution was washed with NaHCO₃ solution, brine, and dried over Na₂SO₄. The solvent was evaporated to afford 1.25 g (40%) of 2-bromo-1-butanol.

General method for the preparation of 2-(alkylcarbonyloxy)butyl aryl ethers 6

In a similar manner to that described in preparation of ETB, 1-bromo-2-butanol was esterified with the corresponding acid chloride to give 2-alkylcarbonyloxy-1-bromo-butane (5). The bromide 5 (1 mmol) was added dropwise to a mixture of the corresponding phenolic compound (1 mmol) and NaH (1 mmol) in DMF. The mixture was heated at 70-80°C for 12 hr. To the mixture was added H₂O, and the product was extracted with ether. The ether solution was washed with brine, dried over Na₂SO₄, and concentrated. Chromatography of the residue on silica gel using hexane-ethyl acetate (16 : 1) afforded the desired compound 6.

Ethyl 3-[2-(*tert*-butylcarbonyloxy)butoxy] benzoate (7) NMR (CDCl₃) δ : 0.98 (2H, t), 1.17 (9H, s), 1.39 (3H, t), 1.76 (2H, m), 4.04 (2H, m), 4.37 (2H, q), 5.12 (1H, m), 6.98-7.68 (4H, m).

Ethyl 2-[2-(*tert*-butylcarbonyloxy)butoxy] benzoate (8) NMR (CDCl₃) δ : 0.97 (3H, t), 1.18 (9H, s), 1.37 (3H, t), 1.58-1.90 (1H, m), 4.07 (2H, d, J=5 Hz), 4.32 (2H, q), 5.08 (1H, m), 6.87-7.77 (4H, m).

4-Ethyl-1-[2-(*tert*-butylcarbonyloxy)butoxy]benzene (9) NMR (CDCl₃) δ : 0.95 (3H, t), 1.00 (3H, t), 1.19 (9H, s), 1.73 (2H, m), 2.56 (2H, q), 3.96 (2H, d, J=5 Hz), 5.07 (8H, m), 6.77 (2H, d, J=8 Hz), 7.05 (2H, d, J=8 Hz).

4-Chloro-1-[2-(*tert*-butylcarbonyloxy)butoxy]benzene (10) NMR (CDCl₃) δ : 0.96 (3H, t), 1.19 (9H, s), 1.73 (2H, m), 3.94 (2H, d, J=5 Hz), 5.06 (1H, m), 6.78 (2H, d, J=8 Hz),

7.18 (2H, d, J = 8 Hz).

5-[2-(*tert*-butylcarbonyloxy)butoxy]-1, 3-benzodioxole (11) NMR (CDCl₃) δ : 0.96 (3H, t), 1.20 (9H, s), 1.72 (2H, m), 3.91 (2H, d, J = 5 Hz), 5.05 (1H, m), 5.88 (2H, s), 6.23-6.70 (3H, m).

Ethyl 4-[2-(isopropylcarbonyloxy)butoxy]benzoate (12) NMR (CDCl₃) δ : 0.98 (3H, t), 1.17 (6H, d, J = 7 Hz), 1.37 (3H, t), 1.76 (2H, m), 2.52 (1H, q), 4.05 (2H, d, J = 5 Hz), 4.31 (2H, q), 5.12 (1H, m), 6.87 (2H, d, J = 9 Hz), 7.95 (2H, d, J = 9 Hz).

Ethyl 4-[2-(isobutylcarbonyloxy)butoxy]benzoate (13) NMR (CDCl₃) δ : 0.92-1.04 (10H, m), 1.36 (3H, t), 1.75 (2H, m), 2.17 (2H, broad s), 4.03 (3H, d, J = 6 Hz), 4.31 (2H, q), 5.14 (1H, m), 6.86 (2H, d, J = 9 Hz), 7.94 (2H, d, J = 9 Hz).

Ethyl 3-[2-(*tert*-butylcarbonyloxy)-1-ethylethoxy]benzoate (14) was synthesized in a similar manner by starting with 2-bromo-1-butanol in place of 1-bromo-2-butanol. NMR (CDCl₃) δ : 1.00 (3H, t), 1.12 (9H, s), 1.38 (2H, t), 1.72 (2H, m), 6.10-6.45 (5H, m), 7.03-7.86 (4H, m).

4-(1, 3-Dioxolan-2-yl)-1-pentanol (15)

A mixture of methyl levulinate (13 g, 0.1 mol), ethylene glycol (9.3 g, 0.15 mol) and *p*-toluenesulfonic acid monohydrate (0.03 g) in benzene was refluxed until 1.8 ml of H₂O was collected in a water separator fitted under a condenser. The mixture was concentrated under reduced pressure and the residue was extracted with ether. The ether solution was washed with brine, and dried over Na₂SO₄. Removal of the solvent gave crude methyl 4-(1, 3-dioxolan-2-yl)levulinate (15.8 g, 91%). A solution of the ketal ester (7.0 g, 0.04 mol) in ether was added to a solution of LiAlH₄ (1.4 g, 0.036 mol) in ether. After stirring for 1 hr at -10 to 0°C, saturated MgSO₄ solution and solid K₂CO₃ were added slowly and the mixture was stirred for 8 hr at room temperature. The precipitate was filtered off and washed with ether. The ether solution was concentrated under reduced pressure. The residue was dissolved in ethyl acetate and the ethyl acetate solution was washed with a small amount of brine, and dried over Na₂SO₄. Removal of the solvent gave compound 15 (3.8 g, 66%). NMR (CDCl₃) δ : 1.35 (3H, s), 1.58-1.78 (4H, m), 1.92-2.12 (1H, broad s), 3.52-3.68 (2H, broad s), 3.96 (4H, s).

Tosylate 16

To a solution of compound 15 (1.46 g, 0.01 mol) in 10 ml of pyridine was added slowly tosyl chloride (1.9 g, 0.01 mol) at 0°C. After stirring for 8 hr at room temperature, the mixture was concentrated and the product was extracted with ether. The ether solution was washed with brine, and dried over Na₂SO₄. After removal of the solvent, the residue was chromatographed on silica gel by elution with hexane-ethyl acetate (4 : 1). Removal of the solvent gave tosylate 16 in 64% yield. NMR (CDCl₃) δ : 1.26 (3H, s), 1.44-1.82 (4H, m), 2.44 (3H, s), 3.87 (4H, d, J = 2 Hz), 4.03 (2H, m), 7.30 (2H, d, J = 8 Hz), 7.74 (2H, d, J = 8 Hz).

General method for the preparation of 5-aryloxy-2-pentanone 17

A mixture of the corresponding phenolic compound (1 mmol) and NaH (1 mmol) in 10 ml of DMF was stirred for 30 min at room temperature. To the mixture was added a solution of tosylate 16 in DMF, and the mixture was stirred for 8 hr at room temperature. To the mixture was added H₂O, and the product was extracted with ether. The ether solution was washed with brine, and dried over Na₂SO₄. After removal of the solvent, the residue was chromatographed on silica gel by elution with

hexane-ethyl acetate mixtures of increasing the polarity. Removal of the solvent gave the corresponding ketal ether. A solution of the ketal ether in acetone was stirred in the presence of *p*-toluenesulfonic acid for 8 hr at room temperature. After concentration under reduced pressure, 30 ml of H₂O was added to the residue. The product was extracted with ether, and the ether solution was washed with brine, dried over Na₂SO₄, and concentrated. Chromatography of the crude product on silica gel using hexane-ethyl acetate (10 : 1) afforded the desired compound 17.

General method for the preparation of ethyl (E)-6-aryloxy-3-methyl-2-hexenoate 18

A mixture of triethyl phosphonoacetate (0.22 g, 1 mmol) and NaH (0.04 g, 1 mmol) in 5 ml of DMF was stirred for 1 hr at room temperature under nitrogen atmosphere. To the mixture was added dropwise a solution of compound 17 (1 mmol) in DMF. The mixture was stirred for 8 hr at 30–40°C. To the mixture was added H₂O, and the product was extracted with ether. The ether solution was washed with brine, and dried over Na₂SO₄. After removal of the solvent, the residue was purified by preparative TLC on silica gel developed with hexane-ethyl acetate (5 : 1) to give (2*E*)-hexenoate 18.

Ethyl (2*E*)-6-(4-ethylphenoxy)-3-methyl-2-hexenoate (19) NMR (CDCl₃) δ: 1.19 (3H, t), 1.26 (3H, t), 1.74–1.99 (2H, m), 2.16 (3H, s), 2.22–2.37 (2H, m), 2.46 (2H, q), 3.90 (2H, t), 4.10 (2H, q), 5.66 (1H, s), 6.76 (2H, d, J=8 Hz), 7.06 (2H, d, J=8 Hz).

Ethyl (2*E*)-6-(4-chlorophenoxy)-3-methyl-2-hexenoate (20) NMR (CDCl₃) δ: 1.27 (3H, t), 1.87–2.08 (2H, m), 2.18 (3H, d, J=1 Hz), 2.24–2.38 (2H, m), 3.91 (2H, t), 4.13 (2H, q), 5.68 (1H, m), 6.77 (2H, d, J=8 Hz), 7.20 (2H, d, J=8 Hz).

Ethyl (2*E*)-6-(3,4-methylenedioxyphenoxy)-3-methyl-2-hexenoate (21) NMR (CDCl₃) δ: 1.26 (3H, t), 1.72–1.96 (2H, m), 2.17 (3H, d, J=1 Hz), 2.12–2.36 (2H, m), 3.85 (2H, t), 4.12 (2H, q), 5.67 (1H, s), 5.87 (1H, s), 6.21–6.70 (4H, m).

Ethyl (E)-4-(3-phenylpropyloxy)-3-methyl-2-butenolate (23)

To a mixture of 3-phenyl-1-propanol (1.0 g, 7.3 mmol) and NaH (0.3 g, 7.3 mmol) in DMF was added dropwise a solution of chloroacetone ethylene ketal (1.0 g, 7.3 mmole) in DMF. The mixture was stirred for 8 hr at room temperature and then 18 hr at 100°C. After cooling, to the mixture was added H₂O, and the product was extracted with ether. The ether solution was washed with brine, and dried over Na₂SO₄. After removal of the solvent, the residue was chromatographed on silica gel by eluting with hexane-ethyl acetate (4 : 1) to give ketal ether in 14% yield. A solution of ketal ether in the presence of small amount of conc. HCl in THF was stirred for 12 hr at room temperature. After concentration under reduced pressure, the product was extracted with ether. The ether solution was washed with brine, and dried over Na₂SO₄. Removal of the solvent gave 0.11 g (59%) of keto ether 22. The following Wittig reaction of compound 22 with triethyl phosphonoacetate was performed in the similar manner as described above to yield 0.13 g (87%, *E*:*Z* = 5 : 2) of ethyl butenoate. The *E* isomer 23 was isolated by preparative TLC on silica gel developed with hexane-ethyl acetate (5 : 1). NMR (CDCl₃) δ: 1.28 (3H, t), 1.91 (2H, m), 2.08 (3H, s), 2.61 (2H, m), 3.44 (2H, t), 3.91 (2H, s), 4.16 (2H, q), 5.94 (1H, s), 7.08–7.24 (5H, m).

Ethyl (E)-4-(1-heptyloxy)-3-methyl-2-butenolate (24) was prepared in the similar method described above by starting with hydroxyacetone ethylene ketal and heptyl bromide in a 66% yield (*E*:*Z* = 5 : 4). NMR (CDCl₃) δ: 0.91 (3H, t), 1.08–1.80 (3H, m), 2.11 (3H, s), 3.43 (2H, t), 3.91 (2H, s), 4.16 (2H, q), 5.94 (1H, s).

Ethyl 4-bromo-3-methyl-2-butenolate (25)

3-Methyl-2-butenic acid (8.0 g, 0.08 mol) in CCl_4 was refluxed with *N*-bromosuccinimide (14.2 g, 0.08 mol) in the presence of azobisisobutyronitrile (0.05 g, 0.3 mmol) for 3 hr. The resulting precipitate was filtered off and the filtrate was concentrated. The residue was extracted with ether and the ether solution was washed with H_2O , brine, and dried over Na_2SO_4 . After removal of the solvent, the residue (crude 4-bromo-3-methyl-2-butenic acid) was dissolved in dichloroethane containing ethanol (11 g, 0.24 mol) and conc. H_2SO_4 (0.3 g, 3 mmol) and then the solution was refluxed for 4 hr. After concentration of the solvent, the product was extracted with ether. The ether solution was washed with 5% NaHCO_3 solution, brine, and dried over Na_2SO_4 . After removal of the solvent, the residue was chromatographed on silica gel by elution with hexane-ethyl acetate (15 : 1) to give 10.5 g (64%) of bromo ester 25 (*E* : *Z* = 85 : 15 by GLC).

General synthesis of ethyl (E)-4-aryloxy-3-methyl-2-butenolate (26)

A solution of the corresponding phenolic compound (1.7 mmol) and NaH (0.07 g, 1.7 mmol) in DMF was stirred for 30 min at room temperature. A solution of compound 25 (0.35 g, 1.7 mmol) in DMF was added dropwise to the mixture, and the mixture was stirred for 3-12 hr at room temperature. To the mixture was added H_2O and the product was extracted with ether. The ether solution was washed with brine, and dried over Na_2SO_4 . After removal of the solvent, the residue was chromatographed on silica gel by elution with hexane-ethyl acetate mixtures of increasing the polarity to give compound 26.

Ethyl (E)-4-substituted amino-3-methyl-2-butenolate (27) was synthesized by the similar manner as described above by using corresponding amines in place of phenols.

Ethyl (*E*)-4-(4-propargyloxyphenoxy)-3-methyl-2-butenolate (28) NMR (CDCl_3) δ : 1.24 (3H, t), 1.73 (3H, d, *J* = 1 Hz), 2.50 (1H, t), 3.20 (2H, s), 4.11 (2H, q), 4.62 (2H, d, *J* = 3 Hz), 6.24 (1H, broad s), 6.83-6.89 (4H, m).

Ethyl (*E*)-4-(3,4-methylenedioxyphenoxy)-3-methyl-2-butenolate (29) NMR (CDCl_3) δ : 1.28 (3H, t), 1.57 (3H, s), 2.17 (3H, s), 4.15 (2H, q), 4.39 (2H, s), 5.89 (2H, s), 5.99 (1H, m), 6.12-6.70 (3H, m).

Ethyl (*E*)-4-(4-propyloxyphenoxy)-3-methyl-2-butenolate (30) NMR (CDCl_3) δ : 1.01 (3H, t), 1.24 (3H, t), 1.73 (3H, d, *J* = 1 Hz), 1.75 (2H, m), 3.19 (2H, s), 3.84 (2H, t), 4.11 (2H, q), 6.23 (1H, s), 6.77-7.12 (4H, m).

Ethyl (*E*)-4-heptylamino-3-methyl-2-butenolate (31) NMR (CDCl_3) δ : 0.82 (3H, t), 1.14-1.28 (15H, m), 2.07 (3H, d, *J* = 1 Hz), 2.49 (2H, t), 3.18 (2H, s), 4.07 (2H, s), 5.79 (1H, m).

Ethyl (*E*)-4-citronellylamino-3-methyl-2-butenolate (32) NMR (CDCl_3) δ : 0.86 (3H, d, *J* = 6 Hz), 1.26 (3H, t), 1.13-1.44 (5H, m), 1.57 (3H, s), 1.66 (3H, s), 1.82-1.97 (2H, m), 2.12 (3H, d, *J* = 1 Hz), 2.56 (2H, t), 3.24 (2H, d, *J* = 1 Hz), 4.12 (2H, q), 5.05 (1H, m), 5.84 (1H, m).

Ethyl (*E*)-4-isobutylamino-3-methyl-2-butenolate (33) NMR (CDCl_3) δ : 0.90 (6H, d, *J* = 7 Hz), 1.26 (3H, t), 1.39 (1H, s), 1.70 (1H, m), 2.11 (3H, d, *J* = 1 Hz), 2.34 (2H, d, *J* = 6 Hz), 3.23 (2H, d, *J* = 1 Hz), 4.12 (2H, q), 5.84 (1H, m).

Ethyl (*E*)-4-(4-butylanilino)-3-methyl-2-butenolate (34) NMR (CDCl_3) δ : 0.90 (3H, t), 1.25 (3H, t), 1.38-1.82 (4H, m), 2.16 (3H, s), 2.47 (2H, t), 3.73 (2H, s), 4.11 (2H, q), 5.92 (1H, m), 6.45 (2H, d, *J* = 9 Hz), 6.96 (2H, d, *J* = 9 Hz).

Bioassays

Bombyx mori (Gunpo×Shugyoku) larvae were reared on artificial diets, Silkmate 1S and 2M (Nippon Nosan Kogyo Co., Ltd) for the 1st instar and from the 2nd to 5th instar larvae, respectively, under a 12 hr light and 12 hr dark photoperiod at $26 \pm 2^\circ\text{C}$. The 3rd instar larvae were topically treated on the dorsal surface with acetone dilutions of test compounds.

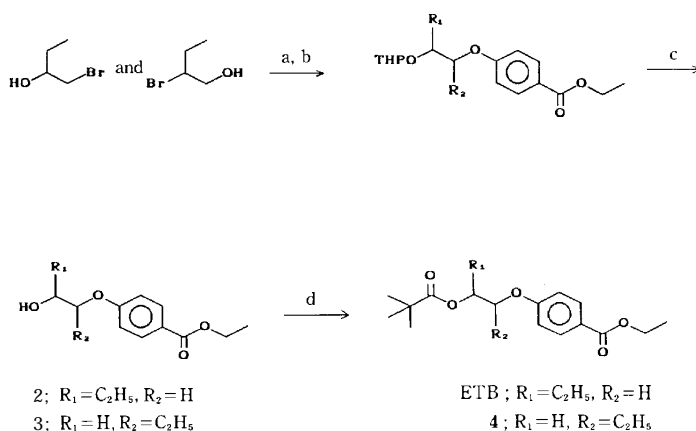
RESULTS AND DISCUSSIONS

Syntheses

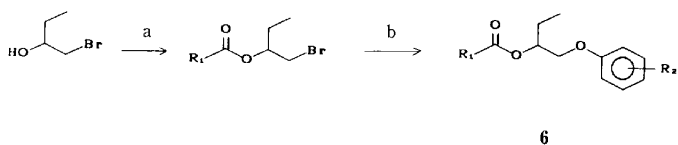
Scheme 1 outlines the synthesis of ETB and its isomer, compound 4. Commercially available 1-bromo-2-butanol containing ca. 20% 2-bromo-1-butanol was used as a starting material. Protection of a hydroxy group was carried out by treating with tetrahydropyran (DHP) in the presence of pyridinium *p*-toluenesulfonate (PPTS) in dichloromethane. Alkylation of ethyl *p*-hydroxybenzoate with the tetrahydropyranyl protected bromide using sodium hydride as a base afforded *p*-alkoxy benzoates **1**. Removal of the tetrahydropyranyl protecting group on compound **1** by acid-catalyzed ethanolysis gave hydroxy compounds **2** and **3** which were separated by column chromatography on silica gel. Esterification of compounds **2** and **3** with pivaloyl chloride using dimethylaminopyridine (DMAP) produced ETB and compound **4**, respectively. Their NMR spectra were in agreement with the assigned structures.

The syntheses of several 2-(alkylcarbonyloxy) butoxy aryl ethers **6** are illustrated in Scheme 2. 1-Bromo-2-butanol was purified as follows; a 5 : 1 mixture of 1-bromo-2-butanol and 2-bromo-1-butanol was treated with 0.4 equivalent of pivaloyl chloride to give unreacted 1-bromo-2-butanol and 2-bromo-1-pivaloyloxybutane which were

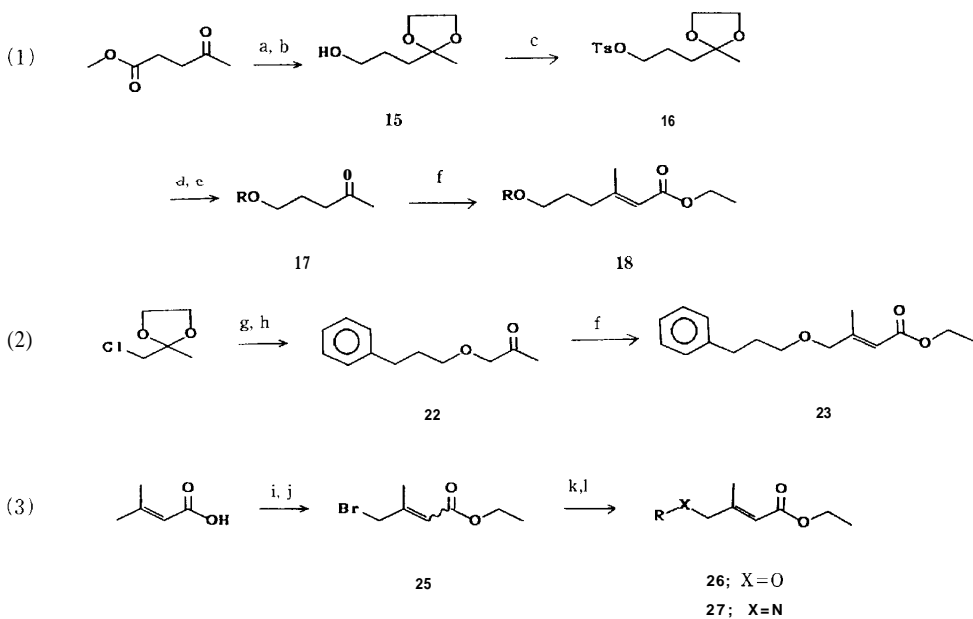
Scheme 1. Synthesis of ETB and compound 4



Reagents ; (a) DHP, PPTS, CH_2Cl_2 ; (b) ethyl *p*-hydroxybenzoate NaH, DMF;
 (c) PPTS, $\text{C}_2\text{H}_5\text{OH}$; (d) $(\text{CH}_3)_3\text{CCOCl}$, DMAP, CH_2Cl_2

Scheme 2. Synthesis of 2-(alkylcarbonyloxy) butyl aryl ethers

Reagents : (a) $R_1\text{COCl}$, DMAP, CH_2Cl_2 ; (b) substituted phenols, NaH, DMF

Scheme 3. Synthesis of ethyl (E)-hexenoate and (E)-butenoate derivatives

Reagents : (a) ethylene glycol, TsOH, benzene ; (b) LiAlH_4 , ether ; (c) TsCl, pyridine ;
 (d) ROH, NaH, DMF; (e) TsOH, acetone ; (f) $(\text{C}_2\text{H}_5\text{O})_2\text{P}(\text{O})\text{CH}_2\text{CO}_2\text{C}_2\text{H}_5$, NaH,
 DMF ; (g) $\text{Ph}(\text{CH}_2)_3\text{OH}$, NaH, DMF ; (h) HCl ; (i) NBS, AIBN, CCl_4 ;
 (j) $\text{C}_2\text{H}_5\text{OH}$, H_2SO_4 , $\text{ClCH}_2\text{CH}_2\text{Cl}$; (k) ROH or RNH_2 , NaH, DMF; (l) chromatographic separation

separated by column chromatography on silica gel. 1-Bromo-2-butanol was esterified in the same manner as compounds 2 and 3 using the corresponding acyl chlorides to provide bromo compound 5. Alkylation of substituted phenols with compound 5 in the presence of sodium hydride gave aryl ether 6.

A large number of EMD analogs were prepared as shown in Scheme 3. Ketalization of methyl levurinate followed by reduction with lithium aluminium hydride gave alcohol 15 which was tosylated to yield compound 16. Treatment of substituted phenols with tosylate 16 in the presence of sodium hydride followed by removal of the protecting group afforded aryloxy ketones 17. The Wittig condensation of compound 17 using triethyl phosphonoacetate provided a mixture of the 2*E* (18) and *Z*-hexenoates which were subsequently separated by chromatography on silica gel.

3-Phenylpropyl ether 23 was prepared by alkylation of 3-phenyl-1-propanol with the ethylene ketal of chloroacetone followed by the Wittig reaction.

Bromination of 3-methyl-2-butenoic acid with *N*-bromosuccinimide (NBS) in the presence of a radical initiator, azobisisobutyronitrile (AIBN), followed by esterification with ethanol in dichloroethane afforded an 85 : 15 mixture of 2*E* and 2*Z*-butenoate 25. Treatment of bromo compound 25 with substituted phenols and amines provided 4-aryloxybutenoate 26 and 4-substituted aminobutenoate 27, respectively.

Benzyl-1, 3-benzodioxole derivatives were prepared by analogy with the method described by Jurd et al., (1979) ; benzylic alcohols condensed with sesamol in the presence of oxalic acid to give the phenolic 1, 3-benzodioxoles, which on treatment with alkyl halides and sodium hydride in dimethylformamide formed alkyl ethers in good yield.

Biological activities

Anti JH activity of ETB analogs was assessed by induction of precocious metamorphosis in *B. mori* larvae. When ETB was applied topically to 3 rd instar larvae, precocious pupation always occurred in the 4 th (penultimate) larval stage. None of the treated 3 rd instar larvae metamorphosed into precocious pupae in the 3 rd larval stage. Table 1 shows the effects of ETB at different ages within the 3 rd instar. A low ETB susceptibility was observed for the strain used in this experiment. The percentage of precocious metamorphosis did not correlate with the dose ; a high dose of ETB

Table 1. Effects of ETB on 3rd instar larvae of *B. mori*.

Time of treatment (hr after ecdysis)	Dose ($\mu\text{g}/\text{larva}$)	Precocious metamorphosis (%)
0	10	5
	50	0
	100	0
	200	0
24	50	20
	100	0
	200	0
48	50	5
	100	20
	200	0

caused no precocious pupation. A similar result has been obtained by Kiguchi *et al.*, (1984) who examined on the different strain of *B. mori*. ETB is well known to show anti **JH** activity at low dose levels and JH activity at higher dose levels against *M. sexta* larvae (Staal *et al.*, 1982).

Since **48** hr-old 3 rd instar larvae seemed to be more susceptible than newly molted larvae, various ETB analogs were tested for 48 hr-old 3 rd instar (Table 2). None of the compounds showed strong anti JH activity. In a series of ethyl alkoxybenzoates, *ortho*-substituted derivative **8** showed lower activity than the *meta* (**7**) or *para* (ETB) isomers. The ethylphenyl ether **9** and the chlorophenyl ether **10** analogs were also effective, but the methylenedioxyphenyl ether **11** was quite inactive. A modification was made in the alkylcarbonyloxy portions (R_1) of the molecule. The activity of isopropyl analog **12** was somewhat higher compared with that of the *t*-butyl analog, while the isobutyl analog **13** was inactive. Interestingly, the ethyl substituent on R_3 , namely compound **4** and **14**, showed slightly greater than ETB or equal activity.

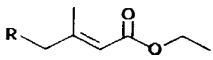

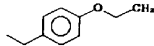
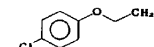
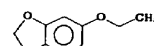
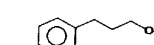
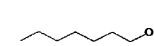

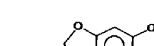
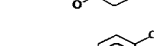
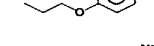
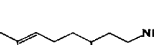

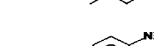
Although EMD has been reported to show anti JH activity on *H. virescens* and *M. sexta* (Staal *et al.*, 1982), no precocious metamorphosis was induced by EMD in the 3-*rd* and 4 *th* instar larvae of *B. mom'*. A number of 2-butenate analogs and related compounds in Table 3 also failed to elicit anti JH effects on *B. mom'*. Some of them caused acute toxicity in the newly molted 3 *rd* instar larvae (Table 3). In the EMD analogs, replacement of the methylene group at C-5 by an oxygen (**24**) or a nitrogen (**31**) atom gave analogs with much higher toxicity. Among the compounds tested, the citronellylaminobutenate **32** showed the highest toxicity. The toxicity in this series was found to fall off sharply with decreasing size of the N substituent (compound **33**). The aniline analog **34** was quite inactive.

Brooks *et al.* (1985) has reported that a series of prop-2-ynyl ethers and compounds containing a 1,3-benzodioxole moiety (e. g. compounds **28** and **29**) inhibited the biosynthesis of JH III in excised corpora allata of the cockroach, *Periplaneta americana*. However, no *in vivo* data has so far been reported on any insect species. We tested prop-2-ynyl ether **28** and 1,3-benzodioxole **29** analog on *B. mom'*. These

Table 2. Effects of ETB analogs on 48 hr-old 3rd instar larvae of *B. mori*.

No.	R_1	R_2	R_3	R_4	$(\mu\text{g/larva})$	Precocious metamorphosis (%)		
						200	100	50
	$t\text{-C}_4\text{H}_9$	C_2H_5	H	4-COOC ₂ H ₅ (ETB)	0	20	5	
7	"	"	"	3-COOC ₂ H ₅	30	10	10	
8	"	"	"	2-COOC ₂ H ₅	0	10	0	
9	"	"	"	4-C ₂ H ₅	0	0	10	
10	"	"	"	4-Cl	20	0	0	
11	"	"	"	3,4-OCH ₂ O-	0	0	0	
12	$i\text{-C}_3\text{H}_7$	"	"	4-COOC ₂ H ₅	20	40	40	
13	$i\text{-C}_4\text{H}_9$	"	"	"	0	0	0	
4	$t\text{-C}_4\text{H}_9$	H	C_2H_5		10	0	20	
14	"	"	"	a - c o o & ,	40	20	10	

Table 3. Insecticidal activity of EMD and related compounds against 3rd instar larvae of *B. mori*.

No.	R	R ($\mu\text{g}/\text{larva}$)	24hr Mortality (%)		
			100	50	5
		(EMD)			
			0		
19			10		
20			50	10	0
21			0		
23			60	0	
24			100	80	0
28			0		
29			0		
30			0		
31			100	30	0
32			100	100	0
33			0		
34			0		

compounds exhibited neither anti JH activity nor acute toxicity at 100 μg .

No precocious metamorphosis was induced by several 1,3-benzodioxoles shown in Table 4 when assayed on the newly molted 3rd instar larvae. The 5-ethoxy analog ($R_1 = \text{C}_2\text{H}_5$, $R_2 = \text{CH}_3$) and J2710 elicited moderate acute toxicity in larval *B. mori*. However, these acute toxicities might be not related to the anti JH action. The toxicity decreased sharply or disappeared with increasing size of the R_1 and R_2 substituents.

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Table 4. Toxicity of 1, 3-benzodioxoles against 3rd instar larvae of *B. mori*.

R ₁	R ₂	(μg/larva)	24hr Mortality (%)			
			10	5	2	1
CH ₃	CH ₃ (J2710)	100	100	20	0	0
C ₂ H ₅	CH ₃	100	100	100	60	0
<i>i</i> -C ₃ H ₇	CH ₃	0	0	0	0	0
H	CH ₃	0	0	0	0	0
CH ₃	C ₂ H ₅	90	90	0	0	0
CH ₃	<i>i</i> -C ₃ H ₇	10	10	0	0	0
CH ₃	CH ₂ -Ph	0	0	0	0	0

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