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Original Article

Synthesis and anti-juvenile hormone activity of ethyl 4-(2-benzylalkyloxy)benzoates and their enantiomers

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A number of ethyl 4-(2-benzylalkyloxy)benzoates were prepared and their activity to induce precocious metamorphosis was evaluated in larvae of the silkworm, *Bombyx mori*, which was clearly recognized as a juvenile hormone (JH)-deficiency symptom. Ethyl 4-(2-benzylhexyloxy)benzoate (**6**) and its 2-benzylheptyloxy analog (**7**) were found to induce precocious metamorphosis at relatively low doses. Both enantiomers of **6** and **7** were prepared using a chiral auxiliary oxazolidinone. (*S*)-Enatiomers were more active than (*R*)-isomers at low doses of 0.1 and 1 μg, but at higher doses their activity was reversed. The activity of compound **6** could be fully counteracted by methoprene, a JH agonist, but not by the dietary administration of 20-hydroxyecdysone. The ester group was important in the ability to induce precocious metamorphosis. The (*S*)-enatiomer of **6** prolonged the duration of the instar and delayed the onset of cocoon spinning when applied to 5th instar larvae, suggesting that this compound might have JH-like activity as well as anti-JH activity.

Keywords: juvenile hormone, anti-juvenile hormone, precocious metamorphosis, silkworm.

Introduction

Since juvenile hormone (JH) is involved in a wide range of physiological processes in both developing and mature insects,[1] compounds leading to JH deficiency symptoms, anti-JH agents, 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.[2] Although several anti-JH agents such as precocenes, fluoromevalonate, dichloroallyl hexanoate, ethyl 4-[2-(*tert*-butylcarbonyloxy)butoxy]benzoate (ETB), 1,5-disubstituted imidazoles, and brevioxime[3] have so far been reported, none of the compounds has been developed for practical use in pest control as yet. Among them, ETB has been found to have both JH-like action and anti-JH action for the tobacco hornworm, *Manduca sexta*[4] and the silkworm, *Bombyx mori*,[5] depending on the dose applied; low doses of ETB induced precocious metamorphosis, a clear sign of JH deficiency, but at higher doses the precocious metamorphosis-inducing activity disappeared and instead, JH-like activity was observed. Riddiford *et al*. have reported that ETB acts as a partial JH antagonist at the target tissue of the larval epidermis.[6] No other anti-JH agents with such action have been found to date.

By modifying the structure of ETB, we have recently found that ethyl 4-[4-methyl-2-(6-methyl-3-pyridyloxy)pentyloxy]-benzoate (**1**)[7] and ethyl 4-(2-phenoxyhexyloxy)benzoate (**2**)[8] showed stronger precocious metamorphosis-inducing activity than ETB against *B. mori* larvae (Fig. 1). In contrast to ETB, their anti-JH activity was correlated with the applied doses to some extent. These findings prompted us to further synthesize and evaluate new ethyl 4-substituted benzoate derivatives as potent anti-JH agents. In the present paper we report the synthesis, precocious metamorphosis-inducing activity, and structure–activity relationships of a novel series of ethyl 4-(2-benzylalkyloxy)benzoates and their optically active compounds.

Materials and Methods

1. *Instrumental analysis*

^{1}H NMR spectra were determined with a JEOL EX-400 (400 MHz) spectrometer, using tetramethylsilane as an internal standard, and all samples were prepared in deuterochloroform. Optical rotation values were measured with a Union

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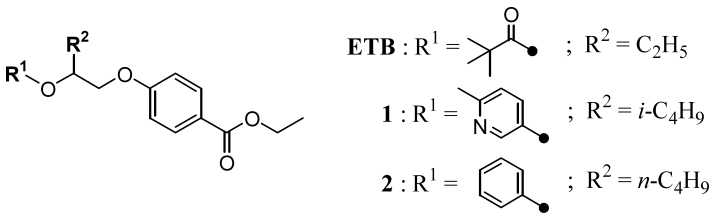


Fig. 1. Structures of ETB and ethyl 4-substituted benzoates.

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.

2. Chemicals

2.1. 2-Benzylhexanoic acid [(*I*): $R=n\text{-}C_4H_9$]

A solution of *n*-hexanoic acid (1.0 g, 8.6 mmol) in 5 ml of tetrahydrofuran (THF) was added dropwise at −40 °C to a solution of lithium diisopropylamide (LDA, 2 M THF solution, 9.5 ml, 19 mmol) dissolved in 10 ml of THF. To the mixture was added hexamethylphosphoramide (HMPA, 1.8 g, 10 mmol) at the same temperature. The mixture was warmed to 50°C and stirred for 40 min. The mixture was cooled to room temperature, and benzyl bromide (1.85 g, 11 mmol) was added to the mixture. The mixture was stirred for 2 hr at 50°C and then for 12 hr at room temperature. After removal of the solvent under reduced pressure, the residue was dissolved in ethyl acetate and the ethyl acetate solution was washed with 2 M HCl 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 : 1) to afford 0.67 g (38%) of 2-benzylhexanoic acid. ^{1}H NMR δ: 0.88 (3H, t, J=6.8 Hz, CH_3), 1.24–1.36 (4H, m, $2CH_2$), 1.55–1.63 (m, 2H, CH_2), 2.35 (1H, t, J=7.1 Hz, CH), 2.76 (1H, dd, J=7.1, 12.2 Hz, CH), 2.98 (1H, dd, J=7.1, 12.2 Hz, CH), 7.17–7.29 (5H, m, phenyl).

2.2. 2-Benzyl-1-hexanol [(*II*): $R=n\text{-}C_4H_9$]

A mixture of 2-benzylhexanoic acid (0.67 g, 3.2 mmol) and lithium aluminum hydride (0.15 g, 4 mmol) in 10 ml of THF was stirred for 12 hr at room temperature. The reaction mixture was quenched with 5 ml of saturated NH_4Cl solution at 0°C. The product was extracted with ethyl acetate and the organic layer was washed with brine and dried over Na_2SO_4. Concentration of the organic layer gave 0.51 g (82%) of crude 2-benzyl-1-hexanol. ^{1}H NMR δ: 0.89 (3H, t, J=6.8 Hz, CH_3), 1.24–1.59 (6H, m, $3CH_2$), 1.78–1.82 (1H, m, CH), 2.64 (2H, δ, J=6.8 Hz, CH_2), 3.53 (2H, d, J=6.8 Hz, CH_2), 7.17–7.28 (5H, m, phenyl).

2.3. Ethyl 4-(2-benzylhexyloxy)benzoate (*6*)

To a solution of the above alcohol in 15 ml of dichloromethane were added triethylamine (0.27 g, 2.7 mmol) and *p*-toluenesulfonyl chloride (0.51 g, 2.7 mmol), and the mixture was stirred for 12 hr at room temperature. After removal of the solvent under reduced pressure, the product was extracted with ethyl acetate. The ethyl acetate solution was washed with water and brine, and dried over Na_2SO_4. Concentration of the organic layer gave 0.98 g (2.8 mmol) of crude 2-benzylhexyl *p*-toluenesulfonate as an oil. To a mixture of ethyl 4-hydroxybenzoate (0.56 g, 3.4 mmol) and potassium carbonate (0.47 g, 3.4 mmol) in 20 ml of dimethylformamide (DMF) was added a solution of the above *p*-toluenesulfonate in 5 ml of DMF. After stirring for 12 hr at room temperature, the product was extracted with ethyl acetate. The organic layer was washed with 2 M NaOH 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 (5 : 1) to give 0.45 g (50%) of **6** as an oil. ^{1}H NMR δ: 0.90 (3H, t, J=6.8 Hz, CH_3), 1.30–1.54 (6H, m, $3CH_2$), 1.38 (3H, t, J=6.8 Hz, CH_3, overlapped), 2.06–2.11 (1H, m, CH), 2.75 (2H, d, J=6.4 Hz, CH_2), 3.83 (2H, d, J=4.9 Hz, CH_2), 4.34 (2H, q, J=6.8 Hz, CH_2), 6.86 (2H, d, J=8.8 Hz, phenyl), 7.17–7.27 (5H, m, phenyl), 7.98 (2H, d, J=8.8 Hz, phenyl).

Compounds **3**–**5** and **7**–**9** were prepared in the same manner as compound **6** using the corresponding alkanoic acid instead of *n*-hexanoic acid.

Ethyl 4-(2-benzylbutyloxy)benzoate (**3**). ^{1}H NMR δ: 0.98 (3H, t, J=7.3 Hz, CH_3), 1.38 (3H, t, J=7.3 Hz, CH_3), 1.45–1.57 (2H, m, CH_2), 1.99–2.05 (1H, m, CH), 2.74–2.76 (2H, m, CH_2), 3.85 (2H, d, J=5.4 Hz, CH_2), 4.34 (2H, q, J=7.3 Hz, CH_2), 6.87 (2H, d, J=8.8 Hz, phenyl), 7.15–7.93 (5H, m, phenyl), 7.97 (2H, d, J=8.8 Hz, phenyl).

Ethyl 4-(2-benzylpentyloxy)benzoate (**4**). ^{1}H NMR δ: 0.92 (3H, t, J=6.8 Hz, CH_3), 1.38 (3H, t, J=6.8 Hz, CH_3), 1.41–1.53 (4H, m, $2CH_2$), 2.08–2.12 (1H, m, CH), 2.72–2.78 (2H, m, CH_2), 3.83 (2H, d, J=5.4 Hz, CH_2), 4.34 (2H, q, J=6.8 Hz, CH_2), 6.86 (2H, d, J=8.8 Hz, phenyl), 7.14–7.28 (5H, m, phenyl), 7.97 (2H, d, J=8.8 Hz, phenyl).

Ethyl 4-(2-benzyl-4-methylpentyloxy)benzoate (**5**). ^{1}H NMR δ: 0.90 (3H, d, J=6.4 Hz, CH_3), 0.91 (3H, d, J=6.4 Hz, CH_3), 1.24–1.34 (2H, m, CH_2), 1.38 (3H, t, J=7.3 Hz, CH_3), 1.70–1.77 (1H, m, CH), 2.13–2.19 (1H, m, CH), 2.74 (2H, d, J=6.8 Hz, CH_2), 3.79 (2H, d, J=5.4 Hz, CH_2), 4.34 (2H, q, J=7.3 Hz, CH_2), 6.86 (2H, d, J=8.8 Hz, phenyl), 7.14–7.28 (5H, m, phenyl), 7.97 (2H, d, J=8.8 Hz, phenyl).

Ethyl 4-(2-benzylheptyloxy)benzoate (**7**). ^{1}H NMR δ: 0.88 (3H, t, J=7.3 Hz, CH_3), 1.27–1.53 (8H, m, $4CH_2$), 1.38 (3H, t, J=7.3 Hz, CH_3, overlapped), 2.05–2.09 (1H, m, CH), 2.75 (2H, d, J=7.8 Hz, CH_2), 3.83 (2H, d, J=5.4 Hz, CH_2), 4.34 (2H, q, J=7.3 Hz, CH_2), 6.86 (2H, d, J=8.8 Hz, phenyl), 7.14–7.27 (5H, m, phenyl), 7.97 (2H, d, J=8.8 Hz, phenyl).

Ethyl 4-(2-benzyloctyloxy)benzoate (**8**). ^{1}H NMR δ: 0.87 (3H, t, J=6.8 Hz, CH_3), 1.27–1.54 (10H, m, $5CH_2$), 1.38 (3H, t, J=7.3 Hz, CH_3, overlapped), 2.05–2.09 (1H, m, CH), 2.72–2.76 (2H, m, CH_2), 3.83 (2H, d, J=5.4 Hz, CH_2), 4.34 (2H, q, J=6.8 Hz, CH_2), 6.86 (2H, d, J=8.8 Hz, phenyl), 7.14–7.28 (5H, m, phenyl), 7.99 (2H, d, J=8.8 Hz, phenyl).

Ethyl 4-(2-benzyl-3-phenylpropyloxy)benzoate (**9**). ^{1}H NMR δ: 1.38 (3H, t, J=7.1 Hz, CH_3), 2.33–2.39 (1H, m, CH), 2.75–2.85 (4H, m, $2CH_2$), 3.76 (2H, d, J=4.4 Hz, CH_2), 4.34 (2H, q, J=6.8 Hz, CH_2), 6.83 (2H, d, J=8.8 Hz, phenyl), 7.14–7.31 (10H, m, phenyl), 7.96 (2H, d, J=8.8 Hz, phenyl).

*2.4. (S)-4-Benzyl-3-hexanoyl-2-oxazolidinone (**IV**)*

n-Butyllithium (1.6 M hexane solution, 5.1 ml, 8.2 mmol) was added dropwise to a solution of (*S*)-4-benzyl-2-oxazolidinone (1.3 g, 7.4 mmol) in 15 ml of THF cooled at −78°C under N_2. After stirring for 30 min, a solution of *n*-hexanoyl chloride (1.0 g, 7.4 mmol) in 5 ml of THF was added dropwise to the mixture at the same temperature. The mixture was stirred for 1 hr at −78°C and the reaction was quenched by the addition of 10 ml of saturated NH_4Cl solution. The product was extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ solution 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 (2 : 1) to give 1.96 g (96%) of (**IV**). ^{1}H NMR δ: 0.92 (3H, t, J=6.8 Hz, CH_3), 1.35–1.41 (4H, m, $2CH_2$), 1.66–1.73 (2H, m, CH_2), 2.77 (1H, dd, J=9.3, 13.2 Hz, CH), 2.85–3.01 (2H, m, CH_2), 3.30 (1H, dd, J=3.4, 13.2 Hz, CH), 4.13–4.22 (2H, m, 2CH), 4.64–4.7 (1H, m, CH), 7.17–7.36 (5H, m, phenyl).

*2.5. (S)-4-Benzyl-3-[(R)-2-benzylhexanoyl]-2-oxazolidinone (**V**)*

LDA (2 M THF solution, 2.7 ml, 5.4 mmol) was added dropwise to a solution of (**IV**) (1.0 g, 3.6 mmol) in 10 ml of THF at −78°C under N_2. After stirring for 30 min, a solution of benzyl bromide (1.8 g, 10 mmol) in 5 ml of THF was added dropwise to the mixture. The mixture was stirred for 30 min at −78°C and then for 2 hr at 0°C. 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 (4 : 1) to give 1.05 g (79%) of (**V**). ^{1}H NMR δ: 0.83 (3H, t, J=6.8 Hz, CH_3), 1.24–1.27 (4H, m, $2CH_2$), 1.37–1.42 (1H, m, CH), 1.64–1.69 (1H, m, CH), 2.64–2.82 (3H, m, CH_2, CH), 2.97 (1H, dd, J=8.8, 12.6 Hz, CH), 4.04–4.13 (2H, m, 2CH), 4.28–4.32 (1H, m, CH), 4.65–4.68 (1H, m, CH), 6.86–6.90 (2H, m, phenyl), 7.19–7.33 (8H, m, phenyl).

*2.6. (R)-2-Benzyl-1-hexanol (**IIR**)*

To a solution of lithium borohydride (0.12 g, 5.5 mmol) in 10 ml of THF was added a solution of (**V**) (0.50 g, 1.4 mmol) in 5 ml of THF at 0°C. After stirring for 24 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 : 2) to give 0.20 g (76%) of (**IIR**): $[\alpha]_D^{16}$ +6.0° (c 0.01, CH_2Cl_2) [lit.[9] +3.5° (c 0.01, CH_2Cl_2)]. The ^{1}H NMR spectrum of (**IIR**) was completely consistent with that of racemic 2-benzyl-1-hexanol.

*2.7. Ethyl 4-[(R)-2-benzylhexyloxy]benzoate (**6R**)*

Compound (**IIR**) was converted to **6R** in the same manner as described in **6**. $[\alpha]_D^{19}$ −41° (c 1, ethanol). The enantiomeric purity was 99% ee by HPLC analysis under the 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.

Ethyl 4-[(*S*)-2-benzylhexyloxy]benzoate (**6S**) was prepared in the same manner as **6R** using (*S*)-2-benzyl-1-hexanol instead of (*R*)-isomer.

Compound **6S:** $[\alpha]_D^{19}$ +40° (c 1, ethanol), enantiomeric purity; 99% ee. The ^{1}H NMR spectra of **6R** and **6S** were completely consistent with that of **6**.

Compounds **7R** and **7S** were prepared in the same manner as **6R** and **6S**, respectively, using *n*-heptanoyl chloride instead of *n*-hexanoyl chloride.

Ethyl 4-[(*R*)-2-benzylheptyloxy]benzoate (**7R**). $[\alpha]_D^{19}$ −38° (c 1, ethanol), 99% ee.

Ethyl 4-[(*S*)-2-benzylheptyloxy]benzoate (**7S**). $[\alpha]_D^{19}$ +43° (c 1, ethanol), 99% ee.

*2.8. 4-(2-Benzylhexyloxy)benzoic acid (**6-acid**)*

A mixture of **6** (0.80 g, 2.4 mmol) and 0.42 g of NaOH (0.38 g, 9.5 mmol) in 5 ml of ethanol and 10 ml of water was refluxed for 6 hr. After removal of the solvent, the residue was dissolved in water. The aqueous solution was washed with ethyl acetate, and acidified with dil. HCl. 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 recrystallized from hexane and ethyl acetate, affording 0.51 g (69%) of **6-acid**, mp 105–107°C. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, t, J =7.3 Hz, CH_3), 1.28–1.55 (6H, m, $3CH_2$), 2.06–2.12 (1H, m, CH), 2.76 (2H, d, J =6.8 Hz, CH_2), 3.85 (2H, d, J =4.9 Hz, CH_2), 6.90 (2H, d, J=8.8 Hz, phenyl), 7.15–7.29 (5H, m, phenyl), 8.04 (2H, d, J=8.8 Hz, phenyl).

3. Biological evaluation

B. mori (Shunrei×Shougetsu) larvae were reared on an artificial diet as previously reported.[10] Test compounds in acetone solution were applied topically to the dorsal abdomen of 72-hr-old 2nd instar, 24-hr-old 3rd instar and newly molted 4th instar larvae. Compounds **6** and **6-acid** were mixed with the artificial diet at concentrations of 50 and 200 ppm according to the procedure reported.[11] The diet containing the test compound was administered for the first 48 hr to newly molted 3rd instar larvae. The diet containing 20-hydroxyecdysone was prepared as described previously[12] and administered for the first 48 hr to 24-hr-old 3rd instar or newly molted 4th instar larvae. The activity of compounds was evaluated by the induction of precocious metamorphosis: spinning a cocoon

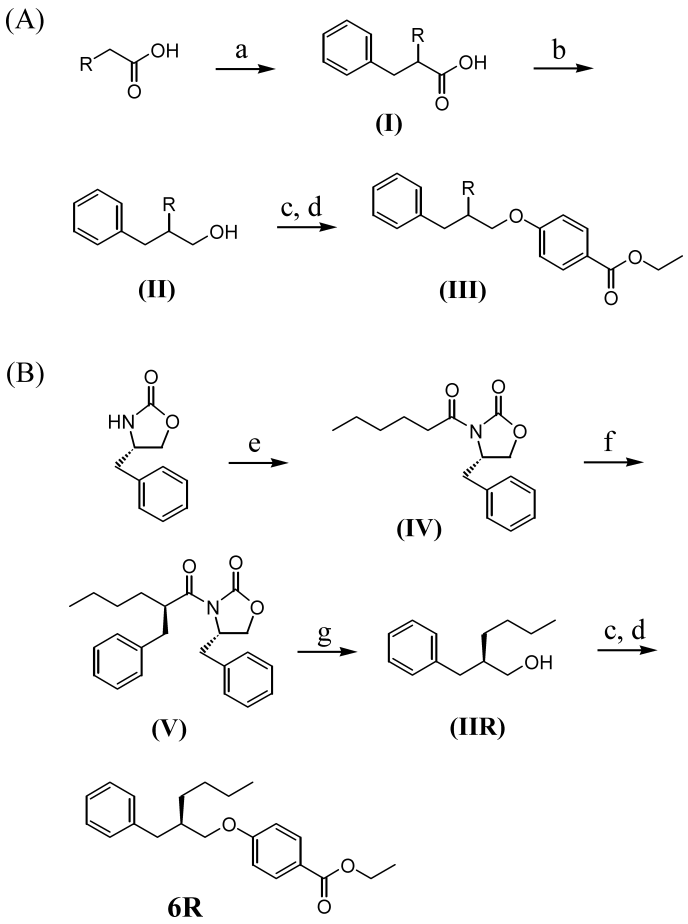


Fig. 2. Synthetic scheme for the preparation of (A) ethyl 4-(2-benzylalkyloxy)benzoates and (B) ethyl 4-[(*R*)-2-benzylhexyloxy]benzoate (**6R**). (a) benzyl bromide, LDA, THF, HMPA; (b) $LiAlH_4$, THF; (c) *p*-toluenesulfonyl chloride, triethylamine, CH_2Cl_2; (d) ethyl 4-hydroxybenzoate, K_2CO_3, DMF; (e) *n*-hexanoyl chloride, *n*-butyllithium, THF; (f) benzyl bromide, LDA, THF; (g) $LiBH_4$, THF.

and subsequent pupation or formation of larval–pupal intermediates from the 4th instar (penultimate) larval period.

Results and Discussion

1. Synthesis

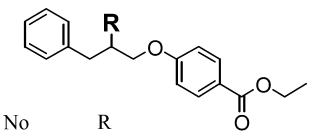
The preparation of a series of ethyl 4-(2-benzylalkyloxy)-benzoates is shown in Fig. 2(A). Reaction of alkanoic acids with benzyl bromide in the presence of LDA gave 2-benzyl-alkanoic acids (**I**), which were reduced to 2-benzylalkanols (**II**) with $LiAlH_4$ in THF. 2-Benzylalkanols (**II**) were converted to the corresponding toluenesulfonates, which were treated with ethyl 4-hydroxybenzoate in DMF in the presence of K_2CO_3 to give ethyl 4-(2-benzylalkyloxy)benzoates (**III**).

Enantiomers of ethyl 4-(2-benzylalkyloxy)benzoates were prepared using a chiral auxiliary oxazolidinone.[13] As a typical procedure, the synthesis of ethyl 4-[(*R*)-2-benzylhexyl-oxy]benzoate (**6R**) is outlined in Fig. 2(B). Acylation of (*S*)-4-benzyl-2-oxazolidinone with *n*-hexanoyl chloride furnished 3-hexanoyloxazolidinone (**IV**) in a 96% yield. Reaction of the lithium enolate derived from imide (**IV**) with benzyl bromide afforded the benzylated product (**V**). Reduction of the compound (**V**) with lithium borohydride afforded optically active 2-benzyl-1-hexanol (**IIR**), the specific rotation of which was +6.0. The absolute configuration of (+)-2-benzyl-1-hexanol has already been established as *R*.[9] Compound (**IIR**) was transformed into **6R** in the same manner as described in (**III**). The enantiomeric excess of **6R** was determined to be 99% by chiral HPLC using a Chiralpac column. Enantiomer **6S** was prepared in an identical manner starting from (*R*)-4-benzyl-2-oxazolidinone and exhibited the opposite specific rotation.

2. Biological activities

Table 1 shows the activity of ETB and ethyl 4-(2-benzylalky-loxy)benzoates to induce precocious metamorphosis in the 4th larval stage of *B. mori* when applied to 24-hr-old 3rd instar larvae. None of the compounds exhibited acute toxicity at the doses applied. Treatment with ETB at 1 μg induced precocious pupation in only 10%, but no activity was observed at higher doses, similar to that reported previously.[5] Analog **3** with an ethyl side chain had low precocious metamorphosis-inducing activity. The introduction of an *n*-propyl group (**4**) increased the degree of activity in comparison with that of **3**.

Table 1. Precocious metamorphosis-inducing activity of ETB and ethyl 4-(2-benzylalkyloxy)benzoates against 3rd instar larvae of *B. mori*[a)]



No	R	Precocious metamorphosis (%) 0.1	1	10	40
			(μg/larva)		
ETB		0	10	0	0
3	C_2H_5	0	0	5	NT
4	*n*-C_3H_7	0	22	37	87
5	*i*-C_4H_9	0	0	22	46
6	*n*-C_4H_9	21	90	34	12
6S	*n*-C_4H_9 *S* (+)	52	96	17	15
6R	*n*-C_4H_9 *R* (−)	0	8	42	67
7	*n*-C_5H_{11}	37	94	48	20
7S	*n*-C_5H_{11} *S* (+)	65	97	39	17
7R	*n*-C_5H_{11} *R* (−)	0	54	83	76
8	*n*-C_6H_{13}	0	20	42	62
9	$CH_2C_6H_5$	0	35	65	95

[a)] Values are the average of 2–3 experiments, and 18–20 individuals were used in each test. The compounds described in this table are racemic unless otherwise specified. NT: not tested.

In contrast to the results found in ethyl 4-[2-(6-methyl-3-pyridyloxy)alkyloxy]benzoate series,[7)] isobutyl analog **5** showed lower activity than *n*-propyl analog **4**. The *n*-butyl group (**6**) gave a remarkable increase in activity at a low dose of 1 μg, but the activity comparatively decreased by increasing the applied doses. The *n*-pentyl analog **7** had almost the same level of activity as **6** and was most effective at treatment of 1 μg. The *n*-hexyl (**8**) and benzyl (**9**) analogs showed lower activity at low doses in comparison with those of **6** and **7**. Only compounds **6** and **7** showed some precocious metamorphosis-inducing activity at a very low dose of 0.1 μg. Compounds **1** and **2** had no activity at this dose.

Since compounds **6** and **7** induced precocious metamorphosis at low doses, the activity of their enantiomers was examined. At lower doses of 0.1 and 1 μg (*S*)-enantiomers were more active than (*R*)-enantiomers, but at higher doses of 10 and 40 μg their activity was reversed. No consistent dose–response relationship was obtained in (*S*)-enantiomers as well as racemates. The activity of racemates at 0.1 μg was approximately half as much as that of the respective (*S*)-enantiomer at 0.1 μg. Thus, the activity of racemates seemed to be due to (*S*)-enantiomers. In the case of ETB, it has been reported that anti-JH activity at low doses and JH activity at higher doses in *M. sexta* larvae are entirely due to the (*S*)-(−)-enantiomer, the (*R*)-(+)-isomer being completely inactive.[14)]

When 3rd instar larvae were treated with ETB or compounds **3**–**9**, precocious metamorphosis always occurred at the 4th larval stage. None of the treated 3rd instar larvae metamorphosed into precocious pupae in the same larval stage. We examined the effects of **6** and its enantiomers on 72-hr-old 2nd instar and newly molted 4th instar larvae (Table 2). When these compounds were applied to 2nd instar larvae, morphologically normal 4th instar larvae were formed and then some 4th instar larvae metamorphosed into precocious pupae. In this case **6S** clearly induced precocious metamorphosis, while **6R** was inactive at the doses applied. Newly molted 4th instar larvae were much less susceptible than 2nd and 3rd instar larvae to these compounds. Similar results have been obtained in the 4-[2-(6-methyl-3-pyridyloxy)-alkyloxy]benzoate series.[7)]

As previously described, precocious metamorphosis is well known to be induced by JH deficiency in the larval stage caused by allatectomy or treatment of anti-JH agents[2)]; however, we have found that the precocious metamorphosis-inducing activity of some 1,5-disubstituted imidazoles is completely reversible by the dietary administration of 20-hydroxyecdysone, suggesting that a temporary decline of ecdysteroid titers in the larval hemolymph might also cause precocious metamorphosis.[12)] We therefore examined the effects of methoprene, a JH agonist, and 20-hydroxyecdysone on the precocious metamorphosis induced by **6** (Table 3). The activity of **6** to induce precocious pupation was completely counteracted by the simultaneous application of methoprene to 24-hr-old 3rd instar larvae or methoprene applied immediately after ecdysis to the 4th stage, but not by the dietary administration of 20-hydroxyecdysone. These results of the rescue experiments indicate that **6** induces precocious metamorphosis in *B. mori* larvae by causing a deficiency of JH titers in the larval hemolymph.

To see whether the ester portion of **6** was necessary for activity, we tested the activity of **6** and the corresponding benzoic acid (**6-acid**) by dietary administration to 3rd instar larvae (Table 4). Compound **6** induced precocious metamorpho-

Table 2. Precocious metamorphosis-inducing activity of **6** and its enatiomers against 2nd and 4th instar larvae of *B. mori*[a)]

Time of treatment	Compound	Precocious metamorphosis (%) 0.1	1	10
			(μg/larva)	
72-hr-old 2nd instar	**6**	0	45	85
	6S (+)	20	89	95
	6R (−)	0	0	0
newly molted 4th instar	**6**	NT	5	10
	6S (+)	NT	10	0
	6R (−)	NT	5	0

[a)] Nineteen to twenty individuals were used in each test. NT: not tested.

Table 3. Effects of methoprene and 20-hydroxyecdysone on precocious metamorphosis induced by **6** in *B. mori* larvae

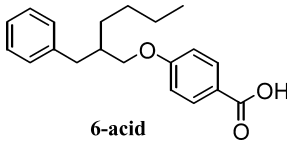
Compound	Time of treatment (larval instar)	Precocious metamorphosis (%)
6[a] (1 μg/larva)	3rd	100
+Methoprene[b] (10 μg/larva)	3rd	0
+Methoprene (10 μg/larva)	4th	0
+20-Hydroxyecdysone[c] (20 ppm)	3rd	95
+20-Hydroxyecdysone (20 ppm)	4th	100

[a] Compound **6** was topically applied to 24-hr-old 3rd instar larvae. [b] Methoprene was topically applied to 24-hr-old 3rd or newly molted 4th instar larvae. [c] The diet containing 20-hydroxyecdysone was administered for the first 48 hr to 24-hr-old 3rd or newly molted 4th instar larvae. Twenty individuals were used in each test.

sis by dietary administration as well as topical application, and there was also no dose–response relationship in this case. Compound **6-acid** did not show any activity at 50 and 200 ppm, indicating that the ethoxycarbonyl group itself is responsible for activity.

As previously described, ETB has been found to act as a JH agonist as well as an anti-JH agent. Kiguchi *et al.* have shown that ETB is an effective JH agonist by bioassay using allatectomized 4th instar larvae.[5] We tested **6** and its enantiomers on 5th instar larvae to see whether they had any JH-like activity (Table 5). It has been reported that in the early stage of the 5th

Table 4. Precocious metamorphosis-inducing activity of **6** and **6-acid** by dietary administration in 3rd instar larvae of *B. mori*

Compound	Concentration (ppm)	Precocious metamorphosis (%)
6	50	58
	200	5
 **6-acid**	50	0
	200	0

A test compound was mixed with the artificial diet at a concentration of 50 or 200 ppm. The diets containing compounds **6** and **6-acid** were administered for the first 48 hr to newly molted 3rd instar larvae. Nineteen to twenty individuals were used in each test.

Table 5. Effects of compounds on the growth of 5th instar larvae of *B. mori*

Compound	Average days required for the onset of cocoon spinning
control	8.0±1.2
methoprene	10.9±0.6
ETB	12.7±1.2
6	10.1±0.7
6S	14.3±1.4
6R	8.4±1.0

One microgram of each compound in acetone solution (1 μl) was topically applied to 24-hr-old 5th instar larvae. Days were counted from the day of ecdysis to 5th instar. Each value represents the means of 10 larvae with S.D.

instar larvae of *B. mori*, JH titers in hemolymph declined to an undectable level, and topical application of a JH agonist during this period delayed the initiation of the larval–pupal transformation.[15] When 1 μg of methoprene was topically applied to 24-hr-old 5th instar larvae, the onset of cocoon spinning was delayed by three days compared with the control period. Larvae treated with ETB started to spin cocoons approximately five days later than control larvae. Compound **6S** also caused a significant delay in the onset of cocoon spinning, while **6R** did not show any effect. The activity of the racemic mixture **6** was less than that of **6S**. These observations suggest that **6S** as well as ETB has some JH-like activity.

In conclusion, we found compounds **6** and **7** derived from ETB as a new class of potent anti-JH agents. These compounds induced precocious metamorphosis at considerably low doses; however, their activity decreased by increasing the applied doses, probably due to their JH-like activity. Although the mode of action of this new series of compounds is presently unknown, they are worthy of further investigation for the development of novel anti-JH agents which strongly induce precocious metamorphosis in proportion to the doses applied.

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