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**Note**

# 2-Methyl-5-pyridyl 2-(Substituted-phenoxy)-ethyl Ethers with Precocious Metamorphosis-Inducing Activity

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## INTRODUCTION

It has been known that juvenile hormone (JH) influences a wide range of physiological processes in both developing and mature insects.[1] JH is secreted throughout immature development and is critical for the regulation of metamorphosis. In addition, JH is required in the adult for several reproductive functions such as pheromone biosynthesis, ovarian development, maturation of eggs in females, and accessory reproductive gland development in males. Diapause in certain insects has also been shown to be regulated by JH. Therefore, an anti-JH agent, which suppresses such JH actions, could be an effective tool for studies on insect physiology as well as a potential insect growth regulator.[2] Although several anti-JH agents have so far been found such as precocenes, fluoromevalonate (FMev), dichloroallyl hexanoate, 1,5-disubstituted imidazoles, and ethyl 4-[2-(*tert*-butylcarbonyloxy)butoxy]benzoate (ETB), their activities were restricted to some insect species and were not sufficiently active for practical purpose.[2]

Precocious metamorphosis is a well-known symptom induced by allatectomy or anti-JH agents in immature insects, penultimate or earlier larval instars. ETB has been found to show JH-like activity as well as anti-JH activity for *Manduca sexta*[3] and *Bombyx mori*,[4] depending on the dose applied; low doses of ETB induced precocious metamorphosis, but at higher doses JH-like activity was observed. No other anti-JH agents with such action have been found to date. Edwards *et al*. have reported that application of ETB resulted in a true reduction of endogenous JH titers in *M. sexta* larvae and have proposed the existence of negative-feedback control of JH biosynthesis.[5] However, the exact mode of action of ETB is still unknown.

We have been interested in ETB as a lead compound for the development of a new anti-JH agent and have reported that ethyl 3-[2-(*tert*-butylcarbonyloxy)butoxy]benzoate (*meta*-ETB) induced precocious metamorphosis in the 3rd instar larvae of *B. mori* at both low and high doses.[6] In our continuing studies on ETB analogs, we have recently described that 2-(3-ethoxycarbonylphenoxy)ethyl 2-methyl-5-pyridyl ether (**1**), having a partial skeleton of *meta*-ETB, induced precocious metamorphosis against 3rd-instar larvae, although its activity was very low.[7] We then synthesized additional pyridyl ether analogs and found 2-(3-chlorophenoxy)ethyl 2-methyl-5-pyridyl ether (**2**) to be more active than compound **1**. This report describes the precocious metamorphosis-inducing activity of compound **2** and briefly discusses the structure-activity relationships.

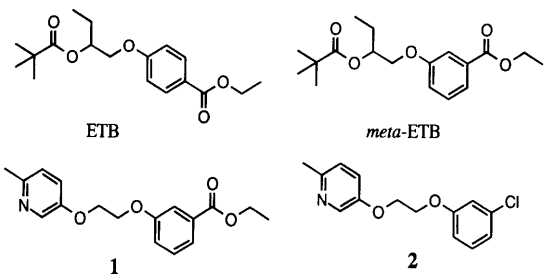


Fig. 1 ETB and related compounds.

## MATERIALS AND METHODS

### *1. Chemicals*

All pyridyl ethers were prepared by Williamson's ether synthesis method and the structures of the compounds were confirmed by $^1$H NMR spectra which were recorded on JEOL EX-400 spectrometer, using tetramethylsilane as an internal standard. The following procedure for the preparation of compound **2** is typical.

A mixture of 3-chlorophenol (2.00 g, 16 mmol) and 1,2-dibromoethane (3.51 g, 19 mmol) was refluxed for 1 hr, and then to this reaction mixture was added 1 N NaOH solution (20 ml, 16 mmol). After refluxing for 5 hr, the mixture was cooled to 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 (8 : 1) to give 2-(3-chlorophenoxy)ethyl bromide (2.20 g, 60%) as an oil. To a suspension of sodium hydride (0.48 g, 12 mmol) in DMF (20 ml) was added portionwise 2-methyl-5-hydroxypyridine (1.08 g, 9.9 mmol), and the mixture was stirred for 30 min at room temperature. The above bromide (2.20 g, 9.9 mmol) in DMF (20 ml) was added dropwise to the mixture. After stirring for 5 hr, to the mixture was added water (30 ml), and the resulting product was extracted with ether. Then the product was extracted with 1 N $H_2SO_4$ (20 ml) solution from the organic layer. After neutralizing the acidic aqueous solution with $K_2CO_3$, the product was extracted with ether again. The organic

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layer was dried over $Na_2SO_4$, and concentrated to give **2** (1.57 g, 60%) as a white solid, mp 64°C. $^1H$ NMR ($CDCl_3$) $\delta$: 2.50 (3H, s, $-CH_3$), 4.31–4.35 (4H, m, $-(CH_2)_2-$), 6.83–6.85 (1H, m, phenyl), 6.95–6.98 (2H, m, phenyl), 7.08 (1H, d, $J=8.8$ Hz, pyridyl), 7.16–7.24 (2H, m, pyridyl and phenyl), 8.25 (1H, d, $J=2.9$ Hz, pyridyl).

### 2. *Biological Evaluation*

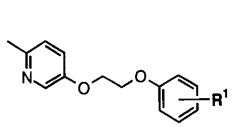
*B. mori* (Shunrei × Shougetsu strain) larvae were reared on artificial diet as previously reported.[8] Test compounds in acetone solution were applied topically to 3rd- or 4th-instar larvae. Compound **2** was mixed with the artificial diet at a concentration of 10, 50 and 200 ppm according to the procedure reported.[9] To newly molted 3rd- and 4th-instar larvae, the diet containing compound **2** was administered throughout 3rd- and 4th-larval period, respectively. 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. There was no induction of precocious metamorphosis in control larvae treated only with the acetone solvent.

## RESULTS AND DISCUSSION

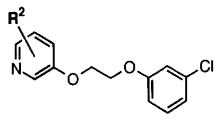
It has been previously reported that ETB induced precocious metamorphosis when applied to 3rd-instar larvae of *B. mori*. In this case, precocious pupation always occurred in the 4th (penultimate) larval stage. Treatment of 4th-instar larvae with ETB never induced precocious metamorphosis.[4] Therefore, we first examined the most sensitive time of the silkworm larvae used in this experiment for the treatment of ETB, *meta*-ETB and compound **2**. The compounds were topically applied to larvae every 24 hr from ecdysis to the 3rd-instar to 48 hr of the 4th-instar (Table 1). ETB at 10 $\mu$g effectively induced precocious pupation when applied to newly molted 3rd instar larvae, but at a high dose of 100 $\mu$g the activity disappeared. This result was in accord with the previous data that the activity of ETB to induce precocious metamorphosis did not correlate with the applied dose.[4,6] An application of ETB at 24 hr of the 3rd-instar or later ages caused no precocious pupation, indicating that the critical periods for ETB in the present strain was very narrow. *Meta*-ETB, which had induced precocious metamorphosis in the different strain (Gunpo x Shugyoku) of *B. mori*,[6] did not show any activity against this strain.

In contrast to ETB, compound **2** was the most effective when applied to 72 hr-old 3rd-instar larvae and showed activity at both 10 and 100 $\mu$g. Treatment of newly molted 3rd-instar larvae with 100 $\mu$g of compound **2** showed insecticidal activity but no mortality was found later than 24 hr of the 3rd-instar. An application of compound **2** till 24 hr of the 4th-instar caused some larvae to undergo precocious pupation. Consequently, there was a quite difference in critical times between ETB and compound **2** treatment for induction of precocious pupation. This result strongly indicates that the mode of action of compound **2** is different from that of ETB, although compound **2** has been led by modifying the structure of ETB.

In order to elucidate structure-activity relationship of 2-methyl-5-pyridyl 2-(substituted phenoxy)ethyl ethers, modifications were made in the substituent on the benzene ring and the pyridyl moiety. Compounds **3**–**8** (Fig. 2) at 100 $\mu$g resulted in less than 30% induction of precocious metamorphosis when applied to 72 hr-old 3rd-instar larvae, being less active than compound **2**. Therefore, the 2-(3-chlorophenoxy)ethyl group in compound **2** was fixed as a partial structure necessary for activity and the 2-methyl-5-



**3**: $R^1$ = 2-Cl **6**: $R^1$ = 3-Br
**4**: $R^1$ = 4-Cl **7**: $R^1$ = 3-$CF_3$
**5**: $R^1$ = 3-F **8**: $R^1$ = 3-$CH_3$



**9**: $R^2$ = H **12**: $R^2$ = 2-Cl
**10**: $R^2$ = 2-$CH_3$ **13**: $R^2$ = 5-Cl
**11**: $R^2$ = 6-$C_2H_5$ **14**: $R^2$ = 6-Cl

Fig. 2 2-(Substituted phenoxy)ethyl pyridyl ethers.

Table 1 Comparison of critical periods of compound **2**, ETB and *meta*-ETB treatment for induction of precocious metamorphosis in the 4th-instar of *B. mori*.

| Time of treatment (hr after ecdysis) | | Precocious metamorphosis (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Compound **2** | | ETB | | *meta*-ETB | |
| | | 10 | 100 | 10 | 100 | 10 | 100 ($\mu$g/larva) |
| 3rd-instar | 0 | 0 | —[a] | 35 | 0 | 0 | 0 |
| | 24 | NT | 10 | 0 | 0 | 0 | 0 |
| | 48 | NT | 25 | 0 | 0 | 0 | 0 |
| | 72 | 10 | 40[b] | 0 | 0 | NT | NT |
| 4th-instar | 0 | NT | 20 | 0 | 0 | 0 | 0 |
| | 24 | NT | 5 | 0 | 0 | NT | NT |
| | 48 | NT | 0 | 0 | 0 | NT | NT |

[a] 100% mortality. [b] At a separate experiment 40% precocious pupae were produced as well. NT : not tested.

Table 2 Precocious metamorphosis-inducing activity of compound **2** by dietary administration against 3rd- and 4th-instar larbae of *B. mori*.

| Treated larvae | Precocious metamorphosis (%) | | |
|---|---|---|---|
| | 10 | 50 | 200 (ppm) |
| 3rd-instar | 0 | 10 | 55 |
| 4th-instar | 5 | 10 | 60 |

pyridyl moiety was replaced with 3-pyridyl or various substituted pyridyl groups. None of the compounds **9**–**14** in Fig. 2 induced precocious pupation when applied to 72 hr-old 3rd-instar larvae at 100 $\mu$g, indicating that the 2-methyl-5-pyridyl moiety was apparently essential for activity.

Since compound **2** did not show enough activity by topical application, its activity was examined by dietary administration (Table 2). When the diet containing compound **2** was administered throughout the 3rd larval period, precocious pupation occurred in the 4th larval stage. Compound **2** showed the almost same activity when administered to 4th-instar larvae.

We have previously reported that 5-(substituted phenoxy) pentyl 3-pyridyl ethers induced precocious metamorphosis in larvae of the silkworm.[10] In this series of compounds, 5-(4-propylphenoxy)pentyl 3-pyridyl ether was the most effective, whereas the introduction of a methyl group at the 6-position of the pyridine ring, that is, 2-methyl-5-pyridyl 5-(4-propylphenoxy)pentyl ether, completely eliminated the activity. Interestingly, Solli *et al*. have found that 6,7-epoxy-3,7-dimethyloct-2-enyl 2-methyl-5-pyridyl ether and the 2-ethyl analog had outstanding JH activity against *Tenebrio molitor*.[11] However, there is no report in the literature of 2-methyl-5-pyridyl ethers inducing precocious metamorphosis. Although compound **2** did not exhibit strong precocious metamorphosis-inducing activity, this series of 2-methyl-5-pyridines is worthy of further investigation for development of new insect growth regulators.

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## 要　　約

### 早熟変態誘起活性を示す2-メチル-5-ピリジル2-(置換フェノキシ) エチルエーテル

金　仁恵，石黒英恵，桑野栄一

幼若ホルモン（JH）様活性とともに抗JH活性を示すことが知られているエチル4-[2-(*tert*-ブチルカルボニルオキシ) ブトキシ] ベンゾエート（ETB）の構造を改変して，カイコ幼虫に対して早熟変態を誘導する2-(3-クロロフェノキシ) エチル2-メチル-5-ピリジルエーテル（**2**）を見いだした．ベンゼン環上の3位の塩素をフッ素，臭素，トリフルオロメチル，メチル基等に換えると活性は低下した．2-メチル-5-ピリジル部位を3-ピリジル基や種々の置換ピリジル基に変換すると活性は消失したことから，2-メチル-5-ピリジル部位は活性発現に必須であった．ETBが3齢起蚕に投与した場合のみ早熟変態を誘導したのに対して，化合物**2**は3齢72時間目や4齢初期に施用しても活性を示したことから，ETBとは作用機構が異なることが示唆された．また，化合物**2**は摂食法でも早熟変態を誘導した．