エチル4-[(6-置換-2, 2-ジメチル-2H-クロメン-7-イル)メトキシ]ベンゾエート類の合成と抗幼若ホルモン活性

<table>
<thead>
<tr>
<th>誌名</th>
<th>Journal of pesticide science</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISSN</td>
<td>1348589X</td>
</tr>
<tr>
<td>著者</td>
<td>古田, 賢次郎</td>
</tr>
<tr>
<td></td>
<td>藤田, 雄大</td>
</tr>
<tr>
<td></td>
<td>飯伏, 翼</td>
</tr>
<tr>
<td></td>
<td>塩月, 孝博</td>
</tr>
<tr>
<td></td>
<td>山田, 直隆</td>
</tr>
<tr>
<td></td>
<td>桑野, 栄一</td>
</tr>
<tr>
<td>巻/号</td>
<td>35巻4号</td>
</tr>
<tr>
<td>掲載ページ</td>
<td>p. 405-411534</td>
</tr>
<tr>
<td>発行年月</td>
<td>2010年11月</td>
</tr>
</tbody>
</table>
Synthesis and anti-juvenile hormone activity of ethyl 4-[(6-substituted 2,2-dimethyl-2H-chromen-7-yl)methoxy]benzoates

Kenjiro Furuta, Norihiro Fujita, Tsubasa Ishushi, Takahiro Shiotsuki, Naotaka Yamada and Eiichi Kuwano

Original Article

Ethyl 4-[(6-substituted 2,2-dimethyl-2H-chromen-7-yl)methoxy]benzoates and their analogues were prepared and the biological activities were evaluated for both anti-juvenile hormone (anti-JH) and JH activity in silkworm larvae, Bombyx mori. Of the compounds tested, ethyl 4-[(6-methoxy-2,2-dimethyl-2H-chromen-7-yl)methoxy]benzoate (3b) showed the most effective precocious metamorphosis-inducing activity in 3rd instar larvae and JH activity in allatectomized 4th instar larvae. Furthermore, JH 1 and 20-hydroxyecdysone (20-E) titers in hemolymph of 3rd instar larvae treated with 3b were measured by liquid chromatography-mass spectrometry (LC-MS) and LC-MS/MS, respectively. The results revealed that compound 3b induced precocious metamorphosis by specifically decreasing JH 1 in hemolymph. © Pesticide Science Society of Japan

Key words: anti-juvenile hormone, juvenile hormone, 20-hydroxyecdysone, precocious metamorphosis.

Introduction

Juvenile hormones (JHs) are a class of regulatory sesquiterpenoids that play a crucial role in metamorphosis, diapause in immature insects and reproduction in adult insects. In the larval stage, molting and metamorphosis are regulated by JHs and ecdysteroid (molting hormone). JHs suppress adult differentiation and favor the maintenance of larval structures. A high titer of JHs must be present in the hemolymph for immature larvae to grow and pass through a series of larval molts induced by ecdysteroid secretion. Since JHs are involved in a wide range of physiological processes in insects, anti-JH agents, which interfere with the actions of JHs in insects, could be an effective tool for studies on insect physiology as well as a potential insect growth regulator (IGR). Many anti-JH agents, including precocenes, ethyl 4-[2-(tert-butylcarbonyloxy)butyloxy]benzoate (ETB) and brevioxime have been reported. We have also found that ethyl 4-(2-benzylhexyloxy)benzoate (KF-13), by modifying the structure of ETB, strongly induced precocious metamorphosis against silkworm larvae, Bombyx mori.

This study is an attempt to design and synthesize a new scaffold of anti-JH agents. We focused on the similarities in structures between JHs and retinoic acid. Interestingly, Palli and et al. have reported that two retinoic acid agonists showed weak JH activity in the black Manduca larval bioassay. A number of compounds having a 1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene ring are known as retinoid agonists or antagonists. Therefore, chromene derivatives having an oxygen atom in a 6-membered ring were designed on the basis of comparison of the structure of retinoids and JHs (Fig. 1), and their precocious metamorphosis-inducing activity against silkworm larvae was evaluated. In this paper, we described the preparation and structure-activity relationship of ethyl 4-

![Fig. 1. Design of the chromene derivatives 1 on the basis of retinoids and JH III.](image-url)
[(6-substituted 2,2-dimethyl-2H-chromen-7-yl)methoxy]benzoates (1) and related derivatives, and furthermore, the effect of a representative compound, ethyl 4-[(6-methoxy-2,2-dimethyl-2H-chromen-7-yl)methoxy]benzoate (3b), on JH I, the major JH in silkworm larvae, and 20-hydroxyecdysone (20-E) titers in hemolymph.

Materials and Methods

1. Instrumental Analysis and Chemicals

1H NMR spectra were determined with a JEOL EX-400 (400 MHz) spectrometer, using tetramethylsilane as an internal standard, and all samples were prepared in deuteriochloroform. Methoprene was kindly supplied by Earth Biochemical Co.

2.2-Dimethyl-2H-chromene-7-carbaldehyde (2a)

To a solution of 2-methyl-3-butyln-2-ol (1.0 g, 12 mmol) in acetonitrile (10 ml) was added DBU (2.71 g, 18 mmol) and trifluoroacetic anhydride (2.75 g, 18 mmol) at −5°C. After the mixture was stirred for 30 min at the same temperature, a prepared solution of 3-hydroxybenzaldehyde (1.45 g, 12 mmol), DBU (2.71 g, 18 mmol) and CuCl2 (10 mg) dissolved in acetonitrile (10 ml) at −5°C was added. Stiring was continued for 3 h at the same temperature, the product was extracted with ethyl acetate, and the extract was washed with 2 N aq. HCl, NaOH and brine, dried over Na2SO4 and concentrated. The residue was purified by column chromatography on silica gel by eluting with n-hexane – ethyl acetate (20:1) to give 0.12 g (6%; in two steps) of 2a as a colorless oil. 1H NMR (CDCl3) δ: 1.37 (3H, t, J=7.3 Hz, CH3), 1.44 (6H, s, CH2), 4.33 (2H, q, J=7.3 Hz, OCH2), 5.03 (2H, s, OCH2), 5.61 (1H, d, J=9.8 Hz, CH), 6.33 (1H, d, J=9.8 Hz, CH), 6.87 (1H, s, phenyl), 6.89 (1H, d, J=7.8 Hz, phenyl), 6.95 (2H, d, J=8.8 Hz, phenyl), 7.99 (2H, d, J=8.8 Hz, phenyl). Anal. Found: C, 74.54; H, 6.55%. Calcd. for C13H12O2: C, 74.15; H, 6.54%.

Compounds 3b–e were prepared in the same manner as described above starting from the corresponding 6-alkoxychromenes.

Ethyl 4-[(6-methoxy-2,2-dimethyl-2H-chromen-7-yl)methoxy]benzoate (3a)

To a solution of 2a (0.12 g, 0.64 mmol) in methanol (10 ml) was added NaBH4 (0.02 g, 0.64 mmol) at room temperature. After stirring for 1 h, the product was extracted with ethyl acetate and the extract was washed with water and brine, dried over Na2SO4 and concentrated to give 0.12 g of crude 2,2-dimethyl-2H-chromene-7-methanol.

To a solution of the above alcohol (0.12 g) in THF (5 ml) was added Ph3P (0.25 g, 0.95 mmol), 40% diisopropyl azodicarboxylate (0.48 g, 0.95 mmol) dissolved in toluene and ethyl p-hydroxybenzoate (0.13 g, 0.95 mmol) at 0°C. After stirring for 16 h at room temperature, the product was extracted with ethyl acetate. The extract was washed with 2 N aq. NaOH and brine, dried over Na2SO4 and concentrated. The residue was purified by column chromatography on silica gel eluting with n-hexane – ethyl acetate (20:1) to give 0.02 g (9%) of 3a as a colorless oil. 1H NMR (CDCl3) δ: 1.37 (3H, t, J=7.3 Hz, CH3), 1.44 (6H, s, CH2), 4.33 (2H, q, J=7.3 Hz, OCH2), 5.03 (2H, s, OCH2), 5.61 (1H, d, J=9.8 Hz, CH), 6.33 (1H, d, J=9.8 Hz, CH), 6.87 (1H, s, phenyl), 6.89 (1H, d, J=7.8 Hz, phenyl), 6.95 (2H, d, J=8.8 Hz, phenyl), 7.99 (2H, d, J=8.8 Hz, phenyl). Anal. Found: C, 74.54; H, 6.55%. Calcd. for C13H12O2: C, 74.15; H, 6.54%.

Compounds 3b–e were prepared in the same manner as described above starting from the corresponding 6-alkoxychromenes.
Ethyl 4-[(6-benzyloxy-2,2-dimethyl-2H-chromene-7-yl)methoxy]benzoate (3e)

Yield: 12% in two steps; ¹H NMR (CDCl₃) δ: 1.36 (3H, t, J=7.3 Hz, CH₃), 1.41 (6H, s, CH₃), 4.33 (2H, q, J=7.3 Hz, OCH₂), 5.06 (2H, s, OCH₂), 5.13 (2H, s, OCH₃), 5.62 (1H, d, J=9.8 Hz, CH), 6.27 (1H, d, J=9.8 Hz, CH), 6.62 (1H, s, phenyl), 6.91 (1H, s, phenyl), 6.97 (2H, d, J=8.8 Hz, phenyl), 7.31–7.40 (5H, m, phenyl), 7.96 (2H, d, J=8.8 Hz, phenyl). Anal. Found: C, 75.29; H, 6.35%. Calcd. for C₂₉H₂₅O₃C: 75.65; H, 6.35%.

Ethyl 4-[(6-methoxy-2,2-dimethylchroman-7-yl)methoxy]benzoate (4)

A solution of 6-methoxy-2,2-dimethyl-2H-chromene-7-methanol (0.15 g, 0.68 mmol) in methanol (5 ml) containing Pd/C (0.01 g) was stirred for 16 h at room temperature under a hydrogen atmosphere. After filtration, the product was extracted with ethyl acetate. The extract was washed with 2 N aq. NaOH and concentrated. The residue was purified by column chromatography on silica gel by eluting with n-hexane–ethyl acetate (7:1) to give 1.05 g (78% in two steps) of 6a as a pale yellow oil. ¹H NMR (CDCl₃) δ: 3.84 (3H, s, OCH₃), 4.51–4.24 (2H, m, OCH₂), 4.31–4.33 (2H, m, OCH₂), 6.46 (1H, s, phenyl), 7.36 (1H, s, phenyl), 10.27 (1H, s, CHO).

Compounds 6b and 6c were similarly prepared by a similar method starting from the corresponding phenol.

3-Methoxy-5,6,7,8-tetrahydronaphthalene-2-carbaldehyde (6b)

Yield: 7% in two steps; ¹H NMR (CDCl₃) δ: 1.74–1.80 (4H, m, CH₂), 2.66–2.74 (4H, m, CH₂), 3.88 (3H, s, OCH₃), 6.66 (1H, s, naphthyl), 7.26 (1H, s, naphthyl), 10.40 (1H, s, CHO).

2,4,5-trimethoxybenzaldehyde (6c)

Yield: 80% in two steps; ¹H NMR (CDCl₃) δ: 3.98 (3H, s, OCH₃), 3.93 (3H, s, OCH₃), 3.98 (3H, s, OCH₃), 6.50 (1H, s, phenyl), 7.73 (1H, s, phenyl), 10.30 (1H, s, CHO).

Compounds 7a–c were prepared in the same manner as described above for the preparation of compound 3a.

Ethyl 4-[(7-methoxy-1,4-benzodioxan-6-yl)methoxy]benzoate (7a)

Yield: 27% in two steps; ¹H NMR (CDCl₃) δ: 1.38 (3H, t, J=7.3 Hz, CH₃), 4.19–4.25 (4H, m, OCH₂), 4.34 (2H, q, J=7.3 Hz, OCH₂), 5.03 (2H, s, OCH₂), 5.10 (2H, s, OCH₂), 6.52 (1H, s, phenyl), 6.95 (1H, s, phenyl), 6.97 (2H, d, J=8.8 Hz, phenyl), 7.36–7.42 (5H, m, phenyl), 7.97 (2H, d, J=8.8 Hz, phenyl). Anal. Found: C, 66.07; H, 5.92%. Calcd. for C₂₉H₂₅O₃C: 66.27; H, 5.85%.

Ethyl 4-[(3-methoxy-5,6,7,8-tetrahydronaphth-2-yl)methoxy]benzoate (7b)

Yield: 36% in two steps; ¹H NMR (CDCl₃) δ: 1.38 (3H, t, J=7.3 Hz, CH₃), 1.77–1.79 (4H, m, CH₂), 2.68–2.76 (2H, m, CH₂), 2.77–2.79 (2H, m, CH₂), 3.82 (3H, s, OCH₃), 4.34 (2H, q, J=7.3 Hz, OCH₂), 5.09 (2H, s, OCH₂), 6.68 (1H, s, naphthyl), 7.05 (2H, d, J=8.8 Hz, phenyl), 7.07 (1H, s, naphthyl), 8.01 (2H, d, J=8.8 Hz, phenyl). Anal. Found: C, 73.88; H, 7.25%. Calcd. for C₂₉H₂₅O₃C: 74.09; H, 7.11%.

Ethyl 4-[(2,4,5-trimethoxybenzyl)oxy]benzoate (7c)

Yield: 12% in two steps; ¹H NMR (CDCl₃) δ: 1.38 (3H, t, J=7.3 Hz, CH₃), 3.84 (3H, s, OCH₃), 3.86 (3H, s, OCH₃), 3.91 (3H, s, OCH₃), 4.34 (2H, q, J=7.3 Hz, OCH₂), 5.10 (2H, s, OCH₂), 6.56 (1H, s, phenyl), 6.97 (1H, s, phenyl), 7.00 (2H, d, J=8.8 Hz, phenyl), 7.99 (2H, d, J=8.8 Hz, phenyl). Anal. Found: C, 65.39; H, 6.45%. Calcd. for C₂₉H₂₅O₃C: 65.88; H, 6.40%.

2. Biological Evaluation

B. norti (Shunrei×Shougetsu) larvae were reared on an artifi-
cial diet. 7) The anti-JH activity of compounds was evaluated by the induction of precocious metamorphosis when topically applied to the dorsal abdomen of 24-hr-old 3rd instar larvae. The JH activity of compounds was evaluated by molting into normal 5th instars when topically applied to allatectomized 24-hr-old 4th instar larvae. 8)

3. Quantification of JH and 20-E in hemolymph

3.1. Reagents and glassware

JH I and II were purchased from SciTech (Prague, Czech Republic) and JH III was from Sigma-Aldrich (St. Louis, MO), respectively, and purified by LC-MS with a silica gel column (SG-80, 10×250 mm; Shiseido, Tokyo, Japan) using 10% diethyl ether in n-hexane as the normal phase. Deuterium-labeled JH III-d3 was used as the internal standard. The LC-MS/MS method reported previously was employed for the preparation according to the previous report. 9) 20-Hydroxyecdysone and ponasterone A were purchased from Sigma-Aldrich.

All solvents used were of residual pesticide analysis grade (5000-fold concentrate guaranteed; Wako Pure Chemicals, Osaka, Japan). All glassware was rinsed sequentially with distilled water, acetone and n-hexane, and heated overnight at 50°C before use.

3.2. Collection of hemolymph samples

Ten micrograms of compound 3b in acetone solution (1 µl) was applied topically to 24-hr-old 3rd instar of B. mori larvae. An abdominal leg was cut and hemolymph was collected in microtubes containing approx. 5% (w/v) phenylthiourea and 1 mM EDTA every 24 hr. Samples were stored at −80°C until used.

3.3. Purification of JH I from hemolymph and analytical conditions for LC-MS

Clean-up was carried out by slightly modifying the method described for JH quantification using GC-MS. 10) Briefly, 10 µl of JH III-d3 in toluene (67.1 pg/µl) as an internal standard was transferred into a clean glass tube, and 0.5 ml methanol was added. To this solution was added the hemolymph (100 µl), the mixture was vortexed vigorously, and 1.5 ml of 2% NaCl solution was added to the sample solution. JHs were extracted by the partition of n-hexane (0.5 ml) three times. The combined extracts (1.5 ml) were purified using a Pasteur pipette packed with 1.0 g aluminum oxide (activity grade III; ICN Biomed, Cleveland, OH) prewashed with n-hexane. After loading the extract, JH I was eluted with 2 ml of 50% ether in n-hexane and then the solvent was removed under a stream of nitrogen. The residue was dissolved in acetonitrile (25 µl).

The Applied Biosystems API2000 LC-MS system was used. The analytical condition was optimized referring to the method reported previously. 11) The samples were separated on a 3×150 mm C18 reversed phase column (UG80; Shiseido) with 70% acetonitrile containing the appropriate sodium salt at a flow rate of 0.4 ml/min. For MS analysis, electrospray ionization (ESI) in the positive mode was used under the conditions of drying gas temperature 400°C with 10 l/min flow rate and 15 V declustering potential (DP). Selected ion masses (SIM) for each JH were monitored as [M+Na]+, that is, m/z 292 and 317 for JH III-d3, and JH I, respectively.

3.4. Purification of 20-E from hemolymph and analytical conditions for LC-MS/MS

Ponasterone A (5 ng/µl) in 10 µl methanol as an internal standard was transferred into a microtube, and 300 µl methanol was added. To this solution was added the hemolymph (20 µl), and the mixture was vortexed vigorously and centrifuged at 13000 rpm for 10 min. The supernatant was collected in new microtubes, and the pellet was re-extracted with 500 µl methanol. The combined methanol extracts (600 µl) were evaporated to dryness under a vacuum. The residue was dissolved in 100 µl of 80% methanol in water.

The Applied Biosystems API2000 LC-MS/MS system equipped with a 3×150 mm C18 reversed phase column (MGB80; Shiseido) was used. The solvents were deionized water containing 0.01% formic acid (solvent A) and acetonitrile. 12) A linear solvent gradient was used starting from 5% acetonitrile in solvent A to 100% acetonitrile over 15 min, followed by elution with 100% acetonitrile for 5 min at a flow rate of 0.3 ml/min. For MS/MS analysis, ESI in the positive mode with nitrogen as collision gas was used under the conditions of drying gas temperature 500°C with 10 l/min flow rate. Then, 20-E and ponasterone A were detected with multiple reaction monitoring (MRM) transition; m/z 481 [M+H]+ → 445 [M+H−2H2O]+ (CE: 66 V, collision energy (CE): 23 V) and m/z 465 [M+H]+ → 429 [M+H−2H2O]+ (DP: 61 V, CE: 23 V), respectively.

Results and Discussion

1. Synthesis

The synthesis of a series of ethyl 4-[(6-substituted 2,2-dimethyl-2H-chromen-7-yl)methoxy]benzoates and related derivatives is outlined in Fig. 2(A) and (B). Chromone 2a was prepared via alkylation of 3-hydroxybenzaldehyde with 2-methyl-3-butyraldoxanhydride in the presence of DBU and CuCl2, and cyclization under heating in N,N-dimethyl acetamide at 180°C. 10) 6-Substituted chromone (I) was obtained by the Wismies reaction, and aldehydes 2b-e were yielded as approximately 5:1 mixtures of 7-CHO and 5-CHO isomers. In the case of phenols 5a-c, formylation 13) was carried out via the magnesium chloride salt of the phenol to yield the o-hydroxyaldehyde exclusively, which was treated with methyl iodide to afford aldehydes 6a-c. Reduction of the aldehyde group in 2a-e and 6a-e with NaBH4, followed by the Mitsunobu reaction with ethyl p-hydroxybenzoate gave compounds 3a-e and 7a-c, respectively.

2. Anti-JH activity of chromenes and related compounds

Table 1 shows the precocious metamorphosis-inducing activity of ethyl 4-[(6-substituted 2,2-dimethyl-2H-chromen-7-yl)methoxy]benzoates and related compounds against 3rd instar larvae of B. mori. Compound 3a showed weak precocious metamorphosis-inducing activity. Introduction of a methoxy
group at 6-position on the chromene ring (3b) led to the marked increase of anti-JH activity, showing 100% induction of precocious metamorphosis at 40 μg; however, when a longer alkoxy substituent, such as an ethoxy (3c) and a n-propoxy (3d) group, was introduced, their anti-JH activity was markedly decreased. Interestingly, the benzyloxy analog 3e exhibited almost the same level of activity as 3b, suggesting that the size of the substituent of C-6 on the chromene ring was significantly not a critical factor for anti-JH activity.

Precocene II possessing a 2,2-dimethyl-2H-chromene skeleton was the first compound to show anti-JH activity. Concerning its mode of action, the double bond of the chromene ring is readily oxidized by P450 in the corpora allata to the epoxide intermediate, a powerful alkylating property, which reacts with cellular macromolecular constituents to destroy the cells of the corpora allata. Therefore, in precocene II, the double bond is indispensable for anti-JH activity. To examine whether the double bond in the chromene ring of 3b was essential for anti-JH activity, the chroman derivative 4 was synthesized. Chroman 4 showed slightly lower anti-JH activity than the corresponding chromene 3b, suggesting that the mode of action of these derivatives is different from that of precocene II. Benzodioxan derivative 7a showed somewhat lower activity than those of 3b and 4. On the other hand, the anti-JH activity of tetraline derivative 7b was markedly decreased, and 4,5-dimethoxy derivative 7c did not show any activity. These results indicate that the ring structure containing an oxygen atom adjacent to the benzene ring is significant for anti-JH activity.

3. JH activity of chromene and chroman derivatives

Table 2 shows the JH activity of methoprene, chromene 3b and chroman 4 against allatectomized 4th instar larvae. All allatectomized and acetone-treated control larvae underwent...
precocious metamorphosis. Compounds 3b and 4 as well as methoprene completely prevented precocious metamorphosis at a high dose of 40 μg so that all treated larvae molted into 5th larvae. The JH activity of compound 3b at 1 μg was lower than that of methoprene. Thus, in analogy with the ethyl 4-(2-benzylalkyloxy)benzoate series,4 these compounds were found to show both anti-JH and JH activity.

4. Changes in JH 1 and 20-E titers in hemolymph after topical application of compound 3b

The JH I titer in hemolymph of larvae treated with 10 μg compound 3b was determined by LC-MS from the day when treated to 3rd instar larvae through pupation (Fig. 3A). In controls, the JH I titer decreased just before ecdysis to 4th instar, and sharply increased to as high as 4.60±2.02 ng/ml after ecdysis. In contrast to the results obtained by radioimmunoassay,16 no JH I was observed in the early stage of 5th larvae. Topical application of compound 3b caused a marked drop in the JH I titer within 24 hr after treatment and JH I was not detected (<0.1 ng/ml) prior to precocious metamorphosis at the end of the 4th instar, but just before pupation, an increase in JH 1 levels (1.68±0.50 ng/ml) was observed. Although, in this case, the JH I titer had already decreased in the hemolymph of 3rd-instar larvae, precocious metamorphosis always occurred in the 4th stadium, suggesting that anti-JH activity of compound 3b was counteracted by JH activity of 3b itself, present in high concentration during the 3rd stadium.

As previously described, precocious metamorphosis is well known to be induced by JH deficiency in the larval stage; 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-E, suggesting that a temporary decline of ecdysteroid titers in the larval hemolymph might also cause precocious metamorphosis.17 We therefore determined the 20-E titer in hemolymph by LC-MS/MS. Compound 3b had no significant effect on the 20-E titer during the 3rd stadium (Fig. 3B). Furthermore, the 20-E titer in 4th instar larvae treated with compound 3b showed a profile very similar to that in control 5th instar larvae before pupation. These results indicate that compound 3b induced precocious metamorphosis by decreasing

---

### Table 1. Precocious metamorphosis-inducing activity of ethyl 4-[(6-substituted 2,2-dimethyl-2H-chromen-7-y)methoxy]benzoates and related compounds against 3rd instar larvae of B. mori.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Precocious metamorphosis&lt;sup&gt;4&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>H</td>
<td>0</td>
</tr>
<tr>
<td>3b</td>
<td>OMe</td>
<td>75</td>
</tr>
<tr>
<td>3c</td>
<td>OEt</td>
<td>0</td>
</tr>
<tr>
<td>3d</td>
<td>O(P-n)</td>
<td>0</td>
</tr>
<tr>
<td>3e</td>
<td>OBn</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>46</td>
</tr>
<tr>
<td>7a</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>7b</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>7c</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>4</sup> Values are the average of two experiments.

---

### Table 2. Effects of methoprene, 3b and 4 on the development of allatectomized 4th instar larvae of B. mori.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (μg/larva)</th>
<th>Precocious pupa</th>
<th>Larval-pupa intermediates</th>
<th>5th instar larva</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allatectomized</td>
<td></td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>+ methoprene</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>+ 3b&lt;sup&gt;4&lt;/sup&gt;</td>
<td>10</td>
<td>2</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>+ 4</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

<sup>4</sup> Number of larvae tested: 10.
JH levels in hemolymph of 3rd and 4th instar larvae. 

In conclusion, we found that ethyl 4-[(6-methoxy-2,2-dimethyl-2H-chromen-7-yl)methoxy]benzoate (3b) was a structurally novel anti-JH agent. Although compound 3b showed JH activity as well as anti-JH activity, it clearly decreased the JH I titer in hemolymph. Compound 3b is therefore a reasonable lead compound for the generation of genuine anti-JH agents showing no JH activity.

Acknowledgments

We are grateful to Prof. Hiromichi Yoshikawa for kindly allowing us the use of the Applied Biosystems API2000. We also thank Ms. Miho Nakamura and Mr. Seiji Nishikawa for technical support. This work was supported by a grant-in-aid to E.K. from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References

報 文

エチル4-[(6-置換-2,2-ジメチル-2H-クロラメン-7-イル)メトキシ]ペンゾエート類の合成と抗幼若ホルモン活性

古田賢次郎、藤田幸太、松村文男、塩月利豊、山田直隆、桑野栄一

幼若ホルモン（JH）とレチノイドの構造を基にして、数種のエチル4-[(6-置換-2,2-ジメチル-2H-クロラメン-7-イル)メトキシ]ペンゾエート類およびその誘導体を合成し、カイコ幼虫に対する抗-JH と JH 活性を調べた。その結果、エチル4-[(6-メトキシ-2,2-ジメチル-2H-クロラメン-7-イル)メトキシ]ペンゾエート（3b）が、カイコ3齢幼虫に対して高い早熟変態誘導活性を示すだけでなく、アラタ体を除去した4齢幼虫に対しては、JH 活性を示すことを見出した。また、化合物3bを3齢幼虫に処理した際の体液中におけるJH1および20-ハイドロキシンフェチソノン（20-E）の濃度変化を調べたところ、JH1は処理後24時間以内に急速に減少し、早熟変態が起きる直前まで検出されなかったのに対して、3齢期の20-E濃度はほとんど変化しないことが明らかになった。

イネのジテルペン型ファイトアレキシン生産に対するサイトカイニンの影響

高 光旭、関田憲文、古賀隆一郎、

ファイトアレキシン生産は、病原菌感染に対するイネの代謝的な防御応答の一つである。本報では、イネの主要なジテルペン型ファイトアレキシンであるファイトカシノ類とモリラクトン類の生産に対するサイトカイニンの関与を検証する。ペンジルアデニン（BA）処理後のイネ培養細胞におけるファイトアレキシン生産を調べた。その結果、BA処理により各種ファイトアレキシンの生産誘導、および非合成酵素遺伝子群の発現誘導が観察された。またイネ植物体を用いた場合でも同様の結果が得られた。イネいもち病菌が感染したイネにおいてはサイトカイニンの産生量が増加していくことが示されていることを考え合わせると以上の結果は、ファイトアレキシン生産誘導に至るシグナル伝達系において、サイトカイニンがシグナル分子として機能している可能性を示唆するものと考えられる。

メトカナゾールに対する日本産実験材料発病菌（Fusarium graminearum 種複合体）の感受性

堅石秀明、三宅泰司、森 静、木村理恵、佐久間美子、賀屋信英

日本産実験材料発病菌（Fusarium graminearum 種複合体）のメトカナゾールに対する MIC 値は 0.20~6.25 mg/l の間に分布し、1.56 mg/l を頂点とする一価の分布を示した。また、PCR 法により F. asiaticum、F. graminearum s. str. の同定や、毒素生産性の判別を行って感受性との関連を調べた。菌の菌種と地域的分布、種ごとの毒素生産性に特徴が見出されなかったが、菌種および異なる毒素生産性株群で MIC は一定の範囲内に収まり、感受性に大きな差はないと思われた。感受性が異なる株の CYP51 遺伝子を PCR により增幅し、塩基配列を比較した結果、感受性の差は塩基配列である CYP51 遺伝子の変異によるものではない可能性が示唆された。感受性の異なる株を接種し、防除効果を調べたところ、メトカナゾールはいずれの感受性の菌株に対しても高い防除効果を示した。

植物病原菌に対するインドール誘導体の抗菌活性

Ahmed S. Abdel-Aty

インドール-3-酢酸とインドール-3-酢酸ならびにインドール誘導体を6種類用いて、真菌の Fusarium culmorum、Pythium debaryanum、Rhizoctonia solani および Macrosporina phaseoli に対する抗菌活性をインビット法で調べた。抗菌活性はテストに使用された真菌およびインドール誘導体の濃度の両方に影響された。それぞれの 50% 阻害濃度（IC_{50}) を計算し、構造活性相関（SAR）を解明した。2-フェニールインドールと1-アセチルインドール-3-酢酸は明確な抗菌活性を示したため、インビットでポリフェノールオキシダーゼ（PPO）、ポリフェノールオキシダーゼ（PO）、DNA、RNA および糖成分に対する効果を調べた。R. solani のポリフェノールオキシダーゼは、2-フェニールインドールに反応し、IC_{50} 値は 80.3 g/ml であった。また、1-アセチルインドール-3-酢酸は2-フェニールインドールよりも活性が高く、F. culmorum と M. phaseoli に対する IC_{50} 値はそれぞれ 41.5 と 80.2 g/ml である。2-フェニールインドールの 87.6 と 117.1 g/ml よりも高かった。P. debaryanum においては、ポリフェノールオキシダーゼは1-アセチルインドール-3-酢酸に阻害され、IC_{50} 値は 45.6 g/ml であった。テストされた化合物のポリフェノールオキシダーゼに対する活性は試験に使用された真菌におけるそれぞれ