J. Pestic. Sci. **48**(3), 99–106 (2023)
DOI: 10.1584/jpestics.D23-010

JOURNAL OF PESTICIDE SCIENCE

Regular Article

Insect growth-regulating activity of 1-benzyl-2-methylbenzimidazole derivatives on silkworms

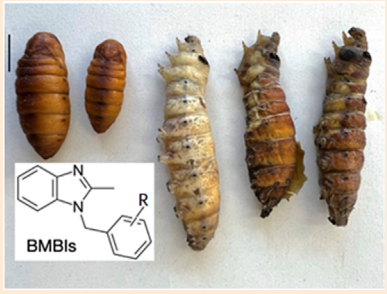
Shuhei Henmi,[1] Ayumu Sueyoshi,[1] Hiroshi Ono,[2] Toshimasa Yamazaki,[2] Konatsu Inoue[3] and Takahiro Shiotsuki[1,3,*]

[1] *Graduate School of Natural Science and Technology, Shimane University, Matsue, Shimane 690–8504, Japan*
[2] *Research Center for Advanced Analysis, National Agriculture and Food Research Organization, Tsukuba, Ibaraki 305–8642, Japan*
[3] *Department of Life Science and Biotechnology, Faculty of Life and Environmental Science, Shimane University, Matsue, Shimane 690–8504, Japan*

(Received February 28, 2023; Accepted June 16, 2023)

Supplementary material

Derivatives of 1-benzyl-2-methylbenzimidazoles (BMBIs) were synthesized to evaluate their biological activities against *Bombyx mori*, a lepidopteran model insect. Synthesized BMBIs exhibited two different biological activities: inhibition of development and acute lethality. From a structural perspective, the activity varied with the position of the substitutions on the 1-benzyl moiety; BMBIs with substitutions on the 2 and/or 4 positions had comparatively high activity in comparison with those with substitutions on the 3-position. There was more activity for the inhibition of development with low doses, and more for acute lethality with high doses. The activity was also affected by the applied stage, that is, application in the 4th instar mostly interfered the larval molting or pupation, whereas that in the 3rd instar caused more acute mortality. Taken together, these results suggest that BMBIs have multiple modes of action.



Keywords: insect growth regulator, juvenile hormone, molting, metamorphosis, Lepidoptera.

Introduction

Lepidoptera includes numerous pest insects practicing agriculture and horticulture, such as diamondback moth (*Plutella xylostella*),[1)] common cutworm (*Spodoptera litura*),[2)] cabbage armyworm (*Mamestra brassicae*),[3)] and tobacco hornworm (*Manduca sexta*).[4)] Insecticides are essential agrochemicals for controlling pest insects; however, resistance has developed by the continued application of insecticides with similar mode of action.[5)] One of the strategies to manage emerging resistant insects is to discover novel insecticides with different modes of action from those of the commonly used insecticides.

Insect growth regulators (IGRs) are promising candidates for this goal because they are specific towards insects. They rely on a mode of action based on affecting the insect endocrine system that is different from the major insecticides targeting the insect nervous system.[6)] Post-embryonic development of insects is regulated by two key hormones, juvenile hormone (JH)[7)] and 20-hydroxyecdysone (20E).[8)] Currently, some IGRs are commonly used as insecticides: JH mimics (IRAC code 7), ecdysone receptor agonists (code 18), and chitin biosynthesis inhibitors (IRAC codes 15 and 16). In addition, some imidazole derivatives have been reported to exhibit IGR activity in Lepidoptera larvae as candidates for novel insecticides. For example, 1-benzyl-5-[(*E*)-2,6-dimethyl-1,5-heptadienyl]imidazole (KK-42) induces precocious metamorphosis in silkworms[9,10)] and it breaks the diapause of Japanese oak silkworm at embryonic stage.[11)] 1-[3-(4-phenoxyphenoxy)propyl]-imidazole (KS-175) inhibits the biosynthesis of ecdysteroids in the prothoracic gland to inhibit molting or pupation.[12)] Furthermore, 1-(3,7-dimethyl-7-ethoxy-2-octenyl)-2-methylbenzimidazole (B-1) possesses larvicidal activity that inhibits cuticular synthe-

* To whom correspondence should be addressed.
E-mail: shiotsuk@life.shimane-u.ac.jp
Published online August 19, 2023

Abbreviations: JH, juvenile hormone; IGR, insect growth regulator; JHBP, juvenile hormone binding protein.

sis.[13] Therefore, the imidazole derivatives are thought to be promising candidates for the development of novel IGRs.

In this study, we synthesized 1-(substituted benzyl)-2-methylbenzimidazoles (BMBIs) to evaluate their activity of acute lethality and inhibition of development in the model insect *Bombyx mori*. We have also shed light on the relationship between the structure of the substituents and biological activity to deduce their modes of action.

Materials and methods

1. Chemicals

The synthetic pathway of BMBIs is shown in Scheme 1. When using benzoic acid or benzaldehyde derivatives to construct benzyl moiety of 1-substituted benzyl-2-methylbenzimidazole, these precursors are reduced using $LiAlH_4$ or $NaBH_4$ and prepared benzyl alcohol derivatives followed by Appel reaction to synthesis benzyl bromide derivatives. Subsequently, these benzyl bromide derivatives were introduced to 2-methylbenzimidazole using S_N2 reactions and yielded 1-benzyl-2-methylbenzimidazole derivatives. When derivatives of benzyl halides were commercially available, the target compounds were synthesized using reactions after these reductions and halogen exchange reactions described above. After extraction with ethyl acetate (EtOAc) following by the dehydration using Na_2SO_4, the crude was purified by silica gel chromatography after each reaction.

1-Benzyl-2-methylbenzimidazole (**1**) was prepared based on procedure B (Scheme 1), 1-benzyl-2-methylbenzimidazole (**1**) was prepared from 2-methylbenzimidazole (0.19 g, 1.4 mmol), NaH (0.06 g, 2.8 mmol; 60% oil suspension) and benzyl chloride (0.20 g, 1.0 mmol). Extraction and washing and purification by silica gel chromatography (19 cm×20 mm chromatographic-tube, *n*-hexane : EtOAc = 1 : 3, v/v) yielded **1** as a yellowish oil (0.24 g, 91%). m.p. 65.5–68.6°C. ^{1}H NMR δ_H ($CDCl_3$): 7.08–7.73 (m, 9H), 5.28 (s, 2H), 2.56 (s, 3H). HRMS *m*/*z* ($[M+H]^+$): Calcd for $C_{15}H_{15}N_2$: 223.1230; Found, 223.1230. The related compounds were prepared in similar procedure as described in Supplement.

Chemical structures were confirmed by ^{1}H-NMR using JEOL JNM A-400 FT NMR spectrophotometer (400 MHz) with tetramethyl silane as an internal standard. High resolution mass spectrometry was conducted with LTQ Orbitrap Velos Pro hybrid mass spectrometer. Melting points were measured with a Yanaco MP-500D melting point apparatus and uncorrected.

KK-42 and methoprene were given by Prof. Kuwano at Kyushu University as gifts.

2. Evaluation of biological activity on silkworms

Bombyx mori (Shunrei×Shougetsu) larvae were reared on Silkmate 2S (Nippon-nosan Kogyo, Japan) under a photoperiodic regime of 12 hr of light and 12 hr of darkness at 25°C.

Each compound in acetone (1 μL) was topically applied to the skin on the dorsal thorax of 1 day-old 3rd or 4th instar larvae. The same volume of acetone was administered as a control. The timing of death, molt, and spinning, and phenotypes were recorded and observed.

The corrected mortality was calculated using the formula shown below.

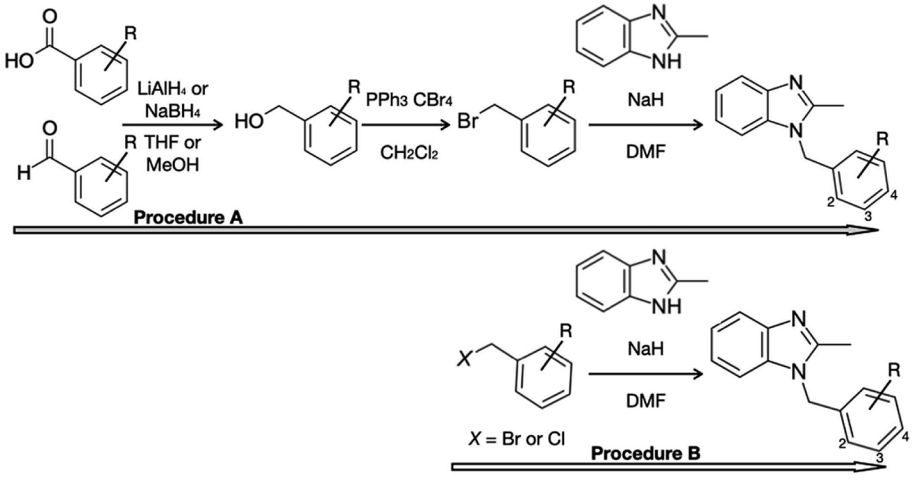
$$\text{Corrected mortality}\,(\%) = (\text{Mt} - \text{Mc})/(100 - \text{Mc}) \times 100$$

where Mc is mortality of the control and Mt is the mortality by each tested chemical. Origin Pro (Light Stone) was used to calculate the median lethal dose (LD_{50}). Excel (Microsoft) was used to conduct multiple comparison, ANOVA test, *F*-test, and *t*-test. The details of statistics are described in the legend of each figure.

Results

1. Analysis of biological activities of BMBIs

The application of BMBIs to silkworm caused multiple outcomes, such as acute larvicidal effect and growth-inhibition affecting the molting and metamorphosis of insects. The visual appearance of larvae after the application differed depending on the structural properties and dosage of the BMBIs, in addition to the developmental stages when the compounds were applied.



Scheme 1. General procedure for the preparation of BMBI derivatives

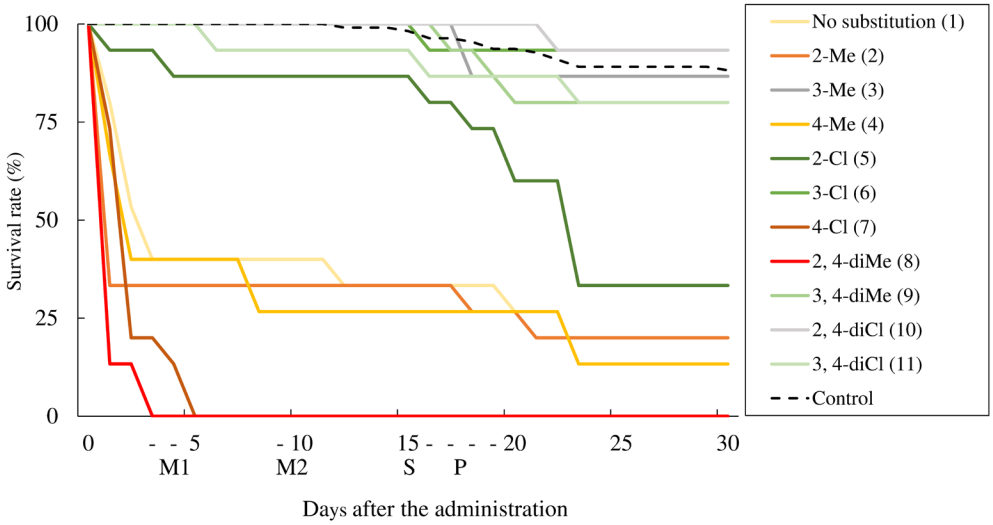


Fig. 1. Survival curves after 10 μg/larva BMBIs were applied to the 3rd instar larvae. The survival rate was followed for 30 days after the application. Each derivative was applied to 15 larvae, except that 110 larvae were used as control. The following letters indicated below the horizontal axis represent the time of the shift of developmental stages of control individuals, M1: molting 3rd to 4th instar, M2: molting 4th to 5th instar, S: spinning, and P: pupation.

Figure 1 shows the survival curves observed, after the application of 10 μg/larvae of each BMBI (**1**–**13**) to the 3rd instar larvae on day 1. The period when the population declines were prominent was within 48 hr after administration and the timing of molting and pupation. The BMBIs caused acute death and mortal growth inhibition. Acute toxicity did not lead to notable convulsions or muscle contractions (Fig. 2, B). In contrast, BMBIs caused multiple phenotypes resulting from growth inhibition,

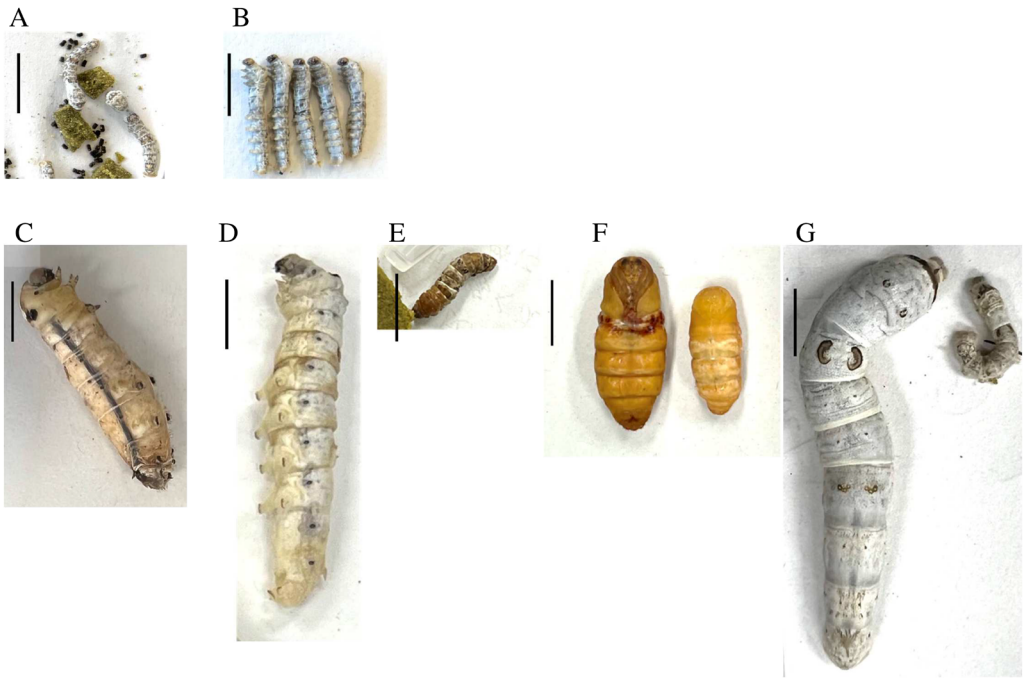


Fig. 2. Typical appearance of individuals affected by administration of BMBIs to 3rd day 1 instar. The bars on the top left side of each picture represent 1 cm. A: The appearance of control individuals (3rd day 1). B: The appearance of individuals dead within 48 hr after application of 4-Me derivative (**4**, 10 μg/larva, 1 day after administration). C: The phenotype of larva-pupa intermediate induced by 4-Me (**4**, 3.0 μg/larvae) occurred in prepupal stage (22 days after administration). D: The larva of abnormal metamorphosis induced by 4-Cl (**7**, 0.10 μg/larva) occurred in the end of the 5th instar (13 days after administration). E: The individual of abnormal precocious metamorphosis induced by non-substituted derivative (**1**, 10 μg/larvae) occurred in 3rd to 4th ecdysis (6 days after administration). F: Right side: the individual of precocious metamorphosis induced by 4-Cl (**7**, 3.0 μg/larvae), left side: the individual of the control (29 days after administration). G: Right side: the dwarfed individual induced by 4-Cl (**7**, 3.0 μg/larvae, 4th day 2), left side: the individual of the control (5th day 2), 11 days after administration.

Table 1. Duration (days) of developmental stage of survived individuals until pupae after treatment on the 3rd day1 larvae.

No.	Compounds	Dosage (μg/larva)	*N*	Duration, days[a]			
				3rd	4th	5th	Total duration[b]
1	H	10	8	4.4±0.7***	5.0±0.5	8.6±1.1	18±14***
2	2-Me	10	4	5.8±0.4***	4.5±0.5	8.8±0.4	19±0.7***
3	3-Me	10	15	3.7±0.7*	4.7±0.4	7.2±0.7	16±1.1
4	4-Me	10	3	5.7±0.5***	4.7±0.5	7.3±0.5	18±0.5
5	2-Cl	10	8	4.0±0.0**	5.0±0.5	7.9±1.1	17±1.4
6	3-Cl	10	14	3.7±0.5*	5.0±0.0	6.9±0.5**	16±0.8
7	4-Cl	10	0	—	—	—	—
8	2,4-diMe	10	0	—	—	—	—
9	3,4-diMe	10	13	4.0±0.0***	4.5±0.5*	7.3±0.5	16±0.7
10	2,4-diCl	10	15	3.8±0.8**	5.5±0.5**	7.6±0.8	17±1.2*
11	3,4-diCl	10	13	4.2±0.4***	5.1±0.5	7.3±0.5**	17±1.0
	KK-42	10	2	7.0	6.5	—	14
		1.0	6	6.2±1.3**	5.7±1.2*	—	12±0.4**
	Methoprene	10	6	4.0±0.0**	5.0±0.0	8.2±0.7	17±0.7
		1.0	9	3.2±0.6	4.7±0.5	8.4±1.0	16±1.1
	Acetone	—	170	3.4±0.6	5.0±0.7	7.8±1.3	16±1.5

[a] Mean±S.D. Homogeneity of variances between control and derivative-treated groups was tested using the *F*-test. The significant differences between the data of control and each compound-treated group were analyzed using Student's *t*-test, as *F*-tests showed equal variances between controls and most groups (***$p<0.001$; **$p<0.01$; *$p<0.05$; not shown, $p>0.05$). [b] The total days until the end of the final instar.

such as inhibition of molt, abnormal pupation, and precocious metamorphosis (Fig. 2, C–E). Additionally, individuals with smaller body size were confirmed by the application of **1**, **2**, **4**, **5**, **7**, and **8** (Fig. 2, F).

Abnormal molting and metamorphosis and dwarfed individuals occurred, suggesting an effect on growth rate. Therefore, the duration of each instar of larvae that survived until pupation after compound administration was determined. The duration from the 2nd molting to the end of the 5th instar was 16±1.5 days in the control group. In contrast, 3 derivatives (**1**, **2**, and **10**) out of the 11 tested BMBIs were found to prolong the total duration of individuals that survived to pupation, 18±14, 19±0.7, and 17±1.2 days, respectively (Table 1). The duration of the 3rd, 4th, and 5th instar stages of the control was 3.4±0.6, 5.0±0.7, and 7.8±1.3 days, respectively. In contrast, 9 of the 11 BMBIs (**1**–**6** and **9**–**11**) resulted the 3rd instar stage elongation significantly, for example, the duration of 3rd instar was 5.8±0.4 days with the application of **2**. In addition, larvae that eventually died due to BMBIs application significantly elongated the total larval stage compared to the control. For example, 25 of the 81 larvae treated with the unsubstituted derivative (**1**, 10 μg/larva) died at the 5th instar stage or later. In this group, the total duration from the 3rd day1 to the end of the 5th instar stage was 21±4.3 days, confirming elongation of 5 days compared to the control ($p<0.001$, Welch's *t*-test). Several BMBIs induced an extension in the instar period.

In terms of structural characteristics of the BMBI for their biological activities, electron-donating 2- and 4-position substituents on the 1-benzyl group showed relatively high activity, whereas low or no activity was found with the BMBIs with 3-position substituents. Interestingly, the phenotypes were different for the positions of chlorine on the 1-benzyl group, that is, acute toxicity with 2-Cl substituent (**5**), inhibition of development with 4-Cl (**7**), and almost no activity with 3-Cl substituent (**6**) (Fig. 3) when applied on day 1of the 3rd instar.

2. Comparison of the phenotype of BMBIs to KK-42 and methoprene

The phenotypes induced by BMBIs were compared with those induced by KK-42 and methoprene. Their biological activities and modes of action have been well studied.[9,14,23,24] In this study, the outcomes caused by BMBIs combined their characteristics induced by KK-42 and methoprene.

When applied to the 3rd and 4th larvae, KK-42 was reported to induce precocious metamorphosis (anti-JH activity). In addition, acute larval death is known to be caused either at a high dose (10 μg/larva) and/or an application administered immediately after molting to 3rd instar. After KK-42 application (1.0 μg/larva) in this experiment, the precocious metamorphosis was induced with 47±38% (Fig. 3, A). In addition, acute death was resulted by 10 μg/larva application at day 1 of 3rd instar with 40±43%. The abnormal pupation and dwarfed individuals were not observed with those applications. Unsubstituted (**1**, 10 μg/larva) and 4-Cl derivatives (**7**, 3.0 μg/larva) caused a precocious metamorphosis at a rate of 9% (7 out of 81 individuals) and 7% (1 out of 15 individuals), respectively. Moreover, these two derivatives (**1** and **7**) resulted in acute death at 10 μg/larva with 47±19% and 80±0% mortality (Fig. 3, A), respectively, which resembled that of KK-42.

In contrast, methoprene (**13**) caused a delay and an arrest of metamorphosis (JH-like activity). Application of BMBIs caused

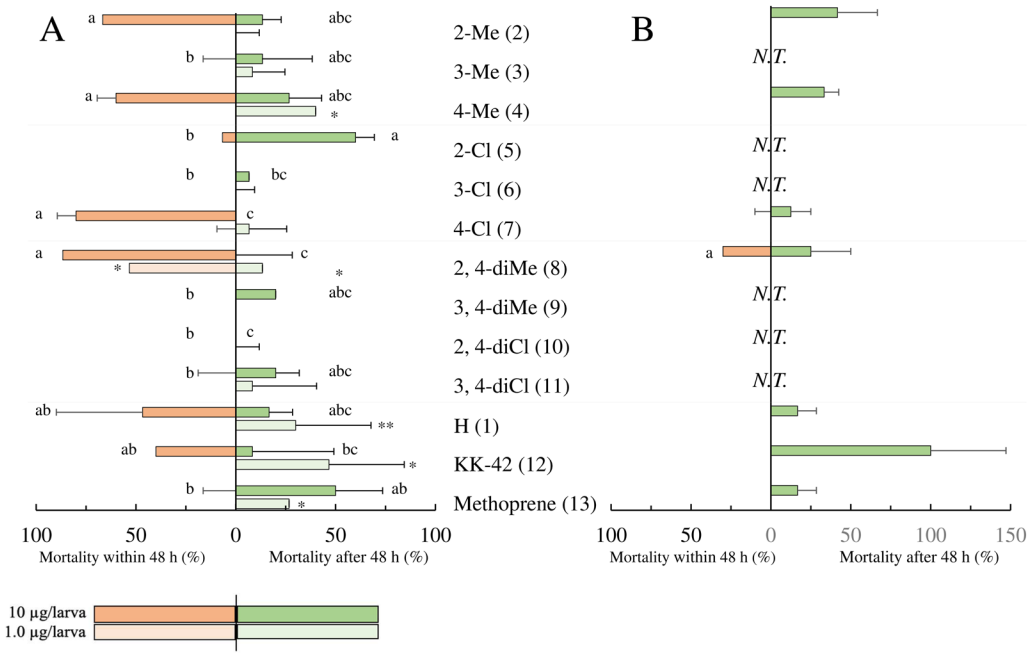


Fig. 3. Mortality after application of BMBIs on 3rd and 4th day1 instar. Data represent corrected mortality (%) and error bars indicate S.D. **A**: Corrected mortality after 1.0 μg/larva (below side in the row of each compound) and 10 μg/larva (above side) compounds to 3 day1 instar application (n=3 with five larvae). Mortality within 48 hr after administration is in the left side, after 48h is in the right side of this figure. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison test (p<0.05). Bars with the same letter are not significantly different (The letters 'a' and 'b' are used to compare acute mortality of 10 μg/larva application. The letters '*' and ' ' are used to compare acute mortality of 1.0 μg/larva application. The letters 'a', 'b', and 'c' are used to compare growth inhibition of 10 μg/larva application. The letters '**', '*', and ' ' are used to compare growth inhibition of 1.0 μg/larvae application.). **B**: Corrected mortality after 4th day1 instar application (Compounds **1**, **2**, **13**, and KK-42: n=3, Compounds **5** and **8**: n=4, Compound **4**: n=6, with five larvae, 10 μg/larva). Data were analyzed by Tukey–Kramer multiple comparison tests (p<0.05). Bars with the same letter are not significantly different (The letters 'a' and ' ' are used to compare acute mortality. No significant difference between each group was detected as a result of comparison of growth inhibition rate.). *N.T.* represents not tested. Incidentally, the difference in corrected lethality after administration of 3rd and 4th instar larvae was compared by Student's t-test (10 μg/larva of **1**, **2**, **4**, **5**, **7**, **8**, KK-42, and methoprene). The significant difference was validated with p<0.05 in the case of acute death rate excepting the KK-42, while no significant difference was confirmed in growth inhibition rate.

delays in molting and pupation; abnormal pupae, such as larvae-pupae intermediates, were also observed. Acute toxicity with methoprene was not observed up to a dose of 10 μg/larva on the 3rd day (Fig. 3). Some BMBIs (**1**, **2**, **4**, **5**, **7**, and **8**) also inhibited pupation, similar to methoprene. Nevertheless, they caused acute death at doses up to 10 μg/larva.

3. Structure–activity relationship of substitution on 1-benzyl group

Electron-donating substitution on the 2- and 4-position of the 1-benzyl group can be crucial for its biological activity. This was observed as a result of comparing the mortality of each BMBI derivative in 3rd instar.

As shown in Fig. 3 (A), the mortalities induced by 2 and/or 4 methyl- or chlorine-substituted BMBIs (**2**, **4**, **5**, and **7**) were higher than those of the non-substituted 1-benzyl compound (**1**). Derivatives with 2- or 4-methyl and 4-chloro substituents (**2**, **4**, and **7**) displayed acute lethality preferentially overgrowth inhibition. Moreover, the use of 2,4-dimethyl di-substituted BMBI (**8**) resulted in an acute lethality for both 1.0 and 10 μg/larva application. However, 2-chloro substituents (**5**) showed a greater growth-inhibiting activity than acute toxicity. Additionally, 2,4-dichloro BMBI (**10**) and 3-substituted derivatives (**3**, **6**, **9**, and **11**) showed neither an acute lethal nor growth inhibition activity.

Our results suggest that the electron-donating methyl substituents exerted positive effects on the biological activity. This is because both 2- and 4-methyl (**2** and **4**) and 2,4-dimethyl (**8**) derivatives induced comparatively high mortality in 3rd instar application. In contrast, the electron withdrawing chloro-substitutions imparted negative effects because the di-substituted derivative (**10**) no longer exerted lethal activity, although the mono-substituted derivatives (**5** and **7**) did. Furthermore, the 3-position substitution reduced the biological activity of both the methyl and chloro substituents. In summary, the electron-donating substituents at the 2- and 4-position of the 1-benzyl group of BMBI are crucial for their biological activity.

4. Dose-dependent effects of the biological activities of BMBIs

The dose-dependent activities of 4-methyl, 4-chloro, 2,4-dimethyl, and unsubstituted benzyl derivatives (**1**, **4**, **7**, and **8**) were investigated. This was performed at a dose from 0.01 to 30 μg/larva with the 3rd instar larvae. Growth inhibition was observed with low doses (≦3 μg/larva), although a high acute death rate was observed at high doses (10 and 30 μg/larva) using all four compounds (Figs. 4 and 5). The 2,4-dimethyl substituted derivative (**8**) showed

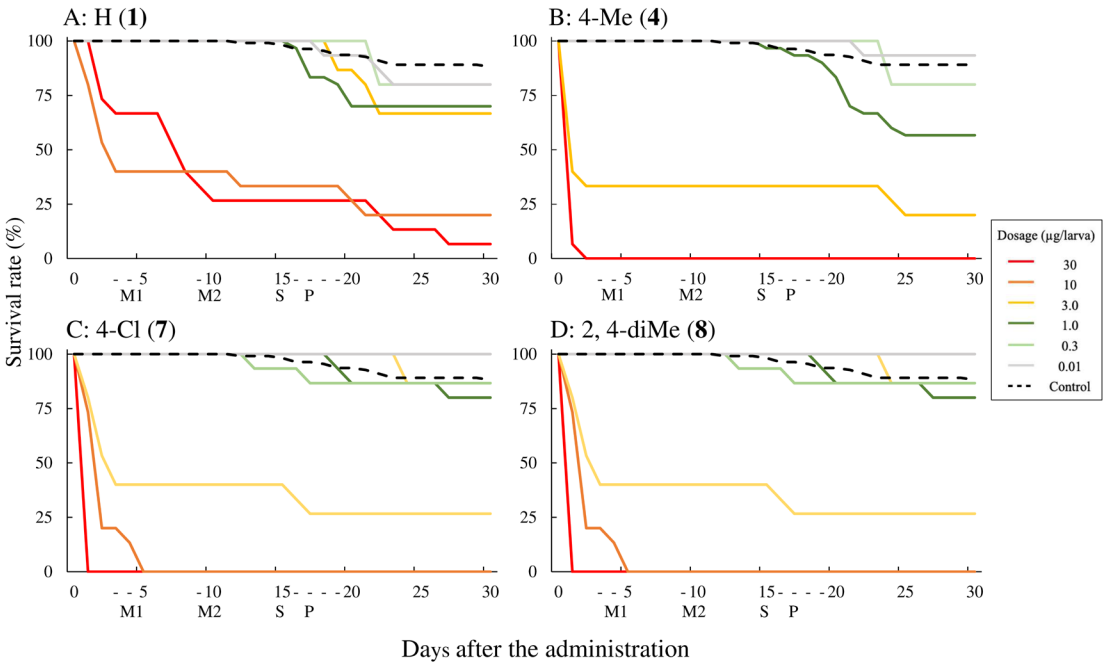


Fig. 4. Dose-dependent fluctuation of the survival curves of BMBIs. The survival rate was followed for 30 days after the application. Each derivative was applied to 15 larvae, except that 110 larvae were used as control. The following letters indicated below the horizontal axis represent the time of the shift of developmental stages of control individuals, M1: molting 3rd to 4th instar, M2: molting 4th to 5th instar, S: spinning, and P: pupation. Dosages are corresponded to the color indicated in the right-side legend.

LD_{50}=0.6±0.2 μg/larva, whereas LD_{50} of **1**, **4**, and **7** were more than 1.0 μg/larva. The 2,4-dimethyl substituted derivative (**8**) exerted an acute lethal activity rather than growth inhibition activity, even at a low dosage of 0.3 μg/larva. In contrast, the larvicidal activity (LD_{50}=7.5±3.1 μg/larva) of **1** was less than that of the other 3 derivatives with LD_{50} lower than about 3 μg/larva (Fig. 5).

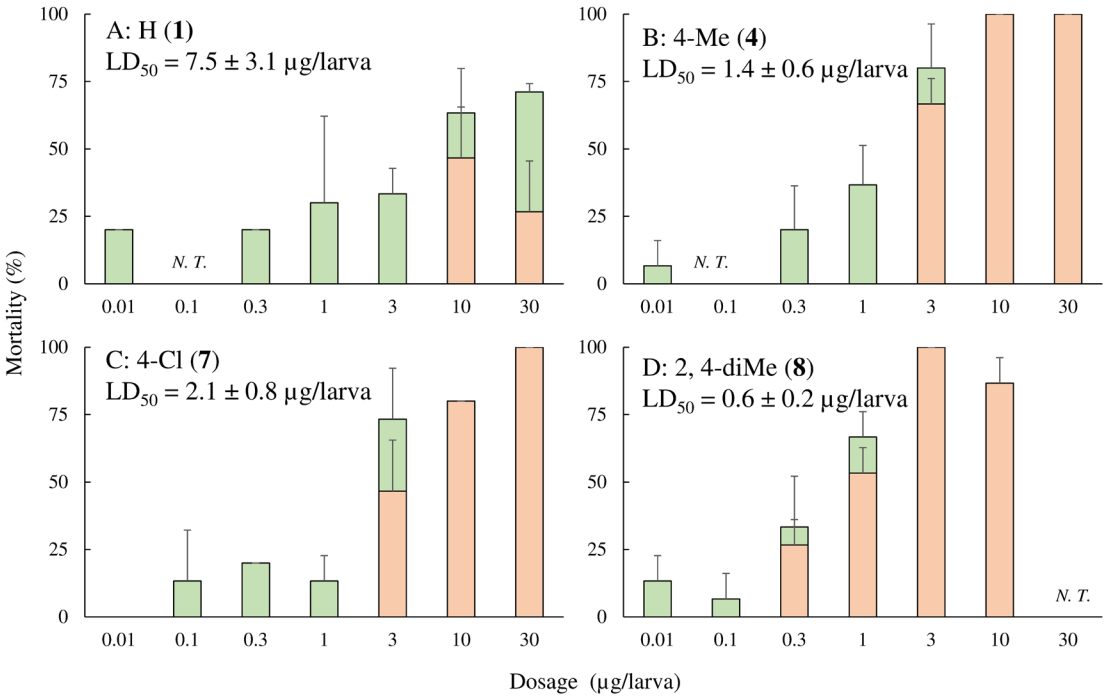


Fig. 5. Dose-dependent fluctuation of the biological activities of BMBIs. Data represent corrected mortality (%) and error bars indicate S.D. (n=3 with five larvae). The color of right green and orange indicates the rate of growth inhibition and acute death respectively. *N.T.* represents not tested.

BMBIs inhibit insect growth or are also highly lethal when administered at high doses. Moreover, among the tested BMBIs, the 2,4-dimethyl derivative (**8**) had comparatively higher biological activity. In contrast, the lethal activity of the unsubstituted derivative (**1**) was the lowest, followed by the derivatives with a substitution at the 3-position (**3**, **6**, **9**, and **11**).

5. Difference of the biological activity of BMBIs between applied stages

Application of 10 μg of BMBIs (**1**, **4**, and **7**) did not cause any acute toxicity on the day 1 of the 4th instar larval stage. In contrast, high acute lethality was observed for the 3rd instar larvae (Fig. 4, B). The 2,4-dimethyl derivative (**8**) showed acute toxicity, whereas the observed mortality was lower than that of the 3rd instar application (Fig. 3, B).

In addition, abnormal pupation and precocious metamorphosis were also induced, as in the 3rd instar application, although the abnormal morphology of the epidermis was confirmed at the time of the 4th to 5th molting caused by 4-chloro BMBI (**7**). This phenotype did not appear after applications to the 3rd instars. Thus, the biological activity of BMBIs on 4th instar larvae was lower than that on 3rd instar larvae.

Discussion

Based on the reported imidazole/benzimidazole structure of several compounds showing IGR activity, novel basic skeleton, 1-benzyl-2-metylbenzimidazole (BMBI) was chosen for derivatization because it was easily prepared by S_N2 reaction of substituted benzyl halides with 2-metylbenzimidazole. The BMBIs tested in this study had two insecticidal properties: acute toxicity and growth inhibition against silkworms. The phenotypes of the growth inhibition were as follows: dwarfing with prolongation of each larval stage, inhibition of pupation, and precocious metamorphoses. Notably, the growth inhibition activity was confirmed after a low-dose administration. This was observed even though the same structural compounds showed acute lethal activity at the high dose when applied to 3rd instar larvae. Moreover, the biological activities of the BMBIs were higher against 3rd instar larvae than against 4th instar. As different phenotypes were observed with different position of chlorine on the 1-benzyl group, the acute toxicity and IGR activity could be separated with derivatization of benzimidazole, the preparation of more related compounds such as different substituted groups on 1- and/or 2-position of benzimidazole are in progress.

Multiple phenotypes induced by BMBIs and their dose and applied stage-dependent change of effects suggest more than two modes of action. BMBIs showed biological activities similar in part to methoprene,[14] KK-42,[9] and B-1.[13] One of the modes of action may be similar to these IGRs. For example, methoprene prolongs the larval stage and inhibits pupation because of its JH agonistic activity.[14] Generally, the JH titers in the hemolymph of insect larvae decrease before pupation.[15] JH is an insect hormone that controls the time of pupation.[16] This occurs by it binding to the nuclear receptor complex, methoprene-tolerant (Met), and steroid receptor coactivator (SRC) to upregulate the transcription factor *Krüppel homolog 1* (Kr-h1), an early response gene.[17,18] *Kr-h1* negatively regulates the ecdysone action by downregulating the expression of *BR-C* and *E93*,[19–21] which transmit signals by 20E to the downstream gene cluster to induce the molting and metamorphosis. Furthermore, transcription of ecdysteroidogenic enzyme genes *Nvd*, *Sro*, *Spok*/*Spo*, *Cyp6t3*, *Phm*, *Dib*, and *Sad* were inhibited in response to the application of methoprene and knockdown of *Kr-h1* in an experiment using cultured *Drosophila* brain–ring gland (RG) complex and *B. mori* prothoracic gland (PG).[22] Thus, the inhibition of pupation by methoprene application was triggered by binding to JH signaling receptor Met/SRC. Thus, BMBIs may be affecting this particular signaling mechanism.

Moreover, KK-42, the imidazole IGR, induces precocious metamorphosis of *B. mori*[9] by inhibiting the biosynthesis of ecdysteroids in PG.[23,24] This causes an early decrease in JH. Precocious metamorphosis is considered to be due to the reduction in the JH titer by the inactivation of JH biosynthesis and induction of the activity of hemolymph JH-degrading esterase. This is attributed to the inhibitory effect of ecdysteroid synthesis.[25] Precocious metamorphosis by BMBIs may be due to the inactivation of 20E.

In the meantime, acute death observed within 48 hr of BMBI application might be attributed to the inhibition of respiration. Because the 2-methylbenzimidazole structure is common with B-1 reported to inhibit the electron transport[13] even though the part of structure on the 1-position of 2- methylbenzimidazole is totally different as branched alkyl chain with double bond in B-1, which is similar to piericidin known for inhibition of electron transport resembled with ubiquinone. Thus, the mode of actions of BMBIs is diverse; it can affect the endocrine system and respiration of insects. However, the similarities between the phenotypes or chemical structures of BMBIs and IGRs cannot explain why dwarfed individuals with prolonged larval stages were observed.

To explain the multiple phenotypes triggered by BMBIs, another mode of action was speculated. It involved octopamine signaling in hormone homeostasis. The significant extension of the larval stage in the insect neuropeptide prothoracicotropic hormone (PTTH) gene-knockout silkworms was found by Daimon *et al.*[26] Ohhara *et al.* revealed that PTTH and insulin-like peptide signaling were impaired by one of the octopamine receptor (*Octβ3R*) knockdown in the RG. This induced defects in the metamorphosis of *D. melanogaster*.[27] The phenotypes with elongating larval stages and deficient pupation by BMBIs were similar in part to those seen in our study. Since it might be that the structural properties caused the different modes of action, more derivatives must be made to extract the factors in the structure for different effects in biological activity. Investigations in expression analyses of insect hormone-related genes by the administration of BMBIs are also to be conducted for efficient molecular design in regards to specific mode of action in insect development.

In conclusion, novel IGRs and BMBIs were deduced to have several possible modes of action. In this study, they include affecting the endocrine system and respiration to cause the multiple phenotypes of growth inhibition and acute toxicity.

Electronic supplementary materials

The online version of this article contains supplementary materials, which are available at https://www.jstage.jst.go.jp/browse/jpestics/.

References

1) M. P. Zalucki, A. Shabbir, R. Silva, D. Adamson, L. Shu-Sheng and M. J. Furlong: Estimating the economic cost of one of the world's major insect pests, *Plutella xylostella* (Lepidoptera: Plutellidae): Just how long is a piece of string? *J. Econ. Entomol.* **105**, 1115–1129 (2012).
2) S.-J. Tuan, C.-C. Yeh, R. Atlihan, H. Chi and L.-C. Tang: Demography and consumption of *Spodoptera litura* (Lepidoptera: Noctuidae) reared on cabbage and taro. *J. Econ. Entomol.* **109**, 732–739 (2016).
3) M. Devetak, M. Vidrih and S. Trdan: Cabbage moth (*Mamestra brassicae* [L.]) and bright-line brown-eyes moth (*Mamestra oleracea* [L.]) - presentation of the species, their monitoring and control measures. *Acta Agric. Slov.* **95**, 149–156 (2010).
4) D. M. Kolodny-Hirsch and F. P. Harrison: Yield loss relationships of tobacco and tomato hornworms (Lepidoptera: Sphingidae) at several growth stages of Maryland tobacco. *J. Econ. Entomol.* **79**, 731–735 (1986).
5) R. Feyereisen, W. Dermauw and T. Van Leeuwen: Genotype to phenotype, the molecular and physiological dimensions of resistance in arthropods. *Pestic. Biochem. Physiol.* **121**, 61–77 (2015).
6) C. M. Williams: Third-Generation Pesticides. *Sci. Am.* **217**, 13–17 (1967).
7) M. Jindra, S. R. Palli and L. M. Riddiford: The juvenile hormone signaling pathway in insect development. *Annu. Rev. Entomol.* **58**, 181–204 (2013).
8) N. Yamanaka, K. F. Rewitz and M. B. O'Connor: Ecdysone control of developmental transitions: Lessons from *Drosophila* research. *Annu. Rev. Entomol.* **58**, 497–516 (2013).
9) E. Kuwano, R. Takeya and M. Eto: Terpenoid imidazoles: New antijuvenile hormones. *Agric. Biol. Chem.* **47**, 921–923 (1983).
10) E. Kuwano, R. Takeya and M. Eto: Synthesis and and-juvenile hormone activity of 1-substituted-5-[(*E*)-2,6-dimethyl-1, 5-heptadienyl] imidazoles. *Agric. Biol. Chem.* **49**, 483–486 (1985).
11) K. Suzuki, T. Minagawa, T. Kumagai, S. Naya, Y. Endo, M. Osanai and E. Kuwano: Control mechanism of diapause of the pharate first-instar larvae of the silkmoth *Antheraea yamamai*. *J. Insect Physiol.* **36**, 855–860 (1990).
12) T. Shiotsuki, F. Yukuhiro, M. Kiuchi and E. Kuwano: Effect of 1-(4-phenoxyphenoxypropyl) imidazole (KS-175) on larval growth in the silkworm *Bombyx mori*. *J. Insect Physiol.* **45**, 1049–1055 (1999).
13) Y. Nakagawa, E. Kuwano, M. Eto and T. Fujita: Effects of insect-growth-regulatory benzimidazole derivatives on cultured integument of the rice stem borer and mitochondria from rat liver. *Agric. Biol. Chem.* **49**, 3569–3573 (1985).
14) H. Akai and M. Kobayashi: Induction of prolonged larval instar by the juvenile hormone in *Bombyx mori* L.: Lepidoptera: Bombycidae. *Appl. Entomol. Zool.* **6**, 138–139 (1971).
15) K. Furuta, A. Ichikawa, M. Murata, E. Kuwano, T. Shinoda and T. Shiotsuki: Determination by LC-MS of Juvenile Hormone titers in hemolymph of the silkworm, *Bombyx mori*. *Biosci. Biotechnol. Biochem.* **77**, 988–991 (2013).
16) E. Dubrovsky: Hormonal cross talk in insect development. *Trends Endocrinol. Metab.* **16**, 6–11 (2005).
17) L. Bittova, P. Jedlicka, M. Dracinsky, P. Kirubakaran, J. Vondrasek, R. Hanus and M. Jindra: Exquisite ligand stereoselectivity of a *Drosophila* juvenile hormone receptor contrasts with its broad agonist repertoire. *J. Biol. Chem.* **294**, 410–423 (2019).
18) T. Kayukawa, C. Minakuchi, T. Namiki, T. Togawa, M. Yoshiyama, M. Kamimura, K. Mita, S. Imanishi, M. Kiuchi, Y. Ishikawa and T. Shinoda: Transcriptional regulation of juvenile hormone-mediated induction of Krüppel homolog 1, a repressor of insect metamorphosis. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 11729–11734 (2012).
19) T. Kayukawa, M. Murata, I. Kobayashi, D. Muramatsu, C. Okada, K. Uchino, H. Sezutsu, M. Kiuchi, T. Tamura, K. Hiruma, Y. Ishikawa and T. Shinoda: Hormonal regulation and developmental role of Krüppel homolog 1, a repressor of metamorphosis, in the silkworm *Bombyx mori*. *Dev. Biol.* **388**, 48–56 (2014).
20) T. Kayukawa, K. Nagamine, Y. Ito, Y. Nishita, Y. Ishikawa and T. Shinoda: Krüppel homolog 1 inhibits insect metamorphosis *via* Direct transcriptional repression of broad-complex, a pupal specifier gene. *J. Biol. Chem.* **291**, 1751–1762 (2016).
21) T. Kayukawa, A. Jouraku, Y. Ito and T. Shinoda: Molecular mechanism underlying juvenile hormone-mediated repression of precocious larval–adult metamorphosis. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 1057–1062 (2017).
22) T. Zhang, W. Song, Z. Li, W. Qian, L. Wei, Y. Yang, W. Wang, X. Zhou, M. Meng, J. Peng, Q. Xia, N. Perrimon and D. Cheng: Krüppel homolog 1 represses insect ecdysone biosynthesis by directly inhibiting the transcription of steroidogenic enzymes. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 3960–3965 (2018).
23) K. Kadono-Okuda, E. Kuwano, M. Eto and O. Yamashita: Anti-ecdysteroid action of some imidazole derivatives on pupal-adult Development of the Silkworm, *Bombyx mori*: Lepidoptera : Bombycidae. *Appl. Entomol. Zool.* **22**, 370–379 (1987).
24) T. Shiotsuki and E. Kuwano: Detection of proteins with a high affinity for imidazole insect growth regulator, KK-42. *J. Pestic. Sci.* **29**, 121–123 (2004).
25) M. Hirai, M. Kamimura, K. Kikuchi, Y. Yasukochi, M. Kiuchi, T. Shinoda and T. Shiotsuki: cDNA cloning and characterization of Bombyx mori juvenile hormone esterase: an inducible gene by the imidazole insect growth regulator KK-42. *Insect Biochem. Mol. Biol.* **32**, 627–635 (2002).
26) M. Uchibori-Asano, T. Kayukawa, H. Sezutsu, T. Shinoda and T. Daimon: Severe developmental timing defects in the prothoracicotropic hormone (PTTH)-deficient silkworm, *Bombyx mori*. *Insect Biochem. Mol. Biol.* **87**, 14–25 (2017).
27) Y. Ohhara, Y. Kayashima, Y. Hayashi, S. Kobayashi and K. Yamakawa-Kobayashi: Expression of β-adrenergic-like Octopamine Receptors during *Drosophila* Development. *Zool. Sci.* **29**, 83–89 (2012).