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Biological activities of juvenile hormone III skipped bisepoxide in last instar nymphs and adults of a stink bug, *Plautia stali*

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ABSTRACT

Juvenile hormone III skipped bisepoxide ($JHSB_3$), methyl (2*R*,3*S*,10*R*)-2,3;10,11-bisepoxyfarnesoate was recently determined as a novel juvenile hormone (JH) in a stink bug, *Plautia stali*. To further confirm the biological function of $JHSB_3$ in this insect, its juvenilizing, reproduction-stimulating and diapause-terminating activities and the presence in the hemolymph were examined. Topical application of $JHSB_3$ to last instar nymphs inhibited their metamorphosis in a dose-dependent fashion. In allatectomized and diapausing adults, $JHSB_3$ application exerted stimulatory effects on the development of ovaries and ectadenia in females and males, respectively. $JHSB_3$ was detected from the hemolymph of reproductively active females by gas chromatography–mass spectrometry analysis while its titer in the hemolymph collected from diapausing adults was too low to be detected. These results demonstrated that $JHSB_3$ has biological function as a JH in *P. stali*. Topical application of $JHSB_3$, its stereoisomers and 10*R*-JH III also indicated that compounds with the 2*R*,3*S*-configuration were more potent than those with the 2*S*,3*R*-configuration and 2,3-double bond.

1. Introduction

The metamorphosis from larvae to adults is an intriguing facet of insect life cycles. The sesquiterpenoid insect hormone, juvenile hormone (JH) is an important regulator of metamorphosis as well as various functions in all insects (Nijhout, 1994). It was in a heteropteran insect, *Rhodnius prolixus*, through his pioneering work that Wigglesworth (1934, 1985) discovered a humoral factor maintaining juvenile characters as the insect grew. Later he referred to this factor as the JH (Wigglesworth, 1940). Since then, heteropteran insects have played important roles in JH research and many findings have been made using these insects as experimental animals. For example, the 'paper factor', juvabione was discovered as a compound with JH activity specific to *Pyrrhocoris apterus* (Bowers et al., 1966; Sláma and Williams, 1965), and the elucidation of hormonal mechanisms of adult diapause, in particular the role of the brain in the diapause induction was elucidated in *P. apterus* (Hodková, 1976, 1977). Migration associated with diapause in *Oncopeltus fasciatus* was shown to be controlled by JH (Rankin and Riddiford, 1977, 1978). The anti-JH compounds, precocenes were identified using these two species (Bowers et al., 1976). However, the form of JH in the Heteroptera remained controversial; the production of JH III and methyl farnesoate by the corpus allatum (CA) (Bowers et al., 1983; Feldlaufer et al., 1982) and the presence of JH I in the hemolymph (Numata et al., 1992) were reported whereas Baker et al. (1988) failed to detect any known JHs in *O. fasciatus*. Instead, the presence of an unknown heteropteran JH was suggested (Hodková et al., 1996; Kotaki, 1993, 1996; Miyawaki et al., 2006). Very recently, we successfully identified the structure of JH in a stink bug, *Plautia stali*, as methyl (2*R*,3*S*,10*R*)-2,3;10,11-bisepoxyfarnesoate, which we have named juvenile hormone III skipped bisepoxide ($JHSB_3$) (**1**) (Fig. 1) (Kotaki et al., 2009). Although it is well known that JH is highly pleiotropic, a juvenilizing effect (inhibition of metamorphosis) and a reproduction-stimulating effect are the typical functions of JH in the larval or nymphal stage, and adult stage, respectively. In our study of structure determination, JH activity was assessed in terms of juvenilizing effect in the nymphal stage, but reproduction-stimulating effect in the adult stage was not explored yet, in spite that the *in vitro* product by the CA from adults was used as the natural sample for the analysis. We also found that isomer **2** has JH activity. Dose–response analysis in nymphal and adult stages, however, has yet to be performed for $JHSB_3$ and its stereoisomers. Furthermore, the use of *in vitro* CA product for structure determination raises another question of whether $JHSB_3$ is actually released into the hemolymph *in vivo*. It is, therefore,

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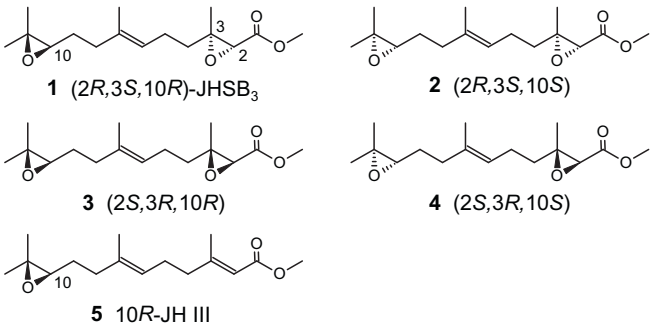

Fig. 1. Structure of $JHSB_3$ (**1**), its stereoisomers (**2–4**) and 10*R*-JH III (**5**).

essential to examine the biological activity and the presence of $JHSB_3$ in the hemolymph to confirm its hormonal function in *P. stali*.

Adults of *P. stali* undergo diapause in response to short-day conditions (Kotaki and Yagi, 1987). Extirpation of the CA from adults reared under long-day conditions mimicked the diapause-inducing effect of short-day conditions, and CA implantation caused the development of reproductive organs in adults kept under short-day conditions (Kotaki and Yagi, 1989). The results of a radiochemical assay developed to measure JH biosynthetic activity *in vitro* (Pratt and Tobe, 1974) supported the hypothesis that JH is a key regulator of adult diapause and the accompanying coloration change in *P. stali* (Kotaki, 1999). Having synthetic $JHSB_3$ in hand, it is now possible to examine the diapause-terminating effect of $JHSB_3$ in *P. stali*.

The purpose of the present study is to examine the juvenilizing and reproduction-stimulating effects of $JHSB_3$ (**1**) and its presence in the hemolymph of adults. The reproduction-stimulating effect was evaluated in allatectomized adults reared under long-day conditions and diapausing adults reared under short-day conditions by assessing ovary and ectadene development in females and males, respectively. The results obtained in the present study demonstrate the hormonal function of $JHSB_3$ in *P. stali*. The JH activity of stereoisomers of $JHSB_3$ (**2–4**) and naturally occurring isomer of JH III, 10*R*-JH III (**5**) was also examined.

2. Materials and methods

2.1. Insects and tests for JH activity

A stock culture of *P. stali* was established from adults collected in Joso (formerly Mitsukaido), Ibaraki, Japan (36.1°N, 140.0°E), in 2001, and kept for more than 30 generations under long-day conditions (16 h light:8 h dark) at 25 °C in the laboratory. Insects were reared on raw peanuts and dry soybeans with water supplemented with 0.05% sodium L-ascorbate and 0.025% L-cysteine. Diapausing insects were obtained by rearing them under short-day conditions (12 h light:12 h dark) at 20 °C (Kotaki and Yagi, 1987).

To examine the juvenilizing effects of $JHSB_3$ (**1**), its stereoisomers (**2–4**) and 10*R*-JH III (**5**) on *P. stali*, an aliquot of 1 μl hexane solution of the test compound was topically applied to the dorsal side of the abdomen of last instar nymphs using a 10 μl-microsyringe on the day of ecdysis to the last nymphal stage. After the final molting, the effect was assessed in terms of forewing and scutellum lengths relative to pronotum width (Fig. 2) and the number of antennal segments as described by Kotaki (1996). Reproduction-stimulating effect was determined in adults whose CA were surgically removed as follows (Kotaki and Yagi, 1989). The corpus cardiacum–corpus allatum complex was removed through a small hole made in the neck membrane from day 4 adults reared under long-day conditions at 25 °C, and those that underwent this operation were referred to as allatectomized. Test compounds dissolved in 1 μl of hexane were topically applied to the dorsal side of the abdomen beneath the wings immediately after allatectomy. As a measure of reproduction-stimulating effect, the diameter of terminal oocyte in female adults and the width of ectadene (ectodermal accessory gland) in male adults were determined using an ocular micrometer under a dissecting microscope 4 days after allatectomy and topical application. It is the ectadene rather than the testis that is under the control of JH in males of *P. stali* (Kotaki, 1999; Kotaki and Yagi, 1989). The effect of $JHSB_3$ in diapausing adults was also examined. Typical diapausing adults are characterized by undeveloped ovaries and ectadenia in females and males, respectively, and brown body coloration (Kotaki, 1999; Kotaki and Yagi, 1987, 1989). Diapausing adults received a topical application of $JHSB_3$ 30–40 days after emergence. They were then kept under short-day conditions at 20 °C and treated with the same amount of $JHSB_3$ 7 days later. Thirty days after the first $JHSB_3$ treatment body coloration and development of reproductive organs were examined. To evaluate the effect of $JHSB_3$ application on body coloration, the body color of insects at the end of the experimental period was classified into 5 grades according to Kotaki and Yagi (1987, 1989): grade 1, green color typically found in reproductively active adults; grade 5, brown color found in diapausing adults; grades 2–4, intermediate colors between grades 1 and 5. Because the color grade is a rank variable, standard deviations for observed values were not calculated.

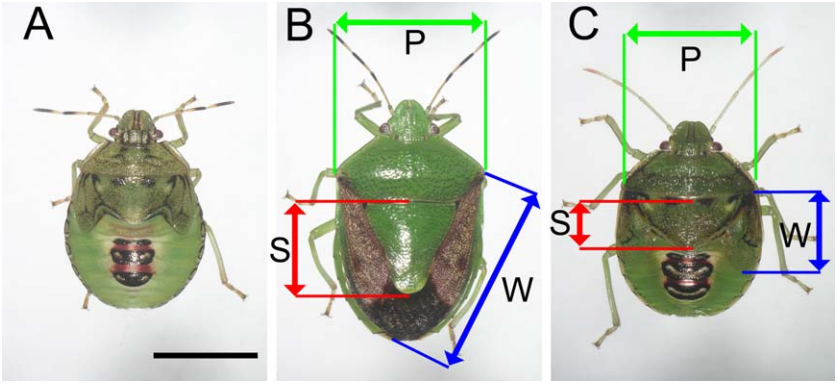

Fig. 2. Bioassay for juvenilizing activity in *P. stali*. A last instar nymph (A), an adult (B) and a nymph-adult intermediate (C) obtained as a result of application of JH-active sample. Scale bar: 5 mm. Arrows with labels W, S and P indicate forewing and scutellum lengths, and pronotum width, respectively.