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Juvenile hormone III skipped bisepoxide, not its stereoisomers, as a juvenile hormone of the bean bug *Riptortus pedestris*

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Highlights	·Juvenile hormone of the heteropteran species has long been controversial. ·We detected JHSB3 in the CC-CA product and in the hemolymph of a bean bug with a chiral UPLC-MS/MS. ·Topical application of JHSB3 effectively averted diapause. ·These results indicate that JHSB3 is the major JH of the bean bug. ·A significance to the configuration of the 2,3-epoxide moiety was found in JHSB3 action.
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Juvenile hormone III skipped bisepoxide, not its stereoisomers, as a juvenile hormone of the bean bug *Riptortus pedestris*

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Abstract

Juvenile hormone (JH) plays a pivotal role in many aspects of insect physiology. Although its presence was first reported in a blood-sucking bug belonging to the suborder Heteroptera (true bugs), JH species in the group has long been controversial. Although some recent studies proposed a putative JH molecular species in several Heteropteran species, it is not conclusive because physicochemical analyses were insufficient in most cases. Here, we studied this issue with an ultraperformance liquid chromatography–tandem mass spectrometer (UPLC-MS/MS) equipped with C18 and chiral columns in the bean bug *Riptortus pedestris* (Heteroptera, Alydidae), in which the JH species has long been controversial. Although a recent study describes $JHSB_3$ as the major JH of this species, that finding was not conclusive because its chirality has not been clarified. In the present study, we detected methyl (2*R*,3*S*,10*R*)-2,3;10,11-bisepoxyfarnesoate, commonly named juvenile hormone III skipped bisepoxide ($JHSB_3$), in the culture media of the corpora cardiaca-corpus allatum (CC-CA) complex and in the hemolymph of this species by a chiral ultraperformance liquid chromatography- tandem mass spectrometer (UPLC-MS/MS). Other $JHSB_3$ stereoisomers were not detected. Topical application of $JHSB_3$ effectively averted diapause. These results indicate that $JHSB_3$ is the major JH of *R. pedestris*. The present study further revealed that $JHSB_3$ and its (2*R*,3*S*,10*S*) isomer are more potent than (2*S*,3*R*,10*R*) and (2*S*,3*R*,10*S*) isomers, which suggests that there is a significance to the configuration of the 2,3-epoxide moiety in JH action. We further found a supplemental significance to the configuration of the 10-position.

Keywords

Chiral chromatography; Diapause; Heteroptera; Juvenile hormone III skipped bisepoxide ($JHSB_3$); Ultraperformance liquid chromatography – tandem mass spectrometer (UPLC-MS/MS)

Abbreviations

CA, corpus allatum (corpora allata)

CC, corpora-cardiaca

JH, juvenile hormone

$JHSB_3$, juvenile hormone III skipped bisepoxide

LC, liquid chromatography

LD, light-dark cycle

MS, mass spectrometer

UPLC, ultraperformance liquid chromatography

1. Introduction

Juvenile hormone (JH), which is synthesized at and secreted from the corpora allata (CA), is an important hormone that plays a role in almost every aspect of insect development and reproduction, including metamorphosis, caste determination in the social insects, regulation of behaviour, polyphenisms, larval and adult diapause regulation, ovarian development, and various aspects of metabolism associated with these functions (Nijhout, 1994; Goodman and Cusson, 2012). Wigglesworth (1934) first described the "inhibitory hormone" that inhibited metamorphosis of the early nymphal stage of the blood-sucking bug *Rhodnius prolixus* (Heteroptera: Reduviidae). The hormone is now termed JH. Since Röller et al. (1967) first identified the chemical structure of one of the JHs, JH I, various chemical structures of JHs, such as JH 0, JH II, JH III, 4-methyl JH I (*iso*-JH 0), and JHB_3, have been identified from various insect orders (Goodman and Cusson, 2012).

The suborder Heteroptera (true bugs) includes many agricultural and hygiene pests and some vectors of human diseases (Schaefer and Panizzi, 2000); thus their effective management is of great importance. A better understanding of their JHs will enable more effective management. However, the JHs of heteropteran species have long been greatly controversial (Baker et al., 1988; Bowers et al., 1983; Kotaki, 1993; Teal et al., 2014). Kotaki et al. (2009, 2011) reported a novel JH, methyl (2*R*,3*S*,10*R*)-2,3;10,11-bisepoxyfarnesoate, commonly named juvenile hormone III skipped bisepoxide ($JHSB_3$) (Fig. 1), in the hemolymph and culture media of the corpora cardiaca-corpus allatum (CC-CA) of *Plautia stali* (Heteroptera: Pentatomidae).

The bean bug *Riptortus pedestris* (formerly *Riptortus clavatus*) (Heteroptera: Alydidae) is known as a serious pest of the soybean (Bae et al., 2014). This species exhibits a clear photoperiodic response, that is, its ovarian development is induced under long-day conditions but is suppressed under short-day conditions (diapause) (Numata and Hidaka, 1982) and the physiological mechanisms underlying the response have been extensively studied (Morita, 1999; Goto, 2013). Numata et al. (1992) revealed JH I as a predominant JH in its hemolymph. This was the first report of the presence of JH I in non-lepidopteran species. However, Kotaki (1993) detected no obvious peak corresponding to JH I in the CC-CA product of *R. pedestris.* Lee et al. (2019) also found no evidence of the presence of JH I in the hemolymph of this species. Alternatively, Kotaki (1993) revealed, via a thin-layer chromatography, that the CC-CA of *R. pedestris* synthesises JH with the same retention factor value as that of the JH of *P. stali*. Recently, Lee et al. (2019) detected a JH with an MS profile corresponding to the $JHSB_3$ standard in the hemolymph of *R. pedestris* with a C18 liquid chromatography–mass spectrometer (LC-MS). Although the paper describes $JHSB_3$ as the major JH of this species, that finding was not conclusive. $JHSB_3$ is a structurally characteristic JH with distal chiral epoxides. There are three stereoisomers, (2*R*,3*S*,10*S*), (2*S*,3*R*,10*R*), and (2*S*,3*R*,10*S*) for $JHSB_3$ (Fig. 1). Because the C18 column does not discriminate between $JHSB_3$ and its stereoisomers, analysis with a chiral column is indispensable for the identification of $JHSB_3$ (Kotaki et al., 2009). The exact physicochemical and biological identification of a hormone is indispensable in the history of endocrinology.

Here we adopted an ultraperformance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS) equipped with C18 and chiral columns to increase the accuracy of the analytical system for investigation of the chemical structure of the JH of *R. pedestris*, with special attention to its chirality. The present study detected $JHSB_3$ in the culture media of the CC-CA complex and in the hemolymph of this species. The biological activity of $JHSB_3$ was tested by applying it topically to females in diapause. In general, inactivation of the CA, and thus the lack of JH, triggers adult diapause (Denlinger et al., 2012). $JHSB_3$ showed very high diapause-averting activity in *R. pedestris* and the effective dose was comparable to that of *P. stali*. The present study demonstrated that $JHSB_3$ is the major JH of *R. pedestris*.

2. Materials and methods

2.1. Insects

Adults of *R. pedestris,* originated from individuals collected at Osaka City, Osaka in 2019 and Fukuyama City, Hiroshima in 2006 (kindly provided by Dr. Y. Suzaki at Okayama University, Japan), were used for JH identification and JH bioassay, respectively. Insects were supplied with water containing 0.05% sodium ascorbate and 0.025% L-cysteine, soybean grain and red clover seeds.

2.2. JH identification by UPLC-MS/MS

Insects were reared in a cylindrical plastic pot (15 cm high and 15 cm diameter) in groups under short-day conditions (LD 12:12 h) at 25 ± 1°C from eggs to adult emergence, according to Numata and Hidaka (1982). Newly emerged adults were individually reared in a small plastic cup (75 mm in diameter, 40 mm in depth) and transferred to long-day conditions (LD 16:8 h) at 25 ± 1°C. A total of 15 females and seven males on days 16-38 (day 0 was defined as the day of adult emergence), that were sexually matured, were individually anesthetized on ice and were immobilized by clay. The CC-CA was then removed from these individuals according to the methods described by Matsumoto et al. (2013) with some modification. In brief, the minimum essential medium (with Hank's salt and L-glutamate and without sodium bicarbonate; GIBCO, Palo Alto, CA, USA) was added with 20 mM of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES; SIGMA, St. Louis, MO, USA) and 5 ppm of Tween 80 (SIGMA) and adjusted to pH 7.2. The CC-CA complexes from all females and those from all males were separately incubated in 30-50 μL of the medium at 30 °C for 5 h. The medium was extracted with 60 μL of hexane four times. The samples were dried up under the stream of argon gas and dissolved again in 30 μL of methanol.

Approximately 15 μL of the hemolymph was collected from sexually-matured females on days 35-40 under long-day conditions. According to Lee et al. (2019), the hemolymph was mixed vigorously with 500 μL of methanol and then added with 2% NaCl solution. The mixed sample was extracted with 250 μL of hexane three times. The

pooled hexane layers were loaded onto a Pasteur pipette column, packed with 1 g of MP alumina N Akt. 1 (MP Biomedicals, Eschwege, Germany). The alumina was activated with 6% weight of water for 2 h at room temperature and prewashed with 1 mL of hexane. After washing with 1 mL of 10% diethyl ether in hexane, the JH was eluted with 50% diethyl ether in hexane. This elute was dried up under the stream of argon gas and dissolved again in 30 μL of methanol.

The UPLC-MS/MS (ACQUITY UPLC H-Class, Xevo TQ-S micro, Waters, Milford, MA) with a C18 column (ACQUITY UPLC BEH C18 Column, 2.1 mm × 100 mm, 1.7 μm particle size, Waters) and a chiral column (CHIRALPAK IA-U, 3.0 mm x 100 mm, 1.6 μm particle size, Daicel, Tokyo, Japan) was used to compare the retention times of $JHSB_3$ and its stereoisomers, JH I, JH III and its stereoisomer (10*S*-JH III), the CC-CA product (equivalent to the product of the CC-CA complexes from 5 females and that from 2.3 males), and the hemolymph (equivalent to the hemolymph of 2.7 females). The system operation, data acquisition, and analysis were controlled and processed using MassLynx software. The flow rate of each sample was 0.2 mL/min and 0.4 mL/min in the solvent for the C18 and chiral columns, respectively. The solvent for the C18 column was 20% water and 80% methanol and that used for the chiral column was 15% water and 85% methanol. The column temperature was 30 °C. The mass spectrometer was operated in the positive ion mode. The tuning parameters were optimized for $JHSB_3$ and JH I: desolvation temperature 400 °C, desolvation gas flow 800 L/h, cone voltage 20 V, collision energy 10 V. Authentic $JHSB_3$ and its stereoisomers, JH I, and JH III and 10*S*-JH III were synthesized as described in Kotaki

et al. (2009) and Manabe et al. (2012). The JH III standard used in the present study was a mixture of JH III and 10*S*-JH III. The MS/MS analysis of the authentic $JHSB_3$ showed the $[M+H]^+$ ion at *m*/*z* 283.2 and the $[M+Na]^+$ at *m*/*z* 305.3. The product ions were detected at *m*/*z* 42.9 and *m*/*z* 233.2 when ions at *m*/*z* 283.2 were used as a precursor, whereas no fragmentation was detected when ions at *m*/*z* 305.3 were used. In the present study, ions at *m*/*z* 283.2 and their product ions at *m*/*z* 233.2 were used as monitor ions for detecting $JHSB_3$ and its stereoisomers. For JH I, ions at *m*/*z* 317.1 were used and no fragmentation was detected. For JH III, ions at *m*/*z* 267.3 and their product ions at *m*/*z* 43.0 were used as monitor ions.

2.3. Diapause-averting activity of $JHSB_3$

Insects were reared in a cylindrical plastic pot in groups under diapause-inducing short-day conditions (LD 12:12 h) at 25 ± 1°C from eggs according to Numata and Hidaka (1982). Newly emerged adult females were individually reared in a small plastic cup under the same photoperiodic and temperature conditions. Seven days after adult emergence, 1 μL of hexane (solvent) or various concentrations of $JHSB_3$ or its stereoisomers were applied topically. Seven days later, they were dissected out to observe the ovarian status. Females with a yolk deposition in the oocytes were judged to be reproductive and those with no deposition were judged to be non-reproductive (diapause), according to Numata and Hidaka (1982).

2.4. Statistical analysis

Diapause incidences were analyzed by Fisher's exact test or Tukey-type multiple comparisons for proportions (Zar, 2010).

3. Results

3.1. UPLC-MS/MS analysis of the CC-CA product

The C18 UPLC-MS/MS data are shown in Fig. 2. The retention time of the main peak of the CC-CA product was identical with that of the authentic $JHSB_3$, indicating that the CC-CA product contains $JHSB_3$ or its stereoisomers (Fig. 2A) because the diastereoisomers could not be separated on the C18-reversed phase (Fig. S1). JH III and 10*S*-JH III were not separated in the C18 column in the same way that $JHSB_3$ and its stereoisomers were, and a small peak corresponding to the JH III and 10*S*-JH III mixture was observed in the CC-CA product (Fig. 2B). A small peak corresponding to JH I was also observed in the CC-CA product of females. The peak corresponding to JH I was very small and almost at the detection limit level in males (Fig. 2C).

The chiral UPLC-MS/MS data are shown in Fig. 3. The chiral-reversed phase clearly separated $JHSB_3$ diastereoisomers (Fig. 3A). The retention time of the peak of the CC-CA product was identical with that of $JHSB_3$, but not with those of the stereoisomers. A small peak corresponding to JH III was observed in the CC-CA

product of females, as in the C18 column; however, the peak was not as clear in males (Fig. 3B). No peak corresponding to JH I was detected in the chiral column, in contrast to the C18 column (Fig. 3C).

3.2. UPLC-MS/MS analysis of the hemolymph

The chiral UPLC-MS/MS data of the hemolymph are shown in Fig. 4. Again, the chiral-reversed phase clearly separated $JHSB_3$ diastereoisomers and we detected a peak corresponding to $JHSB_3$. No peaks corresponding to its stereoisomers were detected.

3.3. Diapause-averting effect of $JHSB_3$

Most intact and hexane-treated females were in diapause (Fig. 5). $JHSB_3$ and its stereoisomer 2 averted diapause in a dose-dependent manner, but their efficacies were distinct. Most females treated with 0.01 μg of $JHSB_3$ averted diapause, whereas those treated with the same amount of stereoisomer 2 were in diapause. Thus, the former is more potent than the latter. Stereoisomer 3 was less effective; i.e. treatment with 1 μg of the chemical had no effect, but treatment with 10 μg averted diapause. Stereoisomer 4 showed no diapause-averting effect.

4. Discussion

In this analytical study, we have established the separation conditions of not only $JHSB_3$, but also JH I and JH III, by the chiral-reversed phase UPLC–MS/MS analytical system for the first time. The new system enabled the rapid and accurate analysis of JHs. The system is also very sensitive. Here, the CC-CA product and the hemolymph from only a few *R. pedestris* individuals were enough to detect $JHSB_3$. The present study shows the result of the CC-CA product equivalent to that from 2.3 males and that of the hemolymph from 2.7 females (approx. 5 μL of the hemolymph). The method was also able to detect the JH from only the single CC-CA complex (data not shown). By contrast, a previous study with *P. stali* required 60 CC-CAs to detect $JHSB_3$ using a chiral gas chromatography–mass spectrometer (Kotaki et al., 2009) and that with *R. pedestris* required 50-100 μL of the hemolymph using a C18 LC-MS. Our new method is beneficial for the detection of JHs in insects, especially in heteropteran species.

The present study unambiguously detected $JHSB_3$ in the CC-CA product and in the hemolymph of *R. pedestris* with a chiral UPLC-MS/MS analysis. $JHSB_3$ is biologically active and it averted diapause effectively. The effective dose is comparable with that in *P. stali*, whose JH was confirmed to be $JHSB_3$ (Kotaki et al., 2011). The present study also revealed that the lowest dose of $JHSB_3$ that averts diapause (0.01 μg) is lower than that of methoprene, one of the well-known JH analogues (JHAs) (0.2 μg; Numata and Hidaka 1984). Dahm et al. (1976) proposed three criteria necessary to be fulfilled to chemically identify JH; i.e., (1) production by the CA; (2) titer fluctuation in

synchrony with the processes controlled by JH; and (3) rescue effect in JH-deprived insects. The first criterion is now fulfilled in the present study. Although Lee et al. (2019) did not focus on the JH chirality, their results together with the present study reveal a close correlation between titer fluctuation of $JHSB_3$ and fecundity; i.e., the fulfilment of the second criterion. Diapause, that is induced by JH deprivation, was effectively averted by topical application of $JHSB_3$ in the present study, fulfilling the third criterion. Therefore, we propose that $JHSB_3$, not $JHSB_3$ stereoisomers 2-4, is the major JH of *R. pedestris*.

Numata et al. (1992) proposed that JH I is the major JH of *R. pedestris*, based on its presence in the hemolymph. However, this is inconsistent with the results reported by Kotaki (1993) and Lee et al. (2019). These papers reported no evidence of the presence of JH I in the CC-CA product or in the hemolymph. The present study detected a peak corresponding to JH I in the CC-CA product in the C18 analysis, but not in the chiral analysis. Thus, the presence of JH I is still ambiguous in *R. pedestris*. The present study further detected a small amount of JH III in the CC-CA product of *R. pedestris* females and males with both C18 and chiral UPLC-MS/MS analyses. The presence of JH III was also proposed in other heteropterans, such as *Halyomorpha halys* (Pentatomidae), *Nezara viridula* (Pentatomidae), and *Oncopeltus fasciatus* (Lygaeidae) (Bowers et al., 1983; Kotaki, 1993), but Lee et al. (2019) detected no peaks corresponding to JH III in *R. pedestris* hemolymph. Further studies are needed to clarify whether *R. pedestris* synthesises JH III.

Distinct biological activity among JH diastereoisomers has been reported (Dahm et al., 1968; Goodman et al., 1978; Kindle et al., 1989; Kotaki et al., 2011; Sakurai et al., 1990). In the present study, we also revealed a difference in JH activity among JH stereoisomers in *R. pedestris*. $JHSB_3$ and its stereoisomer 2 are more potent than stereoisomers 3 and 4 in diapause aversion of *R. pedestris*, as reported in *P. stali* (Kotaki et al., 2009, 2011). The former two stereoisomers have the 2*R*,3*S*-configuration, while the latter two have the 2*S*,3*R*-configuration, which suggests that there is a significance to the configuration of the 2,3-epoxide moiety for JH action. Furthermore, the present study revealed the supplemental significance of the configuration of the 10-position; i.e. isomers possessing the 10*R*-configuration are more potent than those possessing the 10*S*-configuration when the configurations of 2- and 3-positions are identical. These differences in JH activity among JH stereoisomers are likely derived from the ligand acceptance of the $JHSB_3$ receptor. Interestingly, *P. stali* showed no ability to discriminate the configuration of the 10-position, although it clearly discriminated the configurations of 2- and 3-positions as shown in *R. pedestris* (Kotaki et al., 2011). The results suggest that the ligand-binding pocket of the JH receptor in *P. stali* is structurally different from that in *R. pedestris*; thus, these receptors may accept different molecules. Indeed, methoprene effectively acts as a JH analogue in *R. pedestris* (Chinzei et al., 1992; Hirai et al. 2000; Numata and Hidaka 1984), but not in *P. stali* and several pentatomid bugs (Kotaki, 1996). However, this may not be the case. In *Drosophila melanogaster*, not only *Drosophila* JHs (JH III, its precursor MF, and JH III bisepoxide JHB_3) but also lepidopteran JH, JH I, can bind and activate its JH receptor (Bittova et

al., 2019). The receptor also shows less ability to discriminate between natural JH III and its enantiomer 10*S*-JH III (Bittova et al., 2019). A cell line from the beetle *Tribolium castaneum* also responds equally well to either JH III or JH I (Kayukawa et al., 2013). It is of interest to investigate which processes provide the difference in potency. The knowledge will greatly contribute to the development of new insect growth regulators specific to heteropteran species (Kaihara et al., 2012; Ramaseshadri et al., 2012).

Although the JHs of heteropteran species have long been greatly controversial (Baker et al., 1988; Bowers et al., 1983; Kotaki, 1993; Teal et al., 2014), $JHSB_3$ is now unambiguously confirmed as a JH in *P. stali* belonging to Pentatomidae (Kotaki et al., 2009, 2011) and in *R. pedestris* belonging to Alydidae (the present study) in the suborder Heteroptera. Hejnikova et al. (2016) also detected a JH with an MS profile corresponding to $JHSB_3$ standard in the hemolymph and in the extract of the CC-CA in *Pyrrhocoris apterus* (Heteroptera, Pyrrhocoridae) with a triple quadrupole mass spectrometer equipped with a C18 column. Although this study does not tell us the stereoisomer configuration, it suggests a possibility of $JHSB_3$ as the major JH of this species. The next point of discussion is whether $JHSB_3$ is widely distributed among species belonging to Heteroptera. Further studies on the JH of heteropteran insects are now welcomed.

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References

Bae, S.D., Kim, H.J., Mainali, B.P., 2014. Infestation of *Riptortus pedestris* (Fabricius) decreases the nutritional quality and germination potential of soybean seeds. J. Asia Pac. Entomol. 17, 477-481. https://doi.org/10.1016/j.aspen.2014.04.006

Baker, F.C., Tsai, L.W., Reuter, C.C., Schooley, D.A. 1988. The absence of the significant levels of the known juvenile hormones and related compounds in the milkweed bug, *Oncopeltus fasciatus*. Insect Biochem. 18, 453-462. https://doi.org/10.1016/0020-1790(88)90062-5

Bittova, L., Jedlicka, P., Dracinsky, M., Kirubakaran, P, Vondrasek, J., Hanus, R., Jindra, M., 2019. Exquisite ligand stereoselectivity of a *Drosophila* juvenile hormone receptor contrasts with its broad agonist repertoire. J. Biol. Chem. 294, 410-423. https://doi.org/10.1074/jbc.RA118.005992

Bowers, W.S., Marsella, P.A., Evans, P.H., 1983. Identification of an hemipteran juvenile hormone: in vitro biosynthesis of JH III by *Dysdercus fasciatus*. J. Exp. Zool. 228, 555–559. https://doi.org/10.1002/jez.1402280316

Chinzei, Y., Miura, K., Kobayashi, L., Shinoda, T., Numata, H., 1992. Cyanoprotein: developmental stage, sex and diapause-dependent expression, and synthesis

regulation by juvenile hormone in the bean bug, *Riptortus clavatus*. Arc. Insect Biochem. Physiol. 20, 61-73. https://doi.org/10.1002/arch.940200107

Dahm, K.H., Bhaskaran, G., Peter, M.G., Shirk, P.D., Seshan, K.R., Röller, H. 1976. On the identity of the juvenile hormone in insects. In: Gibert, L.I. (ed) The Juvenile Hormone. Plenum Press, New York, pp. 19–47.

Dahm, K.H., Röller, H., Trost, B.M., 1968. The juvenile hormone-IV. Stereochemistry of juvenile hormone and biological activities of some of its isomers and related compounds. Life Sci. 7, 129-137. https://doi.org/10.1016/0024-3205(68)90296-8

Denlinger, D.L., Yocum, G.D., Rinehart, J.P., 2012. Hormonal control of diapause. In: Gilbert, L.I. (ed) Insect Endocrinology. Elsevier BV Academic Press, Waltham, pp. 430-463.

Goodman, W.G., Cusson, M., 2012. The juvenile hormone. In: Gilbert, L.I. (ed) Insect Endocrinology. Elsevier BV Academic Press, Waltham, pp. 310-365.

Goodman, W., Schooley, D.A., Gilbert, L.I., 1978. Specificity of the juvenile hormone binding protein: The geometrical isomers of juvenile hormone I. Proc. Natl. Acad. Sci. USA. 75, 185-189. https://dx.doi.org/10.1073%2Fpnas.75.1.185

Goto, S.G., 2013. Roles of circadian clock genes in insect photoperiodism. Entomol. Sci. 16, 1-16. https://doi.org/10.1111/ens.12000

Hejnikova, M., Paroulek, M., Hodkova, M. 2016. Decrease in *Methoprene tolerant* and *Taiman* expression reduces juvenile hormone effects and enhances the levels of juvenile hormone circulating in males of the linden bug *Pyrrhocoris apterus*. J. Insect Physiol. 93-94, 72-80. http://dx.doi.org/10.1016/j.jinsphys.2016.08.009

Hirai, M., Watanabe, D., Chinzei, Y., 2000. A juvenile hormone-repressible transferrin-like protein from the bean bug, *Riptortus clavatus*: cDNA sequence analysis and protein identification during diapause and vitellogenesis. Arch. Insect Biochem. Physiol. 44, 17–26. https://doi.org/10.1002/(SICI)1520-6327(200005)44:1%3C17::AID-ARCH3%3E3.0.CO;2-O

Kaihara, K., Kotaki, T., Numata, H., Ohfune, Y., Shinada, T., 2012. Structure-activity relationship of novel juvenile hormone, $JHSB_3$, isolated from the stink bug, *Plautia stali*. Tetrahedron 68, 106-113. https://doi.org/10.1016/j.tet.2011.10.080

Kayukawa, T., Tateishi, K., Shinoda, T., 2013. Establishment of a versatile cell line for juvenile hormone signalling analysis in *Tribolium castaneum*. Sci. Rep. 3, 1570, https://doi.org/10.1038/srep01570

Kindle, H., Winistörfer, M., Lanzrein, B., Mori, K. 1989. Relationship between the absolute configuration and the biological activity of juvenile hormone III. Experientia 45, 356-360. https://doi.org/10.1007/BF01957477

Kotaki, T., 1993. Biosynthetic products by heteropteran corpora allata *in vitro*. Appl. Entomol. Zool. 28, 242-245. https://doi.org/10.1303/aez.28.242

Kotaki, T., 1996. Evidence for a new juvenile hormone in a stink bug, *Plautia stali*. J. Insect Physiol. 42, 279-286. https://doi.org/10.1016/0022-1910(95)00113-1

Kotaki, T., Shinada, T., Kaihara, K., Ohfune, Y., Numata, H. 2009. Structure determination of a new juvenile hormone from a heteropteran insect. Org. Lett. 11, 5234–5237. https://doi.org/10.1021/ol902161x

Kotaki, T., Shinada, T., Kaihara, K., Ohfune, Y., Numata, H., 2011. Biological activities of juvenile hormone III skipped bisepoxide in last instar nymphs and adults of a stink bug, *Plautia stali*. J. Insect Physiol. 57, 147–152. https://doi.org/10.1016/j.jinsphys.2010.10.003

Lee, J., Kim, C.-H., Jang, H.A., Kim, J.K., Kotaki, T., Shinoda, T., Shinada, T., Yoo, J.W., Lee, B.L., 2019. *Burkholderia* gut symbiont modulates titer of specific juvenile hormone in the bean bug *Riptortus pedestris*. Dev. Comp. Immunol. 99, 1033399. https://doi.org/10.1016/j.dci.2019.103399

Manabe, A., Ohfune, Y., Shinada, T. (2012) Stereoselective total syntheses of insect juvenile hormones JH 0 and JH I. Synlett 23, 1213-1216. https://doi.org/ 10.1055/s-0031-1290803

Matsumoto, K., Numata, H., Shiga, S. 2013. Role of the brain in photoperiodic regulation of juvenile hormone biosynthesis in the brown-winged green bug,

Plautia stali. J. Insect Physiol. 59, 387–393. https://doi.org/10.1016/j.jinsphys.2013.01.007

Morita, A. 1999. Neural and endocrine mechanisms for the photoperiodic response controlling adult diapause in the bean bug, *Riptortus clavatus*. Entomol. Sci. 2, 579-587.

Nijhout, H.F. 1994. Insect Hormones. Princeton University Press, Princeton.

Numata, H., Numata, A., Takahashi, C., Nakagawa, Y., Iwatani, K., Takahashi, S., Miura, K., Chinzei, Y., 1992. Juvenile hormone I is the principal juvenile hormone in a hemipteran insect, *Riptortus clavatus*. Experientia 48, 606–610. https://doi.org/10.1007/BF01920248

Numata, H., Hidaka, T., 1982. Photoperiodic control of adult diapause in the bean bug *Riptortus clavatus* Thunberg (Heteroptera: Coreidae) I. Reversible induction and termination of diapause. Appl. Entomol. Zool. 17, 530-538. https://doi.org/10.1303/aez.17.530

Numata, H., Hidaka, T., (1984) Termination of adult diapause by a juvenile hormone analogue in the bean bug, *Riptortus clavatus*. Zool. Sci 1, 751-754. http://hdl.handle.net/2433/108638

Ramaseshadri, P., Farkaš, R., Palli, S.R., 2012. Recent progress in juvenile hormone analogs (JHA) research. Advances Insect Physiol. 43, 353-436. https://doi.org/10.1016/B978-0-12-391500-9.00005-X

Röller, H., Dahm, K.H., Sweely, C.C., Trost, B.M., 1967. The structure of the juvenile hormone. Angew. Chem. Int. Ed. Engl. 6, 179-180. https://doi.org/10.1002/anie.196701792

Sakurai, S., Ohtaki, T., Mori, H., Fujiwhara, M., Mori, K., 1990. Biological activity of enantiomerically pure forms of insect juvenile hormone I and III in *Bombyx mori*. Experientia 46, 220–221. https://doi.org/10.1007/BF02027321

Schaefer, C.W., Panizzi, A.R., 2000. Heteroptera of economic importance. CRC Press, Boca Raton.

Teal, P.E.A., Jones, D., Jones, G., Torto, B., Nyasembe, V., Borgemeister, C., Alborn, H.T., Kaplan, F., Boucias, D., Lietze, V.U. 2014. Identification of methyl farnesoate from the hemolymph of insects. J. Nat. Prod. 77, 402−405. https://doi.org/10.1021/np400807v

Wigglesworth, V.B. 1934. The physiology of ecdysis in *Rhodnius prolixus* (Hemiptera). II. Factors controlling moulting and 'metamorphosis.' Q. J. Microsc. Sci. 77, 191–222.

Zar, J.H. 2010. Biostatistical Analysis, Fifth edition. Prentice Hall, Upper Saddle River.

Figure legends

Fig. 1 Structures of JH I, JH III and its stereoisomer (10*S*-JH III), and $JHSB_3$ (1) and its stereoisomers (2-3)

Fig. 2 The C18 UPLC-MS/MS analyses of the CC-CA product of *Riptortus pedestris.* (A) The CC-CA product of females and males and the authentic $JHSB_3$, (B) the CC-CA product of females and males and the mixture of the authentic JH III and 10*S*-JH III, and (C) the CC-CA product of females and males and the authentic JH I

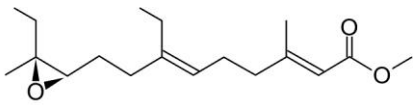
Fig. 3 The chiral UPLC-MS/MS analyses of the CC-CA product of *Riptortus pedestris.* (A) The CC-CA product of females and males, the authentic $JHSB_3$, and its stereoisomers, (B) The CC-CA product of females and males and the mixture of the authentic JH III and 10*S*-JH III, and (C) The CC-CA product of females and males and the authentic JH I. For chemical structures, see Fig. 1

Fig. 4 The chiral UPLC-MS/MS analyses of the hemolymph of *Riptortus pedestris.* The hemolymph, the authentic $JHSB_3$, and its stereoisomers. For chemical structures, see Fig. 1

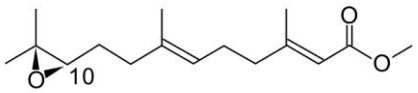
Fig. 6 Effect of $JHSB_3$ (black closed circles) and its stereoisomers 2 (grey open circles), 3 (black closed triangles) and 4 (grey open triangles) on diapause incidence. Diapause incidences of intact and hexane-treated females are also shown. n = 11-63. Diapause incidences were statistically compared at the same dose. Asterisks indicate statistical differences detected by Fisher's exact test ($P < 0.05$). Letters indicate statistical differences among samples by Tukey-type multiple comparisons for proportions ($P < 0.05$)

Fig. S1 The C18 UPLC-MS/MS analyses of the authentic $JHSB_3$ and its stereoisomers (2-
463 4). See Fig. 1 for their chemical structures
464

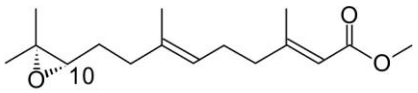
JH I



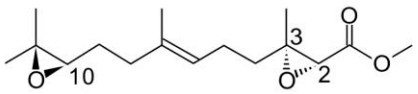
JH III



10*S*-JH III



1. (2*R*,3*S*,10*R*)
$JHSB_3$



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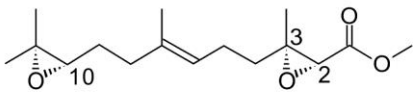
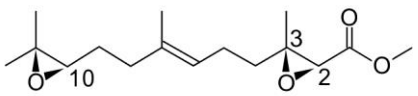


3. (2*S*,3*R*,10*R*)



4. (2*S*,3*R*,10*S*)

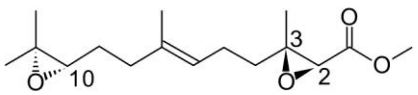


Fig. 1

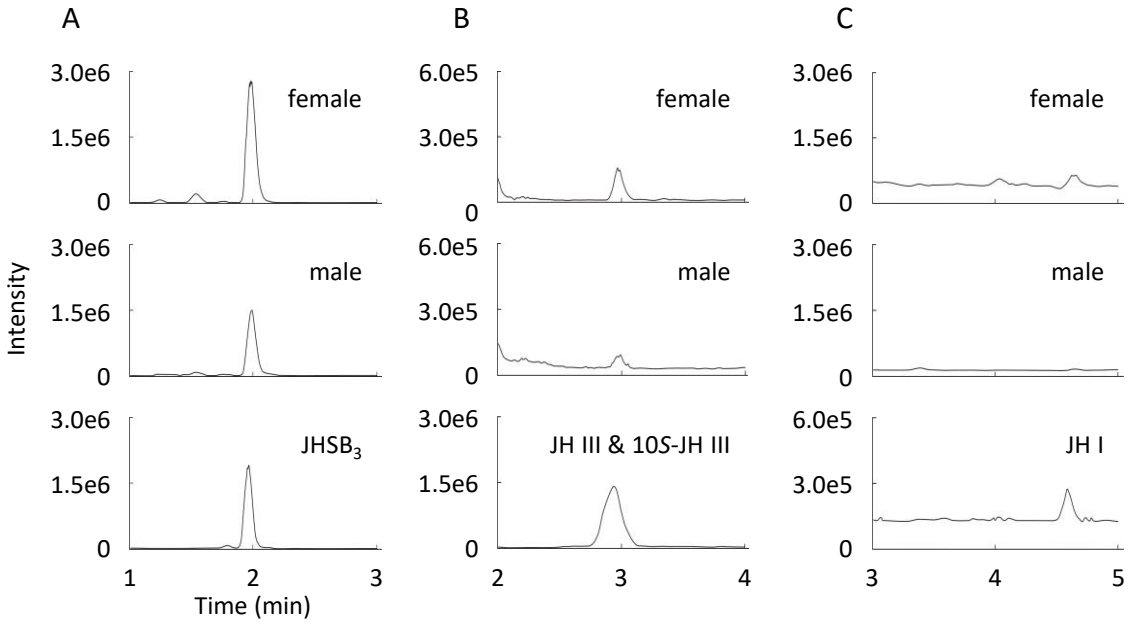

A
B
C
Intensity
3.0e6
1.5e6
0
6.0e5
3.0e5
female
male
JHSB3
JH III & 10S-JH III
JH I
1
2
3
4
5
Time (min)

Fig. 2

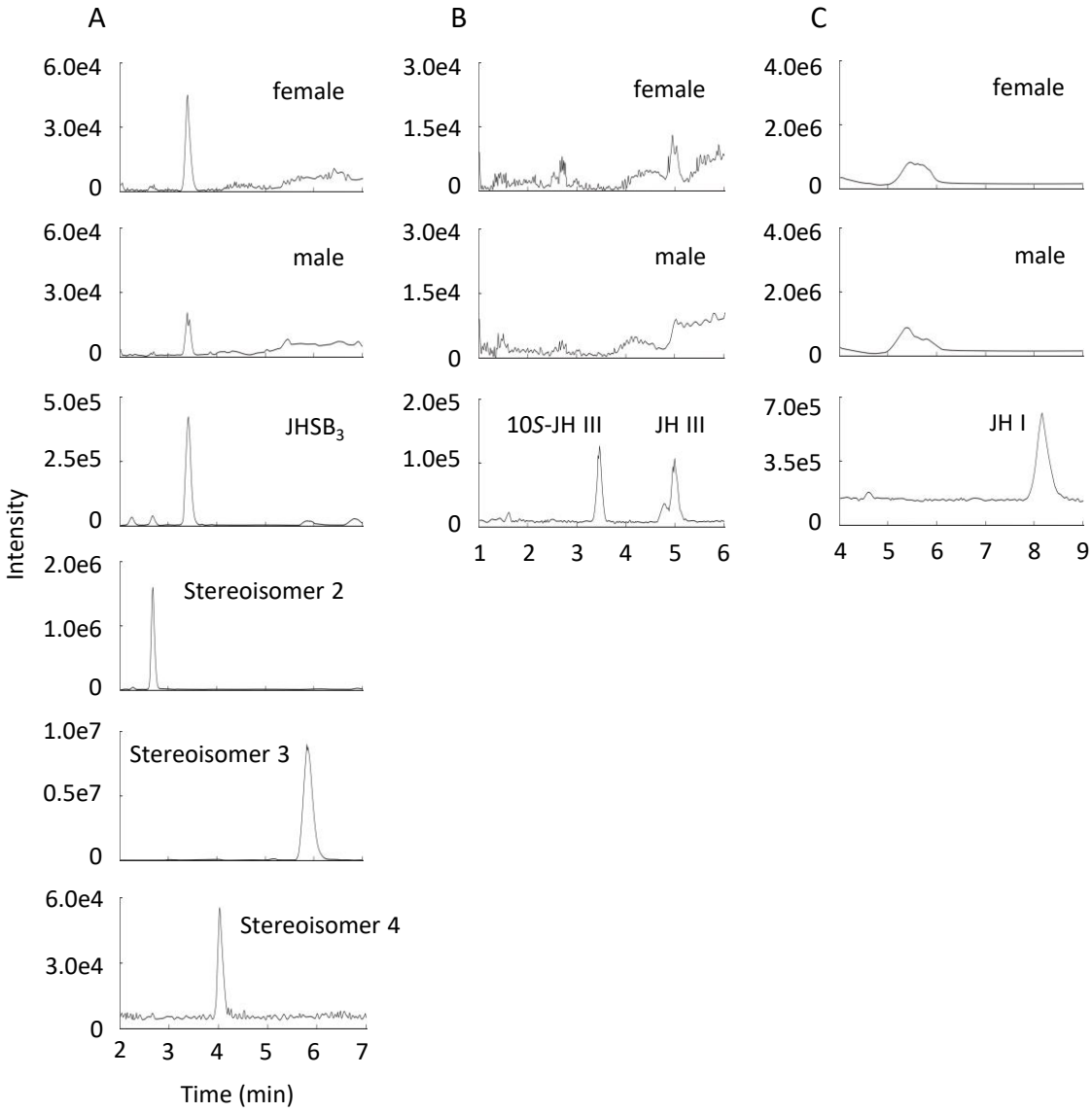

A
B
C
female
male
JHSB3
Stereoisomer 2
Stereoisomer 3
Stereoisomer 4
10S-JH III
JH III
JH I
Intensity
Time (min)

Fig. 3

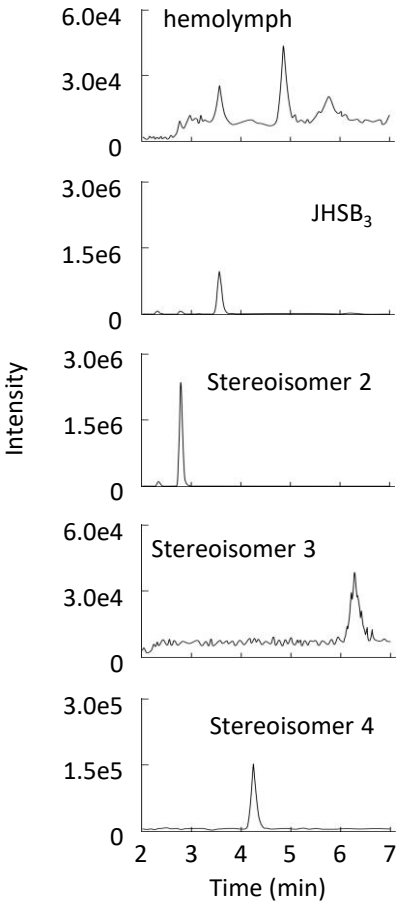

hemolymph
JHSB3
Stereoisomer 2
Stereoisomer 3
Stereoisomer 4
Intensity
6.0e4
3.0e4
0
3.0e6
1.5e6
0
3.0e6
1.5e6
0
6.0e4
3.0e4
0
3.0e5
1.5e5
0
2
3
4
5
6
7
Time (min)

Fig. 4

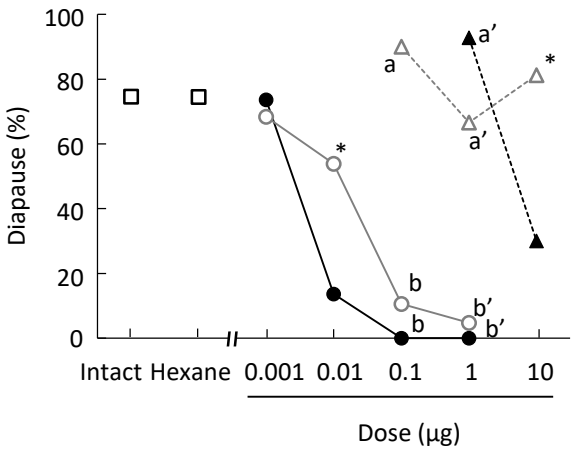

100
80
60
40
20
0
Diapause (%)
a
a′
a′
*
*
b
b
b′
b′
Intact Hexane 0.001 0.01 0.1 1 10
Dose (µg)

Fig. 5

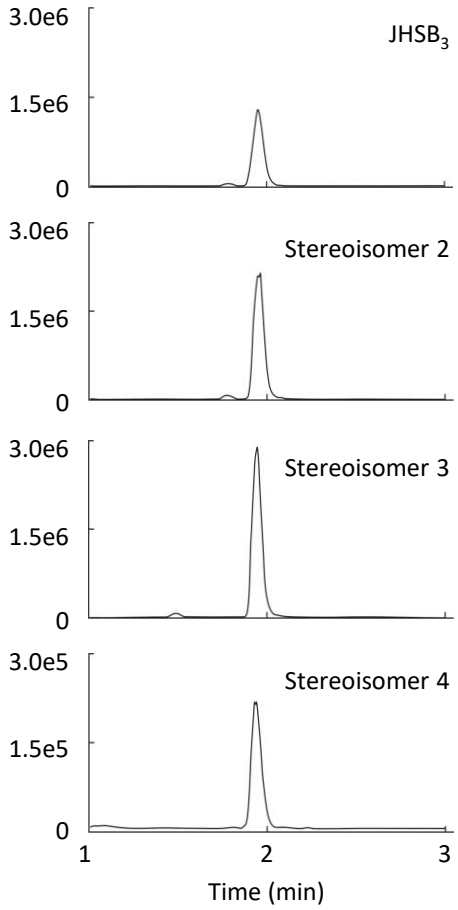

3.0e6
1.5e6
0
JHSB3
3.0e6
1.5e6
0
Stereoisomer 2
3.0e6
1.5e6
0
Stereoisomer 3
3.0e5
1.5e5
0
Stereoisomer 4
1
2
3
Time (min)

Fig. S1

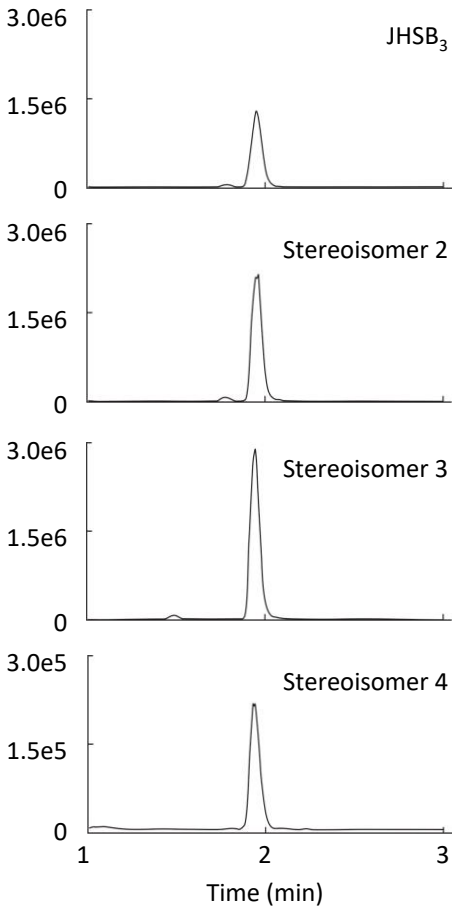


Fig. S1 The C18 UPLC-MS/MS analyses of the authentic JHSB3 and its stereoisomers (2-4). See Fig. 1 for their chemical structures