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Structure Determination of a New Juvenile Hormone from a Heteropteran Insect

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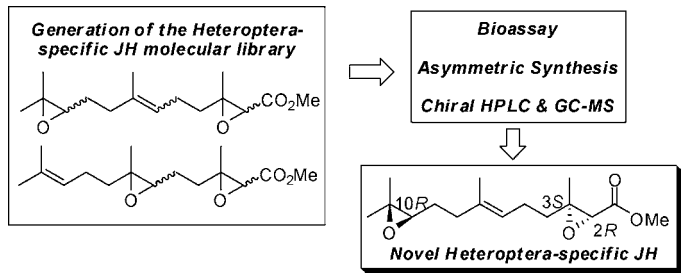
ABSTRACT



The structure of the juvenile hormone (JH) in the suborder Heteroptera, order Hemiptera, has been known for a very long time to be different from the JH of other orders, but the structure has been a matter of controversy. The structure was first elucidated by an unprecedented approach involving the screening of a JH molecular library. The novel Heteroptera-specific JH ($JHSB_3$) is a new category of JH that is featured by the skipped bisepoxide structure.

The juvenile hormone (JH) is an acyclic sesquiterpenoid that regulates many aspects of insect physiology, such as metamorphosis, reproduction, diapause, and polyphenisms (Figure 1).[1] In 1934, V. B. Wigglesworth first discovered JH in the blood-sucking bug, *Rhodnius prolixus* (suborder Heteroptera, order Hemiptera),[1,2] as a humoral factor that prevents metamorphosis of the final instar nymph into an adult. The structure of JH I (**1**) in the cecropia moth was first determined in 1967.[3,4] Since then, the structures of JHs (**2**−**6**) have been reported.[1,5] Heteroptera is a major insect group and includes ca. 40 000 species.[5] Many species in Heteroptera are serious pests of crops and gardens. Moreover, *Triatoma*, *Rhodnius*, and *Panstrongylus* are the most important triatomine vectors of the Chagas parasite. Despite the importance of heteropteran insects as experimental

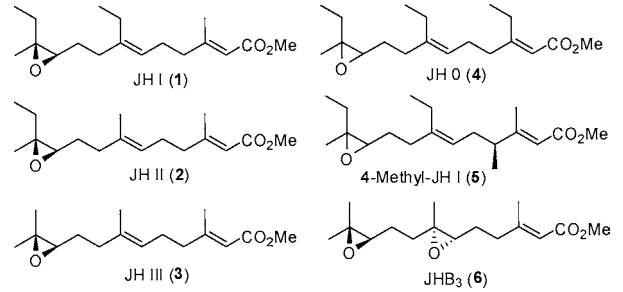


Figure 1. Structures of juvenile hormones.

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(1) Goodman, W. G.; Granger, N. A. *Comprehensive Molecular Insect Science*, Vol. 3; Gilbert, L. I., Iatrou, K., Gill, S. S., Eds.; Elsevier: Oxford, 2005; pp 319−408.
(2) Wigglesworth, V. B. *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 7; Kerkut, G. A., Gilbert, L. I., Eds.; Pergamon Press: Oxford, 1985; pp 1−24.
(3) Wigglesworth, V. B. *Q. J. Microsc. Sci.* **1934**, *77*, 191–222.
(4) Röller, H.; Dahm, K. H.; Sweely, C. C.; Trost, B. M. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 179–180.

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animals and also as agricultural and medical pests, the structure of JH in Heteroptera remains uncertain.[6] Therefore, structure determination of the heteropteran JH has been an important subject promoting not only physiological and biological studies, but also the development of Heteroptera-specific insecticides and growth regulators based on the Heteroptera-specific JH structure. Herein, we report the structure of the JH in *Plautia stali*, a member of the family Pentatomidae, suborder Heteroptera. The structure determination process was characterized by the screening of a JH molecular library. The stereoselective synthesis of the novel JH by catalytic asymmetric syntheses confirmed the absolute chemical structure of the novel skipped bisepoxide JH.

Purification of JH from the crude extract was key to the previous structure determination studies.[5] Extensive efforts have been made for collection of many insect samples and the purification of the crude mixture to provide the JH sample for the next spectroscopic analysis. In this study, we used a novel approach involving the construction of a JH molecular library, followed by its screening with the aim of shortening the structure determination process (Figure 2). In the final

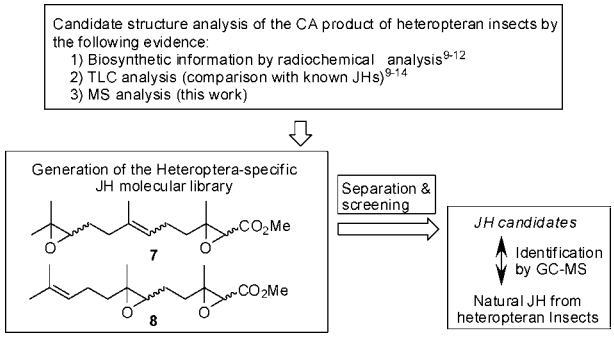


Figure 2. Strategy for structure determination of the Heteroptera-specific JH.

stage, we implemented identification of the final candidates of the natural JH by highly sensitive GC−MS.

Along these lines, we generated the JH molecular library based on the following experimental results.[7−12] JH is produced in the corpus allatum (CA) of insects. The biosynthetic activity of JH in the CA can be analyzed by *in vitro* incubation of the CA in the presence of *S*-[*methyl*-^{3}H] methionine, the ester methyl group precursor of JH.[1,5] This radiochemical assay was adopted to analyze the *in vitro* products of the CA of several heteropteran insects.[5,9−12] Results of radiochemical assay are summarized as follows: (i) the ^{3}H-labeled CA products were obtained from the CA incubation media; (ii) TLC analyses of the heteropteran CA products with the guidance of radioactivity indicated that the CA of these bugs produced ^{3}H-labeled products with the same R_f value, but the R_f values were not identical with those of JH III (**3**) and JHB_3 (**6**); and (iii) the addition of farnesol or farnesoic acid, known as a biosynthetic precursor of JH III (**3**) and JHB_3 (**6**) to the same CA incubation media enhanced the synthetic activity of the ^{3}H-labeled CA products. These results suggested the presence of a farnesene skeleton and a methyl group in the Heteroptera-specific JH.

To gain further structural information, we analyzed the CA product in *P. stali* by GC−MS using a normal capillary column to provide a major peak at 16.2 min. CI−MS (NH_3) analysis of the major peak showed the $[M + NH_4]^+$ ion at *m*/*z* 300 and the $[M + H]^+$ ion at *m*/*z* 283 (Supporting Information Figure S1). The molecular formula of the CA product was estimated by FAB−MS to be $C_{16}H_{26}O_4$ (HRMS (*m*/*z*): $[C_{16}H_{26}O_4 + H]^+$ calcd for 283.1909, found 283.1885), indicating that the molecular formula was the same as that of JHB_3 (**6**) originally isolated from *Drosophila melanogaster*.[13] This result might imply the possibility that the Heteroptera-specific JH is a diastereomer of JHB_3 (**6**). However, this possibility was ruled out by the previous facts, in that the mixture of synthetic racemic JHB_3 (**6**) and its diastereomers prepared by epoxidation of racemic JH III (**3**) had the same R_f values.[13,14] On the basis of these results, we proposed the structure of Heteroptera-specific JH as one of the bisepoxide isomers **7** or **8** (Figure 2).

We constructed the JH molecular library containing **7** and **8** (a mixture of 32 stereoisomers) by the nonselective Darzens reaction[15,16] of a 6:4 geometric mixture of geranyl acetone (**9**) followed by a nonregioselective epoxidation reaction with *m*-CPBA (Figure 3A). The preliminary juvenilizing activity test of the synthetic mixture exhibited a potent juvenilizing effect on *P. stali* at a dose of 1 μg/insect (Figure 3B, Supporting Information Figure S2).[10,11] The presence of the target JH in the molecular library was supported by the GC−MS data of the JH molecular library (Supporting Information Figure S1). The synthetic JH mixture saparated to 5 peaks under the GC conditions. One of the peaks at 16.2 min was identical to that of the CA product by means of the coinjection of the CA product and the synthetic JH mixture.

An efficient separation of the mixture was the key to the next screening stage. We surveyed several separation conditions to facilitate fractionation of the structurally similar **7** and **8**, and found that the use of a chiral normal-phase column

(5) Morgan, E. D.; Wilson, I. D. *Miscellaneous Natural Products Including Marine Natural Products, Pheromones, Plant Hormones, and Aspects of Ecology*, Vol. 8; Mori, K., Eds.; Pergamon Press: Oxford, 1999; pp 263−369.
(6) Schaefer, C. W.; Panizzi, A. R., Eds. *Heteroptera of Economic Importance*; CRC Press: Boca Raton, FL, 2000.
(7) Davey, K. G. *Insect Biochem. Mol. Biol.* **2000**, *30*, 663–669.
(8) Menn, J. J., Beroza, M., Eds. *Insect Juvenile Hormones, Chemistry and Action*; Academic Press: New York, 1972.
(9) Kotaki, T. *Appl. Entomol. Zool.* **1993**, *28*, 242–245.
(10) Kotaki, T. *J. Insect Physiol.* **1996**, *42*, 279–286.
(11) Hodková, M.; Okuda, T.; Wagner, R. *Eur. J. Entomol.* **1996**, *93*, 535–543.
(12) Miyawaki, R.; Tanaka, S. I.; Numata, H. *Formosan Entomol.* **2006**, *26*, 1–10.
(13) Richard, D. S.; Applebaum, S. W.; Sliter, T. J.; Baker, F. C.; Schooley, D. A.; Reuter, C. C.; Henrich, V. C.; Gilbert, L. I. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 1421–1425.
(14) Shiga, S.; Hamanaka, Y.; Tatsu, Y.; Okuda, T.; Numata, H. *Zool. Sci.* **2003**, *20*, 1199–1206.
(15) Hoare, J. H.; Yates, P. *J. Chem. Soc., Chem. Commun.* **1981**, 1126–1128.
(16) Mamaghani, M.; Tabatabaeian, K.; Ghanadzadeh, A.; Habibi, F. *Tetrahedron Lett.* **2003**, *44*, 4775–4777.

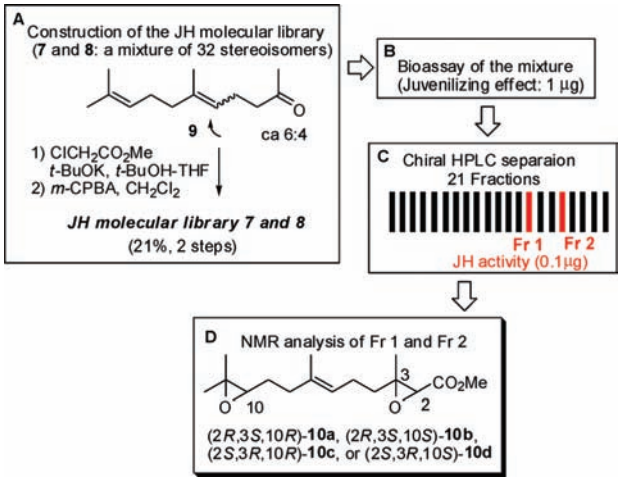
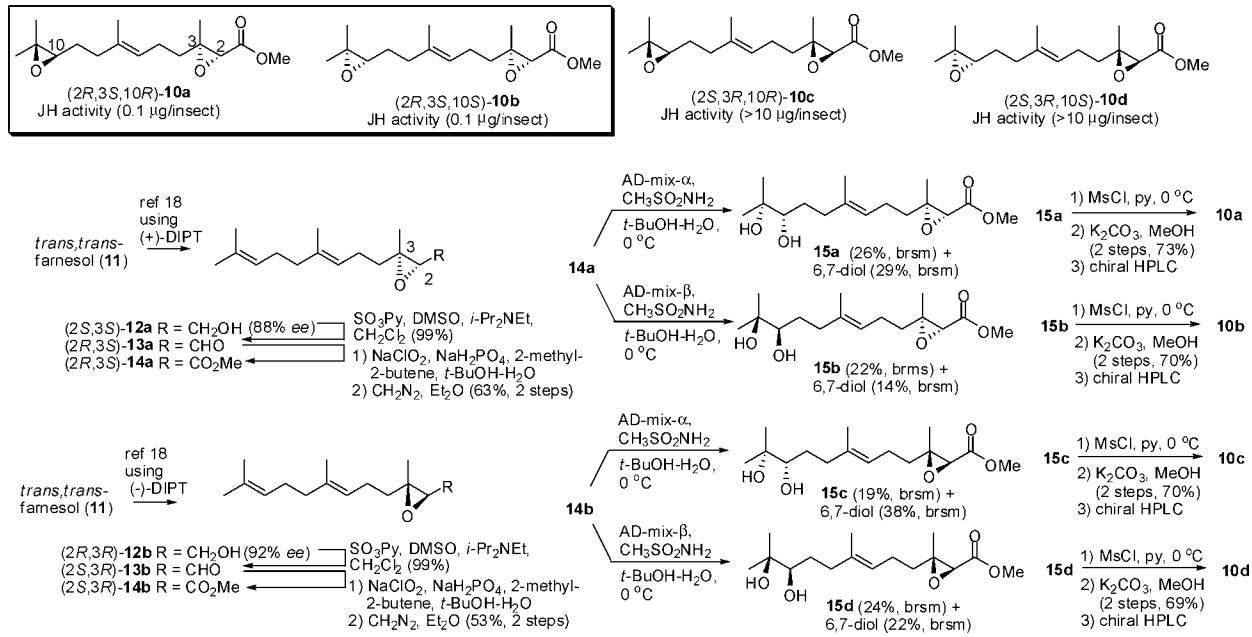


Figure 3. Selection of the candidate structures **10a**−**10d** from the JH molecular library. (A) Construction of the JH molecular library. (B) Juvenilizing effect of the JH molecular library. (C) Separation of the mixture using the chiral HPLC column and screening of each fraction. Fr 1 and Fr 2 showed potent juvenilizing activities (0.1 μg/insect). (D) Candidate structures of the biologically active Fr 1 and Fr 2 by NMR analysis.

significantly improved the fractionation to provide 21 fractions (Figure 3C, Supporting Information Figure S3). The juvenilizing activity test of each fraction led to the discovery of two biologically active fractions, Fr 1 and Fr 2, at a dose of 0.1 μg/insect for the complete inhibition of the metamorphosis. The other fractions did not show any potent juvenilizing activities at the same dose. These results encouraged us to examine the structure of Fr 1 and Fr 2 by NMR spectroscopy (Figure 3D). As a result, we speculated these structures to be **10a**, **10b**, **10c**, or **10d**. However, relative stereochemistry could not be determined because the NMR data of Fr 1 were similar to those of Fr 2 (Supporting Information Figure S4). These spectral similarities favorably supported an enantiomeric combination (**10a** and **10d**, or **10b** and **10c**) for Fr 1 and Fr 2. However, we could not exclude the other possibility of a diastereomeric relationship (e.g., **10a** and **10b**) because of the skipped bisepoxide structure that may not provide distinguishable spectroscopic differences between the diastereoisomers.

To determine the structures of Fr 1 and Fr 2, we carried out a stereoselective synthesis of **10a**-**10d** using catalytic asymmetric synthesis, their juvenilizing activity test, and a chiral HPLC study. *Trans*,*trans*-farnesol (**11**) was subjected to the Katsuki-Sharpless epoxidation[17] reaction to secure chiral stereocenters at C2 and C3 to provide the optically active (2*S*,3*S*)-epoxide **12a**[18,19] and (2*R*,3*R*)-**12b**,[20,21] respectively (>88% *ee*) (Scheme 1, Supporting Information synthetic procedure). We converted the chiral epoxides **12a** and **12b** to their corresponding methyl esters **14a** and **14b** in three steps. The Sharpless asymmetric dihydroxylation reaction using AD-mix-α or AD-mix-β[22,23] was applied to secure chiral stereocenter at C10. The resulting diols **15a**−**15d** were converted to their corresponding bisepoxides which, upon purification under the chiral HPLC condition, produced optically active **10a**−**10d** (Supporting Information Figures S5 and S6). Among them, **10a** and **10b** were identical to the two bioactive Fr 2 and Fr 1, respectively, with regard to their chiral HPLC behaviors and spectroscopic data. The juvenilizing activity study of **10a**−**10d** indicated that (i) **10a** and **10b** exhibited potent juvenilizing effects at a dose of 0.1 μg/insect and those of **10c** and **10d** were much less potent (>10 μg/insect for the complete inhibition of the

Scheme 1. Syntheses of Optically Pure Bisepoxides **10a**−**10d**



metamorphosis). Thus, the structures of Fr 1 and Fr 2 were assigned to be (2*R*,3*S*,10*S*)-**10b** and (2*R*,3*S*,10*R*)-**10a**, respectively.

We determined the structure of the natural JH by GC−MS analysis of **10a**, **10b**, and the CA product in *P. stali*. A total of 60 CA in *P. stali* were incubated to collect the CA product for this analysis (Figure 4).[10] To this end, we applied a chiral

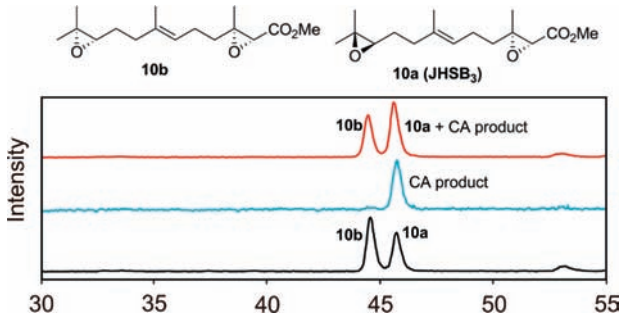


Figure 4. Chiral GC−MS analysis of synthetic **10a**, **10b** and the CA product of *Plautia stali*. Extracted ion chromatograms for $[M + NH_4]^+$ ions at *m*/*z* 300. Co-injection of **10a**, **10b**, and the CA product (red line). The CA product (blue line). Bisepoxides **10a** and **10b** (10 ng each) (black line). The retention time of the CA product was identical to that of **10a**. The MS (CI, NH_3) data are shown in the Supporting Information (Figure S8).

capillary GC column to separate the structurally similar **10a** and **10b**. Under the chiral GC−MS condition, **10a** and **10b** were distinctly separable. The retention time of the CA product was identical with that of **10a**. The MS spectrum pattern of the CA product was in good agreement with that of **10a** (Supporting Information Figure S7). We named the novel JH in *P. stali* as juvenile hormone III skipped bisepoxide ($JHSB_3$). This is a new category of JHs in that its bisepoxide arrangement is different from the precedent JHs. $JHSB_3$ (**10a**) was also detected in the hemolymph of reproductively active females of *P. stali* in our preliminary experiment, suggesting its release into the hemolymph as the biologically functional hormone.

It is suggested that the JH of several heteropteran insects including *P. stali* has been different from the known JHs.[6,9−12] In this context, $JHSB_3$ (**10a**) is a promising JH in heteropteran insects. Using the JH library led to the discovery of not only $JHSB_3$ (**10a**) but also the potentially active diastereomer **10b**. This result is interesting in the structure−activity relationship of the chiral epoxide moiety in JHs. Previously, the juvenilizing activities of JH I (**1**) and JH III (**3**) in Lepidoptera and their enatiomers are investigated. The bioactivities of the *ent*-**1** and *ent*-**3** were much less potent than those of the natural products, indicating that the chiral 10,11-epoxide moieties played a crucial role in the bioactivity.[24] In the case of $JHSB_3$ (**10a**) and **10b**, the stereochemical demand for the juvenilizing activity in *P. stali* was rigorous on the (2*R*,3*S*)-epoxide moiety. In contrast, the chirality of the 10,11-epoxide moiety was not necessarily essential for the bioactivity. This structure−activity relationship would benefit the design of Heteroptera-specific insecticides and growth regulators using the structure of $JHSB_3$ (**10a**) and **10b** as lead molecules.

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Supporting Information Available: Experimental and analytical procedures, and characterization of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) Katsuki, T.; Martin, V. *Org. React.* **1996**, *48*, 1–300.
(18) Syanik, M.; Ishibashi, H.; Ishihara, K.; Yamamoto, H. *Org. Lett.* **2005**, *7*, 1601–1604.
(19) Dittmer, D. C.; Discordia, R. P.; Zhang, Y.; Murphy, C. K.; Kumar, A.; Pepito, A. S.; Wang, Y. *J. Org. Chem.* **1993**, *58*, 718–731.
(20) Kigoshi, H.; Ojika, M.; Shizuri, Y.; Niwa, H.; Yamada, K. *Tetrahedron Lett.* **1982**, *23*, 5413–5414.
(21) Marshall, J. A.; Hann, R. K. *J. Org. Chem.* **2008**, *73*, 6753–6757.
(22) Noe, M. C.; Letavic, M. A.; Snow, S. L. *Org. React.* **2005**, *66*, 109–625.
(23) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483–2547.
(24) Sakurai, S.; Ohtaki, T.; Mori, H.; Fujiwhara, M.; Mori, K. *Experientia* **1990**, *46*, 220–221.