Structure Determination of a New Juvenile Hormone from a Heteropteran Insect

Toyomi Kotaki¹*, Tetsuro Shinada²*, Kanako Kaihara², Yasufumi Ohfune², and Hideharu Numata²

¹National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-8634, Japan.
²Graduate School of Science, Osaka City University, Osaka 558-8585, Japan.

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General method for analysis and synthesis of JH.

Reagents. All reagents and solvents were purchased from Sigma-Aldrich, Nacalai Tesque, or Tokyo Chemical Industry, and used without further purification unless otherwise indicated. Solvents of anhydrous grade were used without distillation.

Analytical instruments and conditions.

Optical rotation: JASCO Polarimeter P-1030. Solvent: CHCl₃ or CH₂OH.
FTIR: JASCO FT/IR-6200 infrared spectrophotometer. Zn/Se cell for neat condition.
NMR: JEOL JNM-LA400 or Bruker AVANCE 600 spectrometer.

Chemical shifts of ¹H-NMR were reported in parts per million (ppm, δ) relative to the residual solvent peaks in CDCl₃ (δ = 7.26) or C₆D₆. (δ = 7.04). Chemical shifts of ¹³C-NMR were reported in ppm (δ) relative to δ = 77.0 for CDCl₃ or δ = 124.0 for C₆D₆.

High resolution mass spectrometry (HRMS): a JEOL JMS-AX500 for fast atom bombardment ionization (FAB), chemical ionization (CI), or electron ionization (EI).

TLC analysis: TLC plates (silica gel 60 F-254, 0.25 mm layer thickness, manufactured by Merck. TLC visualization: UV lamp (254 nm) or developing by a charring solution of ethanoic phosphomolybdic acid.

Silica gel column chromatography: silica gel, Daisogel IR-60 1002W (40/63 mm).

Chiral HPLC analysis: Shimadzu Prominence [column, Chiralpak IA (Daicel, 0.46×25 cm); Solvent, 0.5% or 1% EtOH in hexane; flow rate, 1.0 mL/min; UV detector, 200 nm].

GC-MS analysis with a normal capillary GC column: Shimadzu GC-MS-QP2010 Plus; [column, DB-35MS (0.25 mmI.D., 30 m, 0.25 μm), carrier gas, He (50 cm/s), oven temp. 120-240 °C (7 °C/min); MS (CI, NH₃ or isobutene)].

Chiral GC-MS condition [column, Rt-β DEXcst (0.25 mmI.D., 30 m, 0.25 μm); carrier gas, He (50 cm/s); oven temp. 160 °C; MS (CI, NH₃)].

Animals, corpus allatum (CA) and hemolymph samples. A stock culture of P. stali was established from adults collected in Joso (formerly Mitsukaïdo), Ibaraki, Japan in 2001, and kept for more than 20 generations under controlled light conditions (16 h light: 8 h dark) at 25 °C in the laboratory. The scientific name, Plautia stali is used for...
this species in the present study according to the revision of the genus *Plautia*\(^1\), although the scientific name, *Plautia crossota stali* has been used for several years. Insects were reared on raw peanuts and dry soybeans with water supplemented with 0.05% sodium L-ascorbate and 0.025% L-cysteine\(^2\). The corpus allatum-corpus cardiacum complex with a small piece of aorta was taken from reproductively active adults through a hole made in the neck membrane, and incubated in a group of 5-10 complexes in 50 µl of the minimum essential medium (with Hank’s salt, without L-glutamate and sodium bicarbonate, added 20 mM of HEPES and 5 ppm of TWEEN 80, adjusted to pH 7.2) in a glass tube (6 mm i.d. and 30 mm long). After about 6 h of incubation at 30 °C, the medium was extracted with 50 µl of hexane three times. The pooled hexane layers containing CA product were stored at -20 °C until used.

A total of 50 µl of the hemolymph was collected from reproductively active females. Hexane extract of hemolymph samples was applied to a mini-column containing neutral alumina (activity III). After washing with 10% diethyl ether in hexane, the JH fraction was eluted with 50% diethyl ether, dried under a slow stream of nitrogen, and re-dissolved in a small volume of toluene. An aliquot was subjected to GC-MS analyses.

**Bioassay for juvenilizing activity**\(^2\). An aliquot of 1 µl of hexane solution containing test compound was applied to the dorsal side of the abdomen of last (5th) instar nymphs on the day or following day of the 4th ecdysis. After the next ecdysis, the length of forewing and scutellum as well as the width of pronotum were determined using an ocular micrometer of a dissecting microscope. Juvenilizing activity was assessed by degree of reduction in forewing and scutellum lengths relative to the width of the pronotum, based on 7-14 individuals for each dosage. The lengths of the forewing and the scutellum were reduced in the nymph-adult intermediate induced by applications of test compounds with juvenilizing-activity.

1. Liu, Q. & Zheng, L. *Entomotaxonomia* **16**, 235-248 (1994).
2. Kotaki, T. *J. Insect Physiol.* **42**, 279-286 (1996).
Synthetic procedure

Construction of JH molecular library (7 and 8)

![Diagram of synthetic procedure](image)

**Darzens reaction of 9: synthesis of methyl 3-(4,8-dimethylnona-3,7-dienyl)-3-methyloxirane-2-carboxylate.**

To a mixture of potassium t-butoxide (336 mg, 3 mmol) in t-BuOH (5 mL) and THF (1 mL), E- and Z-6,10-dimethyl undeca-5,9-dien-2-one (9) (288 mg, 2 mmol) was added. After the stirring for 15 min, methyl chloroacetate (238 mg, 2.2 mmol) was added to the mixture. The mixture was stirred for 12 h, quenched with saturated ammonium chloride, and extracted with hexane/ethyl acetate (1:1) (30 mL×2). The organic layers were washed with brine, dried over anhydrous MgSO₄, filtrated through a thin silica gel pad. The filtrate was concentrated under the reduced pressure. The residue was purified by silica...
gel column chromatography (hexane/ethyl acetate = 10:1 then 5:1) to give a mixture of the titled monoepoxides as a mixture of stereoisomers (186 mg, 35%) as pale yellow oil.

\[ ^1\text{H-NMR (400 MHz, CDCl}_3\text{) }\delta = 5.10-5.00 \text{ (m, 2H), 3.78 (s, 3H), 3.77 (s, 3H), 3.76 (s, 3H), 3.40-3.30 \text{ (m, 1H), 2.10-1.90 (m, 6H), 1.80-1.60 (m, 9H), 1.38 (s, 3H), 1.34 (s, 3H), 1.33 (s, 3H)}.\]

**Synthesis of biepoxide mixture 7 and 8.** To a mixture of the resulting mono-epoxide described above (130 mg, 0.5 mmol) in CH\(_2\)Cl\(_2\) (25 mL) and 5% aq NH\(_3\) (10 mL) was added 3-chloroperbenzoic acid (ca. 70%, 86 mg, 0.71 mmol) at 0 °C. The mixture was stirred for 1 h, partitioned with brine (20 mL) and ethyl acetate (20 mL), and extracted. The organic layer was dried over anhydrous MgSO\(_4\) and filtered. The filtrate concentrated under the reduced pressure. The residue was purified by silica gel chromatography (hexane/ethyl acetate = 5:1 to 3:1) to give a mixture of 7 and 8 as pale yellow oil (56.4 mg, 40%) with recovery of the starting monoepoxide (41.2 mg, 32%).

\[ ^1\text{H-NMR (400 MHz, CDCl}_3\text{) }\delta = 5.10-5.00 \text{ (m, 1H), 3.78 (s, 3H), 3.77 (s, 3H), 3.38 (s, 1H), 3.32 (s, 1H), 2.75-2.65 \text{ (m, 1H), 2.48-2.44 (m, 0.5H), 2.30-2.24 \text{ (m, 0.5H), 2.20-2.00 (m, 3H), 2.13 (s, 3H), 1.70-1.18 (m, 13H); HRMS (EI, m/z): [M]\text{]}^+ \text{ calcd for C}_{16}\text{H}_{26}\text{O}_4 282.1831, \text{ found 282.1823}.\]
Synthesis of JHSB₃ (10a) and its stereoisomers 10b-10d.

Synthesis of chiral epoxy esters 14a and 14b.

**Synthesis of (2R,3S)-aldehyde 13a.** According to the literature¹,², (2S,3S)-epoxide 12a [4.2 g, 17.6 mmol, [α]D²⁸ -6.2 (c 0.33, CHCl₃), 88% ee checked by the Mosher’s method using (+)-MTPA¹,²] in CH₂Cl₂ (180 mL) was added DMSO (12.5 mL) and i-Pr₂NEt (15.5 mL). To the mixture was added pyridine sulfate (11.2 g) in several portions at 0 °C with stirring. The mixture was stirred for 1.5 h and diluted with hexane (200 mL) and filtrated through a thin silica gel pad which was washed with hexane/AcOEt (5:1, 300 mL). The filtrate was concentrated under reduced pressure. The residue was purified on silica gel column chromatography (hexane/ethyl acetate = 10:1 then 4:1) to give (2R,3S)-aldehyde 13a: 4.15 g (99%); colorless oil; [α]D²⁶ +71.6 (c 0.70, CHCl₃); IR (neat): 2968, 2920, 2856, 1722, 1450, 1406, 1383, 1236 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃): δ = 9.46 (d, J = 5.0 Hz, 1H), 5.06-5.10 (m, 2H), 3.19 (d, J = 5.0 Hz, 1H), 2.17-2.04 (m, 4H), 2.00-1.97 (m, 2H), 1.78-1.70 (m, 1H), 1.68 (s, 3H), 1.60 (s, 6H), 1.60-1.53 (m, 1H), 1.45 (s, 3H); ¹³C-NMR (100 MHz, CDCl₃): δ = 199.5, 136.4, 131.4, 124.1, 122.4, 64.1, 63.5, 39.6, 38.3, 26.5, 25.6, 23.3, 17.6, 17.2, 16.0; HRMS (CI, m/z): [M+H]⁺ calcd for [C₁₅H₂₄O₂+H]⁺ 237.1854; found: 237.1852.

(2S,3R)-Aldehyde 13b. According to the same procedure described above, (2S,3R)-13b was prepared from (2R,3R)-epoxide 12b³,⁴ [92% ee checked by the Mosher method using (+)-MTPACl, [α]D²⁸ +6.2 (c 0.82, CHCl₃)]. Analytical data of 13b were identical with those of (2R,3S)-13 except for the sign of optical rotation: [α]D²⁷ -73.8 (c 0.98, CHCl₃).

1. Syanik, M.; Ishibashi, H.; Ishihara, K.; Yamamoto, H. Org. Lett. 2005, 7, 1601.
2. Dittmer, D. C.; Discordia, R. P.; Zhang, Y.; Murphy, C. K.; Kumar, A.; Pepito, A.
(2R,3S)-14a. To a solution of (2R,3S)-aldehyde 13a (3.44 g, 14.6 mmol) in t-BuOH-H2O (5:1; 250 mL) was added 2-methyl-2-butene (12 mL, 117 mmol), NaH2PO4·2H2O (6.8 g, 43.8 mmol), and 80% NaClO2 (2 g, 17.52 mmol) at 0 °C with stirring. The mixture was allowed to warm to room temperature, stirred for 30 min, and extracted with Et2O (100 mL×3). The combined organic layers were washed with brine (50 mL×2), dried over anhydrous MgSO4, and filtered. The filtrate was concentrated under reduced pressure to give the corresponding carboxylic acid. The crude carboxylic acid was subjected to the next step without further purification. To a solution of the crude carboxylic acid in Et2O (50 mL) was added a solution of diazomethane in Et2O generated from N-nitrosourea in 40% KOH/Et2O at 0 °C until a slightly excess amount of diazomethane (pale yellow) remained in the solution. The mixture was stirred for 0.5 h and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 20:1 then 1:5) to give (2R,3S)-methyl ester 14a (2.45 g, 63%, 2 steps) as colorless oil: [α]D26 -41.5 (c 1.35, CHCl3); FTIR (neat): 2970, 2918, 2860, 1759, 1441, 1408, 1385, 1292, 1203 cm⁻¹; ¹H-NMR (400 MHz, CDCl3) δ = 5.08 (brt, J = 6.0 Hz, 1H), 5.06 (brt, J = 6.0 Hz, 1H), 3.77 (s, 3H), 3.34 (s, 1H), 2.15-2.02 (m, 4H), 1.98-1.94 (m, 2H), 1.72 (dt, J = 14.5, 7.6 Hz, 1H), 1.66 (s, 3H), 1.58 (s, 3H), 1.58 (s, 3H), 1.57-1.50 (m, 1H), 1.34 (s, 3H); ¹³C-NMR (100 MHz, CDCl3) δ = 169.1, 136.2, 131.4, 124.2, 122.7, 62.6, 58.6, 52.2, 39.6, 37.8, 26.6, 25.7, 23.5, 17.7, 16.2, 16.0; HRMS (CI, m/z): [M+H]+ calcd for [C16H26O3+H]+ 267.1960, found 267.1959.

(2S,3R)-14b. According to the same procedure described above, (2R,3S)-14b (2.4 g, 53%, 2 steps) was synthesized from 13b (4.0 g, 16.9 mmol). Analytical data of (2R,3S)-14b were identical with those of (2R,3S)-14a except for the sign of optical rotation: [α]D26 +43.4 (c 0.75, CHCl3).
Synthesis of chiral bisepoxides 10a-10d

ADmix-α 11 10 7 6 3 2 OMe
(2R,3S)-14a

ADmix-β OH
15a
1) MsCl
2) K₂CO₃, MeOH
3) chiral HPLC

(2R,3S,10R)-10a

ADmix-α OH
15b
1) MsCl
2) K₂CO₃, MeOH
3) chiral HPLC

(2R,3S,10S)-10b

ADmix-β OH
15c
1) MsCl
2) K₂CO₃, MeOH
3) chiral HPLC

(2S,3R,10R)-10c

ADmix-β OH
15d
1) MsCl
2) K₂CO₃, MeOH
3) chiral HPLC

(2S,3R,10S)-10d

General procedure for the Sharpless asymmetric dihydroxylation reaction of 14a and 14b. The suspension of AD-mix-α or AD-mix-β (2.8 g), and CH₃SO₂NH₂ (570 mg, 6 mmol) in t-BuOH-H₂O (1:1, 20 mL) was vigorously stirred at 0 °C for 20 min. After the addition of the methyl ester 14 (532 mg, 2 mmol), the mixture was stirred at 0 °C for 19 h, quenched with Na₂SO₃ (3 g), and extracted with CH₂Cl₂ (10 mL). The aqueous layer was extracted with ethyl acetate (50 mL×3). The combined organic layers were dried over anhydrous MgSO₄ and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 10/1, 4/1, then 3/2) to give the desired chiral 10,11-diol 15a-15d containing other minor 10,11-diols, and the 6,7-diols, and recovery of starting monoepoxides 14a-d.

Product Yields of the epoxide forming process were summarized in Table S1. The diastereomeric ratios of the resulting diols 15a-15d were determined after the conversion to the bisepoxides 10a-d and their chiral HPLC analyses as shown in the next page. The regioselectivities (10,11-diol:6,7-diol, ca. 1:1~1:2) observed were lower than those of the dihydroxylation of farnesol and the related molecules 1,2. We carefully examined the reaction conditions and found that the product ratio of the 6,7-diols were increased even at the lower temperature. These details will be reported in due course.
Table S1

| Substrate | Reagent | Yield (%) of diols and recovery of the starting material (%) |
|-----------|---------|----------------------------------------------------------|
| (2R,3S)-14a | AD-mix-α | 15a + other 10,11-diols (18), 6,7-diols (20), 14a (30) |
|           | AD-mix-β | 15b + other 10,11-diols (19), 6,7-diols (12), 14a (12) |
| (2S,3R)-14b | AD-mix-α | 15c + other 10,11-diols (15), 6,7-diols (30), 14b (21) |
|           | AD-mix-β | 15d + other 10,11-diols (20), 6,7-diols (18), 14b (18) |

15a: other 10,11-diols = 89:11. 15b: other 10,11-diols = 91:9. 15c: other 10,11-diols = 87:13. 15d: other 10,11-diols = 93:7. The product ratios of 15a-15b were determined after conversion of the 10,11-diols to the corresponding bisepoxides 10a-10d, followed by their chiral HPLC analysis (Table S2 in the next page).

1. Crispino, G. A.; Sharpless, K. B. *Synthesis* 1993, 77.

2. Okochi, T., Mori, K. *Eur. J. Chem.* 2001, 2145.

15a: colorless oil; [α]_D<sup>27</sup> -41.2 (c 0.56, CHCl₃); IR (neat): 3425, 2974, 1753, 1442, 1297, 1211 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃): δ = 5.19 (m, 1H), 3.79 (s, 3H), 3.35 (s, 1H), 3.35 (dd, J = 10.5, 1.94 Hz, 1H), 2.27-2.07 (m, 4H), 1.73 (m, 1H), 1.63 (s, 3H), 1.61 (m, 2H), 1.41 (m, 1H), 1.36 (s, 3H) 1.20 (s, 3H) 1.16 (s, 3H); ¹³C-NMR (100 MHz, CDCl₃): δ = 169.1, 135.9, 123.5, 77.8, 73.0, 62.6, 58.5, 52.2, 37.6, 36.5, 29.4, 26.4, 23.4, 23.2, 16.1, 15.8; HRMS-CI: m/z [M+H]⁺ calcd for [C₁₆H₂₈O₅+H]⁺ 301.2015; found: 301.2017.

15b: [α]_D<sup>27</sup> -17.1 (c 0.44, CHCl₃); IR (neat): 3471, 2970, 2866, 1755, 1443, 1387, 1294, 1209 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃): δ = 5.17 (m, 1H), 3.78 (s, 3H), 3.35 (s, 1H), 3.33 (dd, J = 10.5, 1.94 Hz, 1H), 2.24 (m, 1H), 2.03-2.16 (m, 3H), 1.74 (m, 1H), 1.63-1.55 (m, 2H), 1.61 (m, 2H), 1.41 (m, 1H), 1.36 (s, 3H) 1.20 (s, 3H) 1.16 (s, 3H); ¹³C-NMR (100 MHz, CDCl₃): δ = 169.1, 136.0, 123.3, 78.0, 73.0 62.6, 58.6, 52.2, 37.6, 36.7, 29.5, 26.4, 23.4, 23.3, 16.1, 15.9; HRMS-CI: m/z [M+H]⁺ calcd for [C₁₆H₂₈O₅+H]⁺ 301.2015; found: 301.2026.

15c: Analytical data of 15c were identical with those of 15b except for the sign of optical rotation. [α]_D<sup>27</sup> +17.5 (c 0.66, CHCl₃).

15d: Analytical data of 15d were identical with those of 15a except for the sign of optical rotation. [α]_D<sup>27</sup> +43.8 (c 0.84, CHCl₃).
General procedure for the synthesis of bisepoxides 10a-10d.

To a mixture of 10,11-diol 15 (126 mg, 0.42 mmol) and pyridine (1.35 mL, 16.8 mmol) in CH₂Cl₂ (13.5 mL) was added mesyl chloride (0.33 mL, 4.3 mmol) at 0 °C. The mixture was stirred at room temperature for 4 h and extracted with CH₂Cl₂ (13.5 mL). The organic layer was washed with saturated aq CuSO₄ (20 mL×2), H₂O (20 mL×2), and brine (20 mL×2), dried over anhydrous MgSO₄, and filtered. The filtrate was concentrated under reduced pressure to give the corresponding mesylate. The crude mesylate was subjected to the next step without further purification. To a solution of the crude mesylate in MeOH (27 mL) was added K₂CO₃ (673 mg, 4.87 mmol). The suspension was stirred vigorously for 30 min and filtered through a thin Florisil pad. The filtrate was concentrated under reduced pressure. The residue was partitioned with hexane (10 mL) and H₂O (20 mL) and extracted with hexane (30 mL×3). The combined organic layers were dried over anhydrous MgSO₄ and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 30/1, 10/1, then 5/1) to give the corresponding bisepoxides 10a-10d. The product yields were given for the diastereomeric bisepoxide mixtures.

Table S2.

| Starting materials | Product ratio of bisepoxides 10a-10d (Yield)³⁴ |
|--------------------|--------------------------------------------------|
| 15a+other minor 10,11-diols | 10a:10b:10c:10d = 88.9 : 5.7 : 5.1 : 0.3 (86.6 mg, 73%) |
| 15b+other minor 10,11-diols | 10a:10b:10c:10d = 1.3 : 91.5 : 0.2 : 7.0 (83.0 mg, 70%) |
| 15c+other minor 10,11-diols | 10a:10b:10c:10d = 7.0 : 0.7 : 87.0 : 5.3 (83.0 mg, 70%) |
| 15d+other minor 10,11-diols | 10a:10b:10c:10d = 0.1: 5.0 : 1.4 : 93.5 (81.9 mg, 69%) |

³Product ratio was determined by chiral HPLC analysis (Fig. S8). ⁴Product yields for the 2 steps.

A part of the resulting bisepoxide was purified under the chiral HPLC condition (0.5% EtOH/hexane, Fig. S5). Analytical data for the optically active 10a-10d are as follows. ¹H- and ¹³C-NMR spectra were show in Fig. S6.
**JHSB₃ (10a)**: colorless oil; [α] D₂⁵ -42.1 (c 1.00, MeOH); FTIR (neat) 2962, 2931, 1759, 1458, 1387, 1205 cm⁻¹; ¹H-NMR (600 MHz, C₆D₆) δ = 5.06 (dd, J = 6.9, 6.5 Hz, 1H), 3.30 (s, 3H), 3.20 (s, 1H), 2.55 (ddd, J = 6.9, 6.5 Hz, 1H), 2.10 (dd, J = 14.4, 8.8, 6.6 Hz, 1H), 2.00 (dd, J = 14.4, 7.9, 6.6 Hz, 1H), 1.95 (m, 2H), 1.60-1.49 (m, 2H), 1.48 (brdt, J = 14.0, 7.6 Hz, 1H), 1.44 (s, 3H), 1.33 (brdt, J = 14.0, 7.6 Hz, 1H), 1.26 (s, 3H), 1.15 (s, 3H), 1.10 (s, 3H); ¹³C-NMR (150 MHz, C₆D₆) δ = 168.7, 135.3, 123.8, 63.4, 62.0, 58.4, 57.3, 51.4, 37.9, 36.8, 27.9, 24.9, 23.7, 18.9, 16.2, 15.9; HRMS (CI) m/z [M+H]+ calcd for [C₁₆H₂₆O₄+H]+ 283.1909, found 283.1909.

**10b**: colorless oil; [α] D₂⁵ -58.1 (c 0.56, MeOH); FTIR (neat) 2960, 2926, 1759, 1441, 1383, 1290, 1252, 1205, 1034 cm⁻¹; NMR spectra of 10b was superimposable with those of 10a: ¹H-NMR (600 MHz, C₆D₆) δ = 5.06 (dd, J = 6.9, 6.5 Hz, 1H), 3.30 (s, 3H), 3.20 (s, 1H), 2.55 (dd, J = 6.9, 6.5 Hz, 1H), 2.10 (dd, J = 14.4, 8.8, 6.6 Hz, 1H), 2.00 (dd, J = 14.4, 7.9, 6.6 Hz, 1H), 1.95 (m, 2H), 1.60-1.49 (m, 2H), 1.48 (brdt, J = 14.0, 7.6 Hz, 1H), 1.44 (s, 3H), 1.33 (brdt, J = 14.0, 7.6 Hz, 1H), 1.26 (s, 3H), 1.15 (s, 3H), 1.10 (s, 3H); ¹³C-NMR (150 MHz, C₆D₆) δ = 168.7, 135.3, 123.8, 63.4, 62.0, 58.4, 57.3, 51.4, 37.9, 36.8, 27.9, 24.9, 23.7, 18.9, 16.2, 15.9; HRMS (CI) m/z [M+H]+ calcd for [C₁₆H₂₆O₄+H]+ 283.1909, found 283.1899.

**10c**: Spectroscopic data of 10c were identical with those of 10b except for the sign of the optical rotation. colorless oil; [α] D₂⁵ +52.9 (c 0.67, MeOH). HRMS (CI) m/z [M+H]+ calcd for [C₁₆H₂₆O₄+H]+ 283.1909, found 283.1900.

**10d**: Spectroscopic data of 10d were identical with those of 10a except for the sign of the optical rotation. colorless oil; [α] D₂⁵ +40.2 (c 1.00, MeOH). HRMS (CI) m/z [M+H]+ calcd for [C₁₆H₂₆O₄+H]+ 283.1909, found 283.1905.
Figure Legend

Fig. S1 GC-MS (Cl, NH\textsubscript{3}) analysis of CA product, the bisepoxide mixture of 7 and 8, and their co-injection. (A) CA product. (B) Bisepoxide mixture of 7 and 8. (C) co-injection (a mixture of CA product and the bisepoxide mixture). A major peak of the CA product (16.2 min) showed the [M+NH\textsubscript{4}]\textsuperscript{+} ion at \textit{m/z} 300 and [M+H]\textsuperscript{+} ion at \textit{m/z} 283. The GC-MS analysis of the bisepoxide mixture provided 5 major peaks. Each peak showed the [M+NH\textsubscript{4}]\textsuperscript{+} ion at \textit{m/z} 300. Co-injection experiment allowed enhancement of the peak intensity at 16.2 min.

Fig. S2 Juvenilizing activity of the synthetic mixture of 7 and 8. Effect of treatment of young last instar nymphs with the bisepoxide mixture on lengths of forewing (upper) and scutellum (lower) relative to the width of pronotum after the next molt. S on the horizontal axis indicates solvent control. Error bars, S.D (\(n=7\)). Photographs, Normal last instar nymph (left) and normal adult (middle) and a nymph-adult intermediate (right) obtained by mixture treatment. Blue, red, and green arrows indicate forewing and scutellum lengths, and pronotum width, respectively. Scale bar: 5 mm.

Fig. S3 Chiral HPLC analysis of the JH molecular library. Each fraction was subjected to the juvenilizing activity test in a dose of 0.1 \textmu g/insect. Fr 1 and Fr 2 exhibited potent juvenilizing activities. HPLC solvent: 0.5% EtOH/hexane.

Fig. S4 NMR data (600 MHz, C\textsubscript{6}D\textsubscript{6}) of biologically active fractions, Fr 1 and Fr 2. (A) \textsuperscript{1}H-NMR data of Fr 1. (B) \textsuperscript{1}H-NMR data of Fr 2. (C) Selected NOESY data of Fr 1. Correlations are shown as red curves. The NOESY spectrum of Fr 2 was similar to that of Fr 1.

Fig. S5 Chiral HPLC data of bisepoxides 10a-10d. (A) Chiral HPLC analysis of the JH molecular library (the same data shown in Fig. S3). (B) Chiral HPLC data of a mixture of 10a-10d. Retention times of Fr 1 and Fr 2 were identical with those of 10b and 10a, respectively.

Fig. S6 NMR and MS (Cl, isobutene) data of optically active 10a-10d.
**Fig. S7** CI-MS (NH$_3$) data of 10a, 10b, and the CA product. The chiral GC-MS data were depicted in Fig. 4. The [M+NH$_4$]$^+$ ion at m/z 300 and [M+H]$^+$ ions at m/z 283 were observed, respectively.

**Fig. S8** Chiral HPLC analysis of the bisepoxides 10a-10d. HPLC solvent (1% EtOH/hexane).
(A) Chromatogram for the epoxidation product (major isomer: 10a).
(B) Chromatogram for the epoxidation product (major isomer: 10b).
(C) Chromatogram for the epoxidation product (major isomer: 10c).
(D) Chromatogram for the epoxidation product (major isomer: 10d).
Each product ratio was summarized in Table S2 in P10.
Fig. S1 GC-MS (Cl, NH₃) analysis of CA product, the bisepoxide mixture of 7 and 8, and their co-injection. (A) CA product. (B) Bisepoxide mixture of 7 and 8. (C) Co-injection (a mixture of CA product & the bisepoxide mixture). A major peak of the CA product (16.2 min) showed the [M+NH₄]⁺ ion at m/z 300 and [M+H]⁺ ion at m/z 283. The GC-MS analysis of the bisepoxide mixture provided 5 major peaks. Each peak showed the [M+NH₄]⁺ ion at m/z 300. Co-injection experiment allowed enhancement of the peak intensity at 16.2 min.
Fig. S2 Juvenilizing activity of the synthetic mixture of 7 and 8. Effect of treatment of young last instar nymphs with the bisepoxide mixture on lengths of forewing (upper) and scutellum (lower) relative to the width of pronotum after the next molt. S on the horizontal axis indicates solvent control. Error bars, S.D ($n=7$). Photographs, Normal last instar nymph (left), normal adult (middle), and a nymph-adult intermediate (right) obtained by mixture treatment. Blue, red, and green arrows indicate forewing and scutellum lengths, and pronotum width, respectively. Scale bar: 5 mm.
Fig. S3 Chiral HPLC analysis of the JH molecular library. Each fraction was subjected to the juvenilizing activity test in a dose of 0.1 μg/insect. Fr 1 and Fr 2 exhibited potent juvenilizing activities. HPLC solvent: 0.5% EtOH/hexane.
Fig. S4  NMR data (600 MHz, C₆D₆) of biologically active fractions, Fr 1 and Fr 2. (A) $^1$H-NMR data of Fr 1. (B) $^1$H-NMR data of Fr 2. (C) Selected NOESY data of Fr 1. Correlations are shown as red curves. NOESY data of Fr 2 was similar to that of Fr 1.
Fig. S5  Chiral HPLC data of bisepoxides 10a-10d. (A) Chiral HPLC analysis of the JH molecular library (the same data shown in Fig. S3). (B) Chiral HPLC data of a mixture of 10a-10d. Retention times of Fr 1 and Fr 2 were identical with those of 10b and 10a, respectively.
Fig. S6  NMR and MS (CI, isobutene) data of optically active 10a-10d.

$^1$H-NMR of JHSB$_3$ (10a)  

\[
\begin{align*}
(2R,3S,10R)-10a
\end{align*}
\]

$^13$C-NMR of JHSB$_3$ (10a)  

\[
\begin{align*}
(2R,3S,10R)-10a
\end{align*}
\]

MS data of 10a (CI, isobutene: [M+H]$^+$ m/z 283)
(Fig. S6, continued)

**1H-NMR of 10b**

![1H-NMR spectrum of 10b](image)

**13C-NMR of 10b**

![13C-NMR spectrum of 10b](image)

**MS data of 10b (CI, isobutene: [M+H]+ m/z 283)**

![MS spectrum of 10b](image)
(Fig. S6, continued)

$^1$H-NMR of 10c

\[(2S,3R,10R)-10c\]

$^{13}$C-NMR of 10c

\[(2S,3R,10R)-10c\]

MS data of 10c (CI, isobutene: [M+H]$^+$ m/z 283)
(Fig. S6, continued)

$^1$H-NMR of 10d

$^{13}$C-NMR of 10d

MS data of 10d (CI, isobutene: [M+H]$^+$ m/z 283)
MS data of 10a

![Diagram of 10a](image)

MS data of 10b

![Diagram of 10b](image)

The CA product

![Diagram of CA product](image)

Fig. S7 CI-MS (NH₃) data of 10a, 10b, and the CA product. The chiral GC-MS data were depicted in Fig 4. The [M+NH₄]⁺ ion at m/z 300 and [M+H]⁺ ions at m/z 283 were observed, respectively.
Fig. S8 Chiral HPLC analysis of the bisepoxides 10a-10d. HPLC solvent (1% EtOH/hexane). Each product ratio was summarized in Table S2 in P10.

(A) Chromatogram for the epoxidation product (major isomer: 10a).
(B) Chromatogram for the epoxidation product (major isomer: 10b).
(C) Chromatogram for the epoxidation product (major isomer: 10c).
(D) Chromatogram for the epoxidation product (major isomer: 10d).
(Fig. S8, continued)

(C)

(D)
NMR data of JHSB₃ (10a) and its stereoisomers 10b-10d (Enlarge data)

Signal assignments: See p 11.
$^1$H-NMR of JHSB$_3$ (10a)

(2R,3S,10R)-10a
$^{13}$C-NMR of JHSB$_3$ (10a)

(2R,3S,10R)-10a

$\text{HOMO}$

$\text{LUMO}$

Current Data Parameters
NAME: mdm13
EXPGD: R1
PROCNO: 1

F2 - Acquisition Parameters
DATE: 20040113
TIME: 11:49

INSTRUM: spect
PROBID: 5 nm BRD BB-18
FILFUL: 29p+30
TD: 65,536
SOLVENT: CDCl$_3$
NS: 4096
DW: 0
SW1: 0
SWR: 0.000000 Hz
AQ: 0.823600 sec
RS: 6.00 usec
UE: 6.00 usec
TE: 229.000 sec
DELTA: 5.0000000 sec
d1: 0.60000000 sec
DELTA: 4.90000000 sec
TB0: 1

--- CHANNEL 1 ---
BUC1: $^{13}$C
F1: 9.00 usec
F11: 1.00 dB
SF01: 150.9288756 MHz

--- CHANNEL 2 ---
CPDPrC2: wait316
BUC2: 18
CFP02: 9.00 usec
F12: 120.00 usec
F112: 14.40 dB
F1L3: 31.00 dB
SF02: 600.1525316 MHz

F2 - Processing parameters
e: 65256
e: 150.9077183 MHz
WOW: 0
LB: 2.00 Hz
GB: 0
FC: 0.05
$^1$H-NMR of 10b

(2R,3S,10S)-10b

Current Data Parameters
NAME: nmr13
EXPNO: 93
PROCNO: 1

#3 - Acquisition Parameters
Data_ 20000134
Time 9.52
INSTRUM spact
DROBB 5 mm DD1 1H,LB
FIDRES 60 kHz
T1 60000
SOLVENT Pyr
NS 4
DG 4
T2 12376.237 Hz
P2 0.198846 Hz
AQ 2.6477449 sec
BG 114
E2 40.400 usec
OK 6.00 usec
TE 298.0 K
D1 1.00000000 sec
T10 1

----------------- CHANNEL T1 -----------------
NUC1 19
D1 9.30 usec
PL1 3.50 usec
2P01 600.1300006 MHz

#2 - Processing parameters
SI 32768
ZF 600.13000674 MHz
HMD 64
DD 0
LB 0.30 Hz
CB 0
DC 0.00
$^{13}$C-NMR of 10b

(2R,3S,10S)-10b

**Current Data Parameters**
- **NAME**: pmhi13
- **MOD**: 2
- **PROCNO**: 1

**f7 - Acquisition Parameters**
- **Date**: 20200113
- **Time**: 14:02
- **INSTRUM**: spect
- **PRF**: 5000 MHz MMR 1-W
- **PULPROG**: zp9516
- **TD**: 65536
- **SOFT**: 0626
- **NS**: 858
- **DS**: 0
- **DM**: 39370.006 Hz
- **FIDRES**: 6000740 Hz
- **ACQ**: 8323699 sec
- **AQ**: 2894.3
- **WN**: 12.799 use
- **DA**: 6.90 use
- **TE**: 298.0 K
- **DG**: 5.00000000 sec
- **d1**: 0.00000000 sec
- **DELTA**: 4.99000010 sec
- **TOE**: 1

**CHANNEL f1**
- **N1C1**: 13C
- **F1**: 9.90 use
- **P11**: 1.60 dm
- **SF01**: 150.920973 Hz

**CHANNEL f2**
- **S2**: 9536
- **SF02**: 600.132916 MHz

**f7 - Processing parameters**
- **SI**: 8536
- **SF**: 150.927704 MHz
- **WCM**: EM
- **EN**: 0
- **G0**: 0
- **LG**: 2.00 Hz
- **GS**: 0
- **FC**: 0.05
$^{1}$H-NMR of $10c$

(2S,3R,10R)-$10c$
$^{13}$C-NMR of 10c

(2S,3R,10R)-10c
$^1$H-NMR of 10d

(2S,3R,10S)-10d
$^{13}$C-NMR of 10d

\[
\text{(2S,3R,10S)-10d}
\]

Current Data Parameters
- NAME: ndic13
- PROCNO: 6
- END

#2 - Acquisition Parameters
- Date: 20080113
- TIME: 12:07
- INSTRUM: spect
- PROBE: 5HB MBC HS-1H
- PULPROG: zgmp30
- TD: 633/6
- SOLVENT: CDCl3
- NSQ: 658
- DS: 0
- BARKES: 30370.97 Hz
- FIDRES: 0.000940 Hz
- AD: 0.8323699 sec
- DL: 78.6 sec
- CPU: 12.76 sec
- TR: 5.00 use
- TE: 128.0 K
- O1: 5.08269000 sec
- d11: 0.3800000 sec
- DELTA: 4.90000010 sec

--- CHANNEL 1 ---
- MDC1: 14C
- P1: 9.00 use
- ps1: -1.60 da
- SFO1: 150.9299573 MHz

--- CHANNEL 2 ---
- APPHRE2: valx12
- MDC2: 1H
- PCE2: 95.00 use
- P2: 129.00 da
- ps2: 11.60 da
- DL13: 11.00 da
- SFO2: 0.5232916 MHz

#2 - Processing parameters
- SI: 63.3K
- GF: 150.9027770 MHz
- MDS: 0
- SR: 0
- FG: 2.00 Hz
- GC: 0
- FC: 0.05