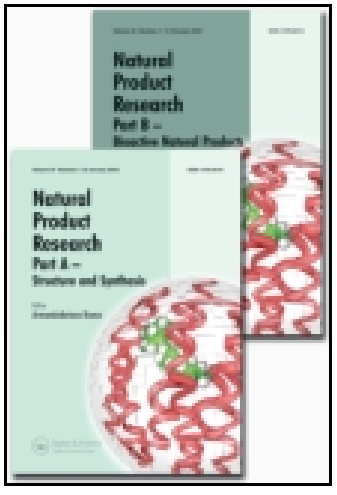

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First preparation of single-enantiomer juvenile hormone III acid and (R)-juvenile hormone III-d $_3$

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First preparation of single-enantiomer juvenile hormone III acid and (*R*)-juvenile hormone III-d_3

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The (*R*)- and (*S*)-enantiomers of juvenile hormone (JH) III acid [(*R*)-**2** and (*S*)-**2**] were prepared by the hydrolysis of (*R*)- and (*S*)-JH III [(*R*)-**1** and (*S*)-**1**], respectively. Each enantiomer of **2** was purified by preparative reversed-phase high performance liquid chromatography in a single operation. (*RS*)-**2** was methylated with CH_3I and K_2CO_3 in MeCN, yielding (*RS*)-**1**. (*R*)-JH III-d_3 [(*R*)-**3**], a single-enantiomer internal standard for quantification, was prepared from (*R*)-**2** with CD_3I and K_2CO_3 in MeCN.

Keywords: enantiomer; juvenile hormone; JH; JH III acid; JH III-d_3

1. Introduction

Juvenile hormone (JH) III [(*R*)-**1**] was first identified from organ cultures of corpora allata of the tobacco hornworm moth, *Manduca sexta* (Judy et al., 1973) (Figure 1). The titres of JHs in haemolymph control insect development and reproduction. In the final step of JH biosynthesis, the JH acids (Ismail, Satyanarayana, Bradfield, Dahm, & Bhaskaran, 1998) are converted to their active forms by JH acid methyltransferase (JHAMT) in Lepidopterans (Goodman & Granger, 2005; Shinoda & Itoyama, 2003). Thus, JHAMT has become an important target for the development of novel insect growth regulators. Currently, we are attempting to supply single-enantiomer JH III acid (**2**) for the kinetic study of the JHAMT-catalysed methylation. For this purpose, enantiopure (*R*)-**2** and (*S*)-**2** were prepared by the hydrolysis of (*R*)-**1** and (*S*)-**1**, respectively. The methylation of (*RS*)-**2** to produce (*RS*)-**1** was accomplished with CH_3I and K_2CO_3 in MeCN (Sekiguchi et al., 2008). Finally, (*R*)-JH III-d_3 [(*R*)-**3**] was also prepared from (*R*)-**2** using CD_3I for liquid chromatography–mass spectrometry (LC–MS) quantification. JHs are fragile biomolecules; therefore, the purification of JHs and their derivatives is a practical problem in insect endocrinology. Here, we report the practical chemical conversion and efficient purification of JH III (**1**) and JH III acid (**2**).

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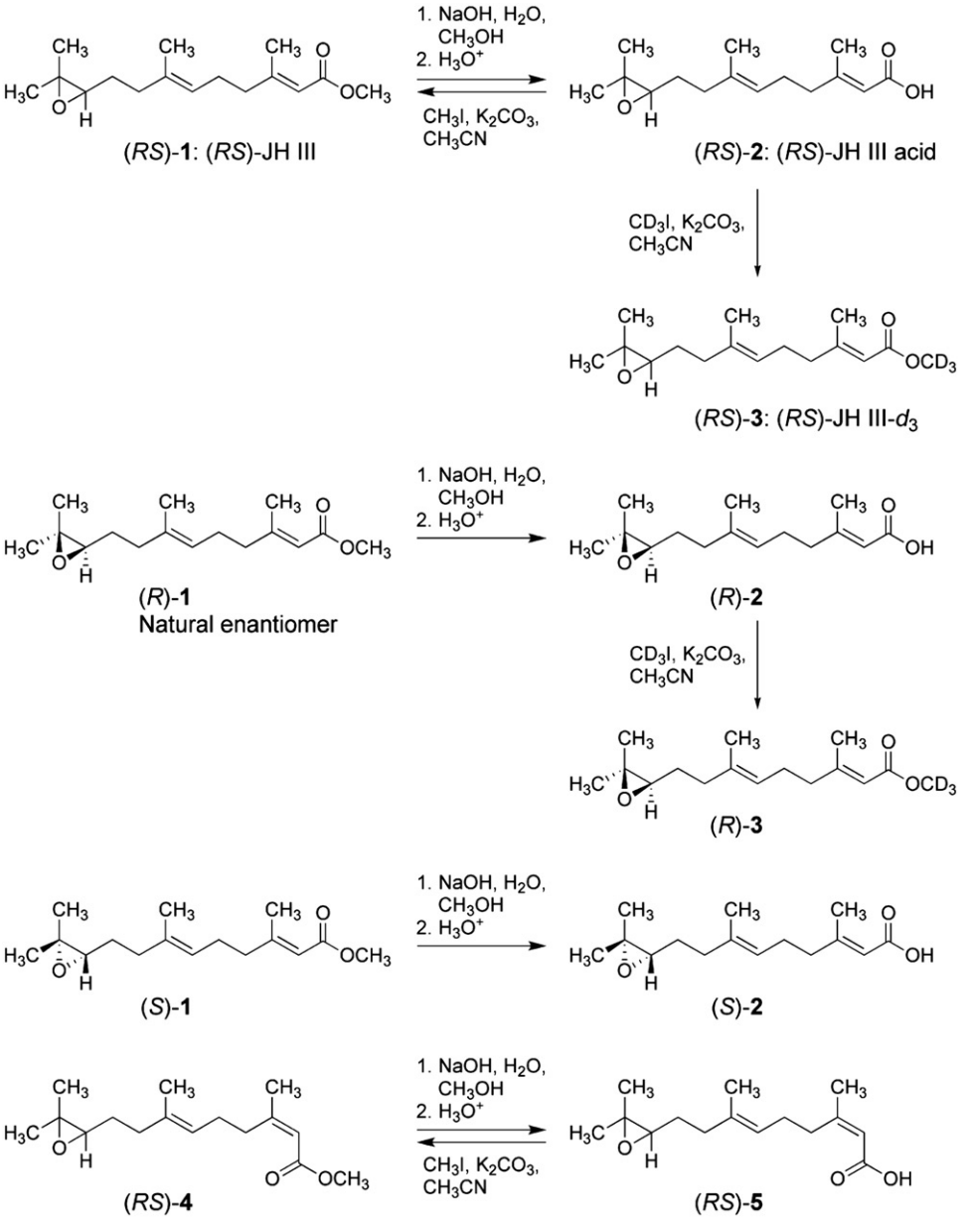
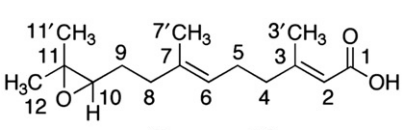


Figure 1. Chemical conversions of JH III (**1**) and related compounds.

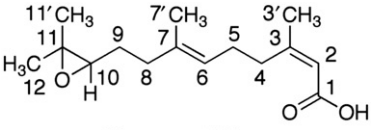
2. Results and discussion

First, the *cis–trans* isomers, (*RS*)-**1** and (*RS*)-**4**, were separated by preparative HPLC using a pre-packed silica gel glass column (100 mm × 22 mm i.d.; hexane/EtOAc = 9 : 1; 2 mL min^{-1}). The third operation gave geometrically pure (*RS*)-**1**. Then, the enantioseparation of (*RS*)-**1** was performed by normal-phase enantioselective HPLC (Chiralpak IA, 250 mm × 10 mm i.d.; hexane/EtOH = 98 : 2; 1.5 mL min^{-1}) (Ichikawa, Ono, Furuta, Shiotsuki, & Shinoda, 2007).

Table 1. NMR data for JH III acid (**2**) and 2*Z*-isomer (**5**).



Compound **2**



Compound **5**

Number	^{13}C-NMR (126 MHz)	^{1}H-NMR (800 MHz)	^{13}C-NMR (126 MHz)	^{1}H-NMR (500 MHz)
1	170.32	–	170.47	–
2	114.81	5.69 (1H, br s)	115.54	5.69 (1H, br s)
3	162.65	–	163.11	–
3′	19.12	2.17 (3H, br d, $J = 1$ Hz)	25.64	1.93 (3H, br d, $J = 1$ Hz)
4	40.98	2.2 (2H, m)	33.49	2.65 (2H, br t, $J = 8$ Hz)
5	25.82	2.2 (2H, m)	26.62	2.18 (2H, br q, $J = 8$ Hz)
6	123.37	5.14 (1H, m)	123.91	5.20 (1H, br t, $J = 7$ Hz)
7	135.44	–	135.10	–
7′	16.01	1.63 (3H, br d, $J = 1$ Hz)	15.88	1.63 (3H, br s)
8	36.33	2.2 (1H, m)	36.26	2.16 (1H, m)
		2.10 (1H, m)		2.10 (1H, m)
9	27.35	1.6 (2H, m)	27.32	1.6 (2H, m)
10	64.17	2.70 (1H, t, $J = 6$ Hz)	64.20	2.71 (1H, t, $J = 6$ Hz)
11	58.40	–	58.48	–
11′	18.74	1.26 (3H, s)	18.72	1.26 (3H, s)
12	24.85	1.30 (3H, s)	24.86	1.30 (3H, s)

Note: The solvent used is $CDCl_3$.

When (*RS*)-**1** was subjected to hydrolysis (Goodman & Adams, 1984), the procedure was frequently accompanied by epoxide ring opening. To prevent this, the acidification procedure was modified as follows: (1) The pH was set at 3 ($\approx pK_a - 2$); (2) 0.1 mol L^{-1} hydrochloric acid was used instead of 1 mol L^{-1}; and (3) the pH was monitored with a pH indicator. In addition, trace hydrochloric acid remaining after solvent extraction was removed with brine. With these modifications, (*RS*)-**2** was routinely obtained as a single major product. Previously, acid **2** had been purified using a LiChrosorb Diol column with $CHCl_3$/EtOH (99 : 1). Considering the toxicity of $CHCl_3$, the crude (*RS*)-**2** obtained was purified by preparative octadecylsilane (ODS) HPLC with MeCN/H_2O in a single operation. The yield of (*RS*)-**2** was 88% in hydrolysis. Subsequently, (*R*)-**1**, (*S*)-**1** and (*RS*)-**4** were subjected to hydrolysis, yielding (*R*)-**2**, (*S*)-**2** and (*RS*)-**5**, respectively. Table 1 shows the NMR signals of **2** and **5** assigned on the basis of the 2-D NMR spectra (i.e. DQFCOSY, NOESY, HSQC and HMBC). JH acids have also been prepared by enzymatic hydrolysis. It was reported that the incubation of (*RS*)-**1** with crude haemolymph of the adult Colorado potato beetle, *Leptinotarsa decemlineata*, resulted in the preferential formation of (*S*)-**2** (up to 55.4% ee) (de Kort, Peter, & Koopmanschap, 1983).

Next, (*RS*)-**2** was methylated with CH_3I and K_2CO_3 in MeCN, to produce (*RS*)-**1**. This methylation would be useful for the stereochemical analysis of natural **2**. Previously, diazomethane had been used for the methylation of **2** (de Kort et al.,

1983). However, because of its instability and toxicity, the storage and transport of diazomethane is not advisable. Moreover, Kagei et al. (1981) reported the 1,3-dipolar additions of diazomethane for conjugated carbonyl compounds (Kagei et al., 1981). The *cis–trans* isomer (*RS*)-**5** was also methylated, giving (*RS*)-**4**. Similarly, (*RS*)-**2** and (*R*)-**2** were methylated with CD_3I and K_2CO_3 in MeCN, yielding (*RS*)-**3** (Ichikawa et al., 2007) and (*R*)-**3**, respectively. Considering the interactions between JHs and proteins, the single-enantiomer (*R*)-**3** would be desirable over (*RS*)-**3** as an internal standard for the LC–MS quantification of (*R*)-**1**.

Both enantiomers of JH III acid (**2**) were prepared by hydrolysis and purified by preparative ODS HPLC. The NMR signals of acid **2** and its *cis–trans* isomer **5** were unambiguously assigned. (*RS*)-**2** was methylated with CH_3I and K_2CO_3 in MeCN to obtain (*RS*)-JH III [(*RS*)-**1**]. Similarly, (*R*)-JH III-d_3 [(*R*)-**3**] was prepared from (*R*)-**2** using CD_3I in MeCN in the presence of K_2CO_3. To the best of our knowledge, this is the first preparation of enantiopure (*R*)-**2**, (*S*)-**2** and (*R*)-**3**. These procedures will contribute to the biosynthetic and physiological studies of JHs.

3. Experimental

3.1. *General*

The NMR spectra were obtained with Bruker Avance 800 and Avance 500 spectrometers (Bruker BioSpin, Rheinstetten, Germany), equipped with cryoprobes. The ^{1}H-NMR chemical shifts were reported relative to the residual protons of $CDCl_3$ (7.26 ppm). The ^{13}C-NMR spectra were obtained with ^{1}H composite pulse decoupling. The infrared (IR) spectra were recorded as the chloroform solutions in a potassium bromide cell with an FT-IR-8200 spectrophotometer (Shimadzu, Kyoto, Japan). The MS data were obtained with an HP1100 LC/MSD instrument (Agilent, Palo Alto, CA, USA) attached to a Zorbax SB-C18 column (50 mm × 2.1 mm i.d.; Agilent) using electrospray ionisation (ESI). HPLC was performed using the LC10AT VP systems (Shimadzu) and the following columns: a pre-packed silica gel column (100 mm × 15 or 22 mm i.d.; Kusano, Tokyo, Japan), a pre-packed ODS column (100 mm × 15 mm i.d.; Kusano), a silica SG80 column (250 mm × 10 or 4.6 mm i.d.; Shiseido, Tokyo, Japan), a Capcell Pak C18 MG column (150 mm × 4.6 or 3 mm i.d.; Shiseido), and a Chiralpak IA column (250 mm × 10 or 4.6 mm i.d.; Daicel, Tokyo, Japan). Crude (*RS*)-**1** was purchased from Sigma–Aldrich (St. Louis, MO, USA). All procedures were conducted in semi-darkness.

3.2. *Preparation of (RS)-2*

A mixture of (*RS*)-**1** (8.0 mg, 30 μmol), methanol (0.3 mL) and aqueous NaOH solution (1 mol L^{-1}, 0.3 mL; Wako, Osaka, Japan) was kept at 40°C for 6 h (Goodman & Adams, 1984). After dilution with 2 mL of ice water, a drop of pH indicator (0.04%, w/v%, bromocresol purple solution; Wako) was added to the mixture. While stirring the mixture on ice, iced hydrochloric acid (0.1 mol L^{-1}, ca 0.26 mL; Wako) was added dropwise until the colour of the iced solution changed from bluish-purple to yellow. The solution was extracted four times with dichloromethane (4 mL × 4). After each extraction, a few drops of hydrochloric acid were added to gradually lower the pH. The organic layer was washed three times with brine and dried

over anhydrous Na_2SO_4. After filtration, the Na_2SO_4 was washed with dichloromethane four times. The combined solution was evaporated under reduced pressure, yielding 7.1 mg of crude (*RS*)-**2**. The crude product was purified by preparative HPLC in a single operation using a pre-packed ODS column (100 mm × 15 mm i.d.), which was eluted with MeCN/H_2O 65 : 35 at a flow rate of 1 mL min^{-1} and with detection at 250 nm. The fraction containing (*RS*)-**2** was extracted three times with dichloromethane. The organic layer was washed twice with brine, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure, giving an 88% yield of (*RS*)-**2** (6.7 mg, 27 μmol). A trace amount of (*RS*)-**1** was recovered. Acid (*RS*)-**2** was kept at −20°C as an MeCN solution under argon.

3.2.1. *(2E,6E)-(RS)-10,11-Epoxy-3,7,11-trimethyldodeca-2,6-dienoic acid [(RS)-2]*

For ^{1}H-NMR (800 MHz, $CDCl_3$) and ^{13}C-NMR (126 MHz, $CDCl_3$), see Table 1; LC–MS [ESI, negative ion mode, (MeCN, 0.1% w/v NH_4OAc; Sigma–Aldrich)/H_2O = 7 : 3]: 251 ($[M-H]^-$, 100%).

3.3. *Preparation of (R)-2*

(*R*)-**1** (5.4 mg, 20 μmol) was converted to (*R*)-**2** (4.0 mg, 16 μmol) by incubation for 7 h with 1 mol L^{-1} aqueous NaOH solution (0.3 mL) and methanol (0.3 mL) in a 78% yield (see Section 3.2 for details). The (*R*)-**2** was enantiopure (>99% ee) (Section 3.9).

3.3.1. *(2E,6E)-(R)-10,11-Epoxy-3,7,11-trimethyldodeca-2,6-dienoic acid [(R)-2]*

LC–MS [ESI, negative ion mode, (MeCN, 0.1% w/v NH_4OAc)/H_2O = 7 : 3]: 251 ($[M-H]^-$, 100%).

3.4. *Preparation of (S)-2*

(*S*)-**1** (4.8 mg, 18 μmol, >99% ee) was converted to (*S*)-**2** (3.0 mg, 12 μmol) by incubation for 6.3 h with 1 mol L^{-1} aqueous NaOH solution (0.3 mL) and methanol (0.3 mL) in a 66% yield (see Section 3.2 for details).

3.4.1. *(2E,6E)-(S)-10,11-Epoxy-3,7,11-trimethyldodeca-2,6-dienoic acid [(S)-2]*

LC–MS [ESI, negative ion mode, (MeCN, 0.1% w/v NH_4OAc)/H_2O = 7 : 3]: 251 ($[M-H]^-$, 100%).

3.5. *Preparation of (RS)-5*

(*RS*)-**4** (2.4 mg, 9 μmol) was converted to (*RS*)-**5** (1.1 mg, 4 μmol) by incubation for 4 h with 1 mol L^{-1} aqueous NaOH solution (0.4 mL) and methanol (0.4 mL) in 48% yield (see Section 3.2 for details).

3.5.1. *(2Z,6E)-(RS)-10,11-Epoxy-3,7,11-trimethyldodeca-2,6-dienoic acid [(RS)-**5**]*

For ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (126 MHz, $CDCl_3$), see Table 1; LC–MS [ESI, negative ion mode, (MeCN, 0.1% w/v NH_4OAc)/$H_2O = 7:3$]: 251 ($[M-H]^-$, 100%); IR (KBr, $CHCl_3$): 2964 cm^{-1}, 2928, 1693, 1647, 1456, 1379, 1117 and 866.

3.6. *Methylation of (RS)-2*

A mixture of (*RS*)-**2** (ca 1 mg, ca 4 μmol), K_2CO_3 (151 mg, 1.09 mmol), CH_3I (0.5 mL, 8 mmol, use with caution) and MeCN (1 mL) was stirred at room temperature for 3 h. The mixture was subjected to chromatography using a short column of silica gel (Wakogel C-200, CH_2Cl_2). The eluent was evaporated under reduced pressure, and the mixture obtained was chromatographed on Wakogel C-200 eluting successively with CH_2Cl_2 and hexane : EtOAc (7 : 3). The hexane : EtOAc solution was evaporated under reduced pressure. The crude product was purified by preparative HPLC (column, pre-packed ODS; eluent, MeCN : H_2O; flow rate, 1 mL min^{-1}; detection, 240 nm). This procedure yielded 0.9 mg of (*RS*)-**1** (ca 3 μmol). The structure of (*RS*)-**1** was confirmed by ^{1}H-NMR (800 MHz, $CDCl_3$), ^{13}C-NMR (201 MHz, $CDCl_3$) and LC–MS (*m*/*z* 289 $[M+Na]^+$).

3.7. *Methylation of (RS)-5*

(*RS*)-**5** (1.0 mg, ca 4 μmol) was converted to (*RS*)-**4** (0.7 mg, ca 3 μmol) by stirring for 2.5 h with K_2CO_3 (149 mg, 1.08 mmol), CH_3I (0.5 mL, 8 mmol) and MeCN (1 mL) (see Section 3.6 for details). The structure of (*RS*)-**4** was confirmed by ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (126 MHz, $CDCl_3$) (see Ichikawa et al. (2007) for spectral data of (*RS*)-**4**).

3.8. *Preparation of (RS)-3*

(*RS*)-**2** (ca 1 mg, ca 4 μmol) was converted to (*RS*)-**3** (0.6 mg, ca 2 μmol) by stirring for 4 h with K_2CO_3 (242 mg, 1.75 mmol), CD_3I (0.5 mL, 8 mmol; 99.5+ at.% D; Isotec, OH, USA; use with caution) and MeCN (0.9 mL). The reaction time was 4 h (see Section 3.6 for details). The structure of (*RS*)-**3** was confirmed by ^{1}H-NMR (800 MHz, $CDCl_3$), ^{13}C-NMR (201 MHz, $CDCl_3$) and LC–MS (*m*/*z* 292 ($[M+Na]^+$) (see Ichikawa et al. (2007) for spectral data of (*RS*)-**3**).

3.9. *Preparation of (R)-3*

(*R*)-**2** (0.4 mg, ca 2 μmol) was converted to (*R*)-**3** (0.3 mg, ca 1 μmol; >99% ee, enantioselective HPLC) by stirring for 3.5 h with K_2CO_3 (250 mg, 1.81 mmol), CD_3I (0.5 mL, 8 mmol) and MeCN (0.75 mL) (see Section 3.6 for details).

3.9.1. *[$^2H_3$]Methyl (2E,6E)-(R)-10,11-epoxy-3,7,11-trimethyldodeca-2, 6-dienoate [(R)-**3**]*

^{1}H-NMR (500 MHz, $CDCl_3$): see Ichikawa et al. (2007) for the spectral data of (*RS*)-**3**; ^{13}C-NMR (126 MHz, $CDCl_3$): see Ichikawa et al. (2007); LC–MS [ESI, positive ion mode, (MeCN, 0.1% w/v NH_4OAc)/$H_2O = 85:15$]: *m/z* 328 ($[M + NH_4 + MeCN]^+$, 40), 292 ($[M + Na]^+$, 10), 270 ($[M + H]^+$, 47), 235 ($[M - OCD_3]^+$, 100).

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