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ARTICLE

# Regioselective Biotransformation of (+)- and (−)-Citronellene by the Larvae of Common Cutworm (*Spodoptera litura*)

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Terpenoids, which have many biological activities and have occurred widely in nature, can be artificially synthesized. However, regioselective oxidation of terpenoids is difficult by chemical methods. In this study, (+)- and (−)-citronellene were biotransformed with *Spodoptera litura* to define the mechanism of metabolism of citronellene and gain a new natural terpenoid. (+)-Citronellene was converted to (2*S*,3*S*)-3,7-dimethyl-6-octene-1,2-diol and (2*R*,3*S*)-3,7-dimethyl-6-octene-1,2-diol (89.7%), (3*S*,6*S*)-(−)-3,7-dimethyl-1-octene-6,7-diol (3.8%), (3*S*)-(6*E*)-(+)-3,7-dimethyl-1,6-octadien-8-ol (4.2%), and (3*S*)-(6*E*)-(+)-3,7-dimethyl-1,6-octadien-8-oic acid (2.3%). In contrast, (−)-citronellene was converted to (2*R*,3*R*)-3,7-dimethyl-6-octene-1,2-diol and (2*S*,3*R*)-3,7-dimethyl-6-octene-1,2-diol (56.3%), (+)-iridan-7,8-diol (3.5%), and (3*R*)-(6*E*)-(−)-3,7-dimethyl-1,6-octadien-8-oic acid (40.2%). The main metabolic pathway of (+)- and (−)-citronellene by larvae of *S. litura* was oxidized at the terminal double bond and *trans*-allylic methyl position. Particularly on (+)-citronellene, the regioselective reaction was shown. On the oxidation of C-6, C-7, and C-8 positions, four new compounds (3*S*,6*S*)-(−)-3,7-dimethyl-1-octene-6,7-diol, (3*S*)-(6*E*)-(+)-3,7-dimethyl-1,6-octadien-8-oic acid, (+)-iridan-7,8-diol, and (3*R*)-(6*E*)-(−)-3,7-dimethyl-1,6-octadien-8-oic acid were produced in regioselective oxidation. It noted that stereoselective oxidation occurred between the enantiomers. The C-6 position was oxidized on the (+)-(3*S*) form, whereas cyclized and the C-7 position were oxidized on the (−)-(3*R*) form.

**KEYWORDS: Biotransformation; citronellene; *Spodoptera litura*; oxidation; cyclization**

## INTRODUCTION

Terpenoids have been known as not only raw materials for flavor and fragrance but also biologically active substances. A great majority of biologically active terpenoids are produced as secondary metabolites of plants, and these terpenoids have been shown to have biological activity against plants, microorganisms, and insects. However, even now, there have been few reports of the naturally metabolic mechanism of terpenoids. Creatures have a number of enzymes to transform materials for living or adapting to an environment. The biotransformation has features as follows: regio- and stereoselective reactions under mild conditions produced optically active compounds easily, which makes it possible to synthesize difficult products when compared to the corresponding chemical method. Various attempts have been made to search for new biologically active terpenoids today.

The investigation in the field of biotransformation of monoterpenoids is gaining more interest: these reactions are performed by bacteria, fungi, yeasts, and even algae. However, there are few reports in the literature on the biotransformation of terpenoids by Lepidoptera insects. The reasons for using the larvae of *Spodoptera litura* as a biological catalyst are as follows: Lepidoptera larvae feed on plants containing terpenoids as their diet and, therefore, possess a high level of enzymatic activity against terpenoids; the larvae consume a large amount of plants, making it possible to obtain more metabolites; and the larvae are easy to rear on a laboratory scale.

Citronellene is the widespread terpene known; however, there is one report on the biotransformation of (+)-citronellene (compound **1**) and (−)-citronellene (compound **2**) by other organisms (*1*). In the past, the biotransformation of geraniol, (−)-dihydromyrcenol, (−)-dihydromyrcenyl acetate, etc. by *S. litura* larvae were investigated (*2*−*13*). Geraniol was oxidized at the C-1 and C-8 positions. Therefore, this indicates that *trans* positions were preferentially oxidized. $\beta$-Myrcene was oxidized at the 3,10 and 1,2 double bonds by the larvae of *S. litura*. (−)-Dihydromyrcenol and (−)-dihydromyrcenyl acetate were oxidized at the 1,2 double bond and C-3 position by the larvae of *S. litura*.

In this paper, the biotransformation of (+)-citronellene (compound **1**) and (−)-citronellene (compound **2**) by the larvae of *S. litura* was investigated for the purpose of estimating possible metabolic pathways in insects.

## MATERIALS AND METHODS

**Rearing of Larvae.** The larvae of *S. litura* were reared in plastic cases (200 × 300 mm wide, 100 mm high, 100 larvae/case) covered with a

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**Table 1.** $^{1}$H NMR Spectral Data for Metabolites **3**−**7** (500.00 MHz, $CDCl_3$)[a]

| | compounds | | | |
|---|---|---|---|---|
| carbon | **3** and **4** | **5** | **6** | **7** |
| 1 | 3.67−3.65 m | 4.92 (1H, dd, *J* = 10, 1.2) | 4.93 (1H, dd, *J* = 10.3, 1.4) | 4.55 (1H, dd, *J* = 9.5, 1.2) |
| | 3.56−3.54 m | 4.97 (1H, dd, *J* = 17, 1.2) | 4.96 (1H, dd, *J* = 17.5, 1.4) | 4.98 (1H, dd, *J* = 17.5, 1.2) |
| 2 | 3.53−3.50 m | 5.70 (1H, ddd, *J* = 17, 10, 7.7) | 5.69 (1H, ddd, *J* = 17.5, 10.3, 7.5) | 5.67 (1H, ddd, *J* = 17.5, 9.5, 7.8) |
| 3 | 1.59−1.54 m | 2.13 (1H, *m*) | 2.16−2.09 (1H, m) | 2.16−2.13 (1H, m) |
| 4 | 1.19−1.15 m | 1.52−1.46 (2H, m) | 1.37−1.33 (2H, m) | 1.44 (2H, m) |
| | 1.59−1.54 m | 1.65−1.57 (1H, m) | | |
| 5 | 2.00−1.92 m | 1.36−1.27 (1H, m) | 2.07−1.98 (2H, m) | 2.21−2.17 (2H, m) |
| | 2.10−2.01 m | | | |
| 6 | 5.11−5.07 m | 3.44 (1H, dd, *J* = 9.8, 2.0) | 5.39−5.36 (1H, m) | 6.90 (1H, td, *J* = 7.5, 1.4) |
| 7 | | | | |
| 8 | 1.68 brs | 1.20 (3H, s) | 3.99 (2H, brs) | |
| 9 | 1.60 brs | 1.15 (3H, s) | 1.65 (3H, s) | 1.82 (3H, d, *J* = 1.4) |
| 10 | 0.94 d (6.9) | 1.01 (3H, d, *J* = 6.9) | 0.99 (3H, d, *J* = 6.6) | 1.01 (3H, d, *J* = 6.6) |

[a] NMR spectra were recorded at 500 MHz ($^{1}$H) in $CDCl_3$ solution using TMS as an internal standard.

nylon mesh screen. The rearing conditions were as follows: 25 °C, 70% relative humidity, and constant light. A commercial diet (Insecta LF; Nihon Nosan Kogyo Co., Ltd., Japan) was given to the larvae from the first instars. From the fourth instars, the diet was changed to an artificial diet composed of kidney beans (100 g), agar (12 g), and water (600 mL) (*14*).

**Materials.** (+)-Citronellene and (−)-citronellene were purchased from Fluka (Tokyo, Japan).

**General Experimental Procedures.** Gas chromatography (GC) was performed on a Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector (FID). The column was a fused silica capillary column (DB-5, 30 m length, 0.25 mm inner diameter). Chromatographic conditions were as follows: the oven temperature was programmed from 80 to 270 at 4 °C/min; injector and detector temperatures were 270 and 280 °C, respectively; split injection was 25:1; the flow rate of helium gas was 30.0 cm/s. Electron impact−mass spectrometry (EI−MS) measurements were obtained using gas chromatography−mass spectrometry (GC−MS). GC−MS was performed on a Hewlett-Packard 5972A mass selective detector interfaced with a Hewlett-Packard 5890A gas chromatograph fitted with a capillary column (HP-5MS, 30 m length, 0.25 mm inner diameter). Chromatographic conditions were the same as described above DB-5. The temperature of the ion source was 230 °C, and the electron energy was 70 eV. The IR spectra was obtained with a JASCO FT/IR-470 plus Fourier transform infrared spectrometer. The NMR spectra was obtained with a JEOL FX-500 (500.00 MHz, $^{1}$H; 125.65 MHz, $^{13}$C) spectrometer. Tetramethylsilane (TMS) was used as the internal standard in $CDCl_3$. Multiplicities were determined by the distortionless enhancement by polarization transfer (DEPT) pulse sequence. The specific rotations were measured on a JASCO DIP-1000 digital polarimeter.

**Administration of the Substrate.** The artificial diet without the agar was mixed with a blender. Compound **1** (2000 mg) was then added directly into the blender at 3 mg/g of diet. After agar was dissolved in water, it was boiled and then added into the blender. The diet was then mixed and cooled in a stainless-steel tray (220 × 310 mm wide, 30 mm high). The fourth and fifth instar larvae (average weight = 0.5 g) were moved into new cases (100 larvae/case), and the diet was fed to the larvae in limited amounts. Groups of 800 larvae were fed the diet containing compound **1** (actually 1400 mg, about 1.8 mg/body) for 2 days, and then the artificial diet not containing compound **1** was fed to the larvae for an additional 2 days. Frass were collected every 5 h (total of 4 days) and stored in a solution of diethyl ether (300 mL). Compound **2** was administered to 800 larvae in the same manner. For diet and frass separation, the fresh frass was extracted as soon as the fourth and fifth instar larvae excreted. For the quantitative analysis of metabolites, GC analysis was used as an internal standard with the substrate.

**Isolation and Identification of Metabolites from Frass.** The frass were extracted with diethyl ether (300 mL, 2 times) and then ethyl acetate (300 mL, 2 times). The extract solution was gathered and evaporated under reduced pressure, and 2.8 g of extract was obtained. The extract was dissolved in ethyl acetate and then was added to the 5% $NaHCO_3$ solution. After shaking, the neutral fraction (1353 mg) was obtained from the ethyl acetate layer. The aqueous layer was separated, then acidified with 1 N HCl (acidic fraction), and extracted with ethyl acetate. After shaking, the acidic fraction (1258 mg) was obtained from the aqueous layer. The neutral fraction was subjected to silica-gel open-column chromatography (silica gel 60, 230−400 mesh, Merck) with a hexane/ethyl acetate gradient (9:1−1:1) system. Compound **1** was recovered at 211.4 mg. Metabolic compounds **3** and **4** (756.7 mg), **5** (34.3 mg), and **6** (37.8 mg) were isolated in the neutral fraction, and metabolites **7** (15.4 mg) were isolated in the acidic fraction. In the cace of compound **2**, the same procedure as described for **1** was used. Compound **2** was transformed to compounds **8** and **9** (467.6 mg), **10** (29.4 mg), and **11** (333.2 mg). The amount of metabolites was isolated from the extract of frass (total extract of 3.0 g), and compound **2** was recovered at 207.2 mg. Metabolic compounds from the frass were identified by a comparison of established GC−MS, IR, and NMR data.

**Table 2.** $^{13}$C NMR Spectral Data for Metabolites **3**−**7** (125.00 MHz, $CDCl_3$)[a]

| | compounds | | | |
|---|---|---|---|---|
| carbon | **3** and **4** | **5** | **6** | **7** |
| 1 | 64.6/65.1 ($CH_2$) | 112.8 ($CH_2$) | 112.7 ($CH_2$) | 113.3 ($CH_2$) |
| 2 | 76.1/75.7 (CH) | 144.6 (CH) | 144.5 (CH) | 143.9 (CH) |
| 3 | 35.7/32.3 (CH) | 38.0 (CH) | 37.4 (CH) | 37.6 (CH) |
| 4 | 32.5/33.0 ($CH_2$) | 33.7 ($CH_2$) | 36.3 ($CH_2$) | 35.1 ($CH_2$) |
| 5 | 25.3/25.5 ($CH_2$) | 29.4 ($CH_2$) | 25.3 ($CH_2$) | 26.7 ($CH_2$) |
| 6 | 124.4/124.3 (CH) | 78.9 (CH) | 126.4 (CH) | 145.2 (CH) |
| 7 | 131.6 (C × 2) | 73.2 (C) | 134.7 (C) | 127.0 (C) |
| 8 | 25.7 ($CH_3$ × 2) | 23.1 ($CH_3$) | 69.0 ($CH_2$) | 173.4 (C) |
| 9 | 17.7 ($CH_3$ × 2) | 26.6 ($CH_3$) | 13.7 ($CH_3$) | 12.0 ($CH_3$) |
| 10 | 15.1 ($CH_3$) | 20.2 ($CH_3$) | 20.2 ($CH_3$) | 20.2 ($CH_3$) |

[a] NMR spectra were recorded at 125 MHz ($^{13}$C) in $CDCl_3$ solution using TMS as an internal standard.

**Structure of Metabolic Products.** (2*S*,3*S*)-3,7-Dimethyl-6-octene-1,2-diol (compound **3**) and (2*R*,3*S*)-3,7-dimethyl-6-octene-1,2-diol (Compound **4**): colorless oil. EI−MS, *m*/*z* (relative intensity): 172 $[M]^+$ (8), 141 $[M-CH_2OH]^+$ (6), 123 $[141-H_2O]^+$ (21), 101 (20), 82 (100), 69 (62), 67 (45), 55 (48), 41 (91), 39 (27). IR (film, $\nu_{max}$, $cm^{-1}$): 3374, 2964, 2927, 1064. $^{1}$H NMR: see **Table 1**. $^{13}$C NMR: see **Table 2**.

(3*S*,6*S*)-(−)-3,7-Dimethyl-1-octene-6,7-diol (compound **5**): colorless oil. $[\alpha]^{24}_{D}$ −6.3 ($CHCl_3$, c 0.4). EI−MS, *m*/*z* (relative intensity): 154 $[M-H_2O]^+$ (0.5), 139 $[154-CH_3]^+$ (0.5), 121 (0.5), 114 (2), 96 (6), 95 (5), 81 (9), 71 (8), 59 (100), 55 (11), 43 (17), 41 (13). IR (film, $\nu_{max}$, $cm^{-1}$): 3404, 2955, 2927, 1639, 1069. $^{1}$H NMR: see **Table 1**. $^{13}$C NMR: see **Table 2**.

(3*S*)-(6*E*)-(+)-3,7-Dimethyl-1,6-octadien-8-ol (compound **6**): colorless oil. $[\alpha]^{24}_{D}$ +9.0 ($CHCl_3$, c 0.2). EI−MS, *m*/*z* (relative intensity): 136 $[M-H_2O]^+$ (5), 121 $[136-CH_3]^+$ (14), 107 (12), 95 (15), 93 (17), 84 (24), 81 (41), 79 (24), 71 (26), 69 (34), 68 (44), 67 (45), 55 (74), 53 (26), 43 (100), 41 (77).

**Table 3.** $^1$H NMR Spectral Data for Metabolites **8**−**11** (500.00 MHz, $CDCl_3$)[a]

| | compounds | | |
|---|---|---|---|
| carbon | **8** and **9** | **10** | **11** |
| 1 | 3.67−3.65 m | 0.94 (3H, d, *J* = 6.9) | 4.55 (1H, dd, *J* = 9.5, 1.2) |
| | 3.56−3.54 m | | 4.98 (1H, dd, *J* = 17.5, 1.2) |
| 2 | 3.53−3.50 m | 1.75−1.71 (1H, m) | 5.67 (1H, ddd, *J* = 17.5, 9.5, 7.8) |
| 3 | 1.59−1.54 m | 1.69−1.60 (1H, m) | 2.16−2.13 (1H, m) |
| 4 | 1.19−1.15 m | 1.14−1.07 (1H, m) | 1.44 (2H, m) |
| | 1.59−1.54 m | 1.98−1.92 (1H, m) | |
| 5 | 2.00−1.92 m | 1.69−1.60 (2H, m) | 2.21−2.17 (2H, m) |
| | 2.10−2.01 m | | |
| 6 | 5.11−5.07 m | 1.98−1.92 (1H, m) | 6.90 (1H, td, *J* = 7.5, 1.4) |
| 7 | | | |
| 8 | 1.68 brs | 3.36 (1H, d, *J* = 10.6) | |
| | | 3.54 (1H, d, *J* = 10.6) | |
| 9 | 1.60 brs | 1.26 (3H, s) | 1.82 (3H, d, *J* = 1.4) |
| 10 | 0.94 d (6.9) | 0.97 (3H, d, *J* = 7.0) | 1.01 (3H, d, *J* = 6.6) |

[a] NMR spectra were recorded at 500 MHz ($^1$H) in $CDCl_3$ solution using TMS as an internal standard.

**Table 4.** $^{13}$C NMR Spectral Data for Metabolites **8**−**11** (125.00 MHz, $CDCl_3$)[a]

| | compounds | | |
|---|---|---|---|
| carbon | **8** and **9** | **10** | **11** |
| 1 | 64.6/65.1 ($CH_2$) | 17.7 ($CH_3$) | 113.3 ($CH_2$) |
| 2 | 76.1/75.7 (CH) | 42.1 (CH) | 143.9 (CH) |
| 3 | 35.7/35.3 (CH) | 41.4 (CH) | 37.6 (CH) |
| 4 | 32.5/33.0 ($CH_2$) | 30.9 ($CH_2$) | 35.1 ($CH_2$) |
| 5 | 25.3/25.5 ($CH_2$) | 23.3 ($CH_2$) | 26.7 ($CH_2$) |
| 6 | 124.4/124.3 (CH) | 47.5 (CH) | 145.2 (CH) |
| 7 | 131.6 (C × 2) | 74.3 (C) | 127.0 (C) |
| 8 | 25.7 ($CH_3$ × 2) | 70.3 ($CH_2$) | 173.4 (C) |
| 9 | 17.7 ($CH_3$ × 2) | 22.8 ($CH_3$) | 12.0 ($CH_3$) |
| 10 | 15.1 ($CH_3$) | 22.3 ($CH_3$) | 20.2 ($CH_3$) |

[a] NMR spectra were recorded at 125 MHz ($^{13}$C) in $CDCl_3$ solution using TMS as an internal standard.

IR (film, $\nu_{max}$, $cm^{-1}$): 3334, 2958, 2925, 1639, 995. $^1$H NMR: see **Table 1**. $^{13}$C NMR: see **Table 2**.

(3*S*)-(6*E*)-(+)-3,7-Dimethyl-1,6-octadien-8-oic acid (compound **7**): colorless oil. $[\alpha]^{24}_D$ +11.3 ($CHCl_3$, c 0.2). EI−MS, *m*/*z* (relative intensity): 153 $[M - CH_3]^+$ (4), 139 (8), 123 $[M - COOH]^+$ (9), 111 (21), 100 (47), 95 (30), 87 (17), 82 (29), 69 (56), 67 (48), 55 (49), 43 (29), 41 (100), 39 (51). IR (film, $\nu_{max}$, $cm^{-1}$): 3300−2500, 1701, 1643. $^1$H NMR: see **Table 1**. $^{13}$C NMR: see **Table 2**.

(2*R*,3*R*)-3,7-Dimethyl-6-octene-1,2-diol (compound **8**) and (2*S*,3*R*)-3,7-dimethyl-6-octene-1,2-diol (compound **9**): colorless oil. EI−MS, *m*/*z* (relative intensity): 172 $[M]^+$ (12), 141 $[M - CH_2OH]^+$ (7), 123 $[141 - H_2O]^+$ (21), 101 (16), 82 (100), 69 (51), 67 (50), 55 (43), 43 (48), 41 (90), 39 (35). IR (film, $\nu_{max}$, $cm^{-1}$): 3388, 2964, 2959, 2926, 1051. $^1$H NMR: see **Table 3**. $^{13}$C NMR: see **Table 4**.

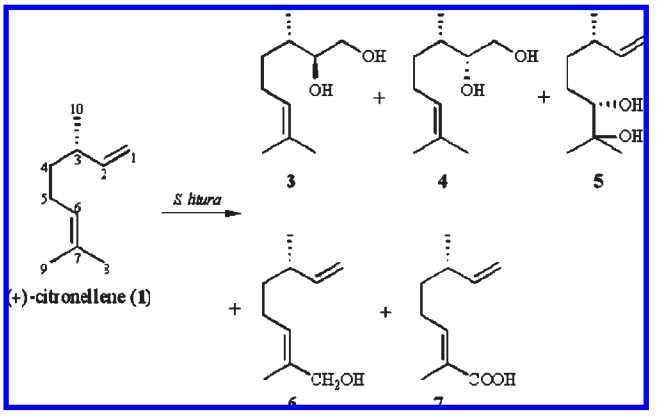
(+)-Iridan-7,8-diol (compound **10**): colorless oil. $[\alpha]^{24}_D$ +3.1 ($CHCl_3$, c 0.6). HREI−MS, *m*/*z*: 172.1456 $[M]^+$, calcd for $C_{10}H_{20}O_2$: 172.1464. EI−MS, *m*/*z* (relative intensity): 157 $[M - CH_3]^+$ (2), 141 $[M - CH_2OH]^+$ (20), 123 (91), 97 (26), 95 (13), 81 (30), 75 (100), 57 (35), 55 (59), 43 (57), 41 (28), 31 (13). IR (film, $\nu_{max}$, $cm^{-1}$): 3408, 2952, 2871, 1041. $^1$H NMR: see **Table 3**. $^{13}$C NMR: see **Table 4**.

(3*R*)-(6*E*)-(−)-3,7-Dimethyl-1,6-octadien-8-oic acid (compound **11**): colorless oil. $[\alpha]^{24}_D$ −12.3 ($CHCl_3$, c 0.5). EI−MS, *m*/*z* (relative intensity): 153 $[M - CH_3]^+$ (3), 123 $[M - COOH]^+$ (9), 41 (100). IR (film, $\nu_{max}$, $cm^{-1}$): 3300−2500, 1701, 1643. $^1$H NMR: see **Table 3**. $^{13}$C NMR: see **Table 4**.

## RESULTS

**Biotransformation of Compound 1 by the Larvae *S. litura*.** In the biotransformation of compound **1**, the five metabolites isolated from the frass were identified (**Scheme 1**). Compounds **3** and **4** (89.7%; the percentage was calculated from the total metabolite weight), **5** (3.8%), and **6** (4.2%) were isolated in the neutral fraction, and compound **7** (2.3%) was isolated in the acidic fraction. Although alcohol and carboxylic acid were detected by GC analysis, intermediary metabolites (epoxide and aldehyde) were not isolated. This suggested that intermediary metabolites were hardly excreted into the frass. The larvae that were fed the diet without substrate were used as the control, and the extract from the frass was analyzed by GC. Calculated from the peak area in the gas chromatogram in the frass extract, the pecentage of recovered compound **1** was 18.6% and the pecentage of metabolic compounds **3**, **4**, **5**, **6**, and **7** were 22.7, 45.4, 3.1, 3.4, and 1.4%, respectively (**Table 5**). The result was that substrates and metabolites were not observed in the frass. The structures of metabolites **3**, **4**, and **6** were determined by interpretation of their $^1$H NMR, $^{13}$C NMR, and mass spectra and a comparison to reported data (*15*−*17*).

**Scheme 1.** Metabolites of (+)-Citronellene (**1**) by the Larvae of *S. litura*




Compound **5** had a molecular formula of $C_{10}H_{20}O_2$ that was estimated by the EIMS spectra. The IR spectrum contained a new hydroxyl band at 3404 $cm^{-1}$. The proton and carbon NMR spectra were similar to that of **1**, except for the C-6 and C-7 positions. In the characteristic HMBC spectrum, a correlation was obtained for two methyl groups with methine carbon (78.9 ppm; C-6) and two methyl groups with quaternary carbon (73.2 ppm; C-7). The absolute configuration of compound **5** was determined by the modified Mosher ester method. The (*R*)- and (*S*)-MTPA esters of compound **5** were obtained by treating compound **5** with (*R*)- and (*S*)-MTPA chloride, respectively. The proton resonances of the (*R*)- and (*S*)-MTPA esters of compound **5** were assigned on the basis of $^1$H−$^1$H correlations, and the differences in the proton chemical shifts of these MTPA ester derivatives, particularly for H-8 ($\Delta\delta = \delta_S - \delta_R + 0.06$), H-9 ($\Delta\delta + 0.03$), H-3 ($\Delta\delta - 0.08$), and H-2 ($\Delta\delta - 0.04$) are shown in **Table 6**. The specific rotation shows the (−) form. From these date, it was concluded that the structure of compound **5** is (3*S*,6*S*)-(−)-3,7-dimethyl-1-octene-6,7-diol.

With regard to compound **7**, the IR spectrum contained a wide band at 3300−2500 $cm^{-1}$. The proton and carbon NMR spectra were similar to that of **1**, except for the existence of a new quaternary carbon and the disappearance of a methyl group. In the characteristic HMBC spectrum, correlations were obtained of the methine group with quaternary carbon (127.0 ppm; C-7), with quaternary carbon (173.4 ppm; C-8), and with methyl group (12.0 ppm; C-9). The geometry of the carbonyl group was achieved by observation of nuclear Overhauser effect (NOE). Irradiation of the signal at 1.82 ppm (H-9) caused the enhancement of the H-5 signal. Thus, geometry of the carboxyl

group is the *trans* position. The specific rotation shows the (+) form. From these data, it was concluded that the structure of compound **4** is (3*S*)-(6*E*)-(+)-3,7-dimethyl-1,6-octadien-8-oic acid.

**Biotransformation of Compound 2 by the Larvae *S. litura*.** The same procedure as described for compound **1** was used. The four metabolites isolated from the frass were identified (**Scheme 2**). Compound **2** was transformed to compounds **8** and **9** (56.3%; the percentage was calculated from the total metabolite weight), compound **10** (3.5%), and compound **11** (40.2%). The amount of metabolites was isolated from the extract of frass. Calculated from the peak area in the gas chromatogram in the frass extract, the pecentage of recovered compound **2** was 18.0% and the pecentage of metabolic compounds **8**, **9**, **10**, and **11**, were 27.1, 13.5, 2.6, and 28.9%, respectively (**Table 5**).

The structures of compounds **8** and **9** were determined by interpretation of their $^1$H NMR and mass spectra and a comparison to reported data (*15*−*17*).

HR−EIMS of compound **10** had a peak at *m*/*z* 172.1456, which was calculated for the molecular formula of $C_{10}H_{20}O_2$. The IR spectrum contained a new hydroxyl band at 3408 cm$^{-1}$. Compound **10** by the proton and carbon NMR spectra contained the signals of one methyl group attached to the quaternary carbon

**Table 5.** Recovery and Yield of Metabolites **1**−**11** by the *S. litura* Larvae[a]

| substrate | substrate in the artificial diet (g) | metabolite[b] (g) | recovery (%) | yield[c] (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | **1** | **3** | **4** | **5** | **6** | **7** |
| (+)-citronellene (**1**) | 1.40 | 1.06 | 75.4 | 18.6[d] | 22.7 | 45.4 | 3.1 | 3.4 | 1.4 |
| | | | | **2** | **8** | **9** | **10** | **11** | |
| (−)-citronellene (**2**) | 1.40 | 1.04 | 74.3 | 18.0[d] | 27.1 | 13.5 | 2.6 | 28.9 | |

[a] Metabolites were obtained from the frass of *S. litura* larvae, which were orally administered compounds **1** and **2**. [b] Calculated from the peak area in the gas chromatogram used as an internal standard {(+)- and (−)-citronellene}. [c] Percentage estimated by GC. [d] Recovered substrate.

**Table 6.** Determination of Absolute Comfiguration for Compound **5** by the Modified Mosher Method Using $^1$H NMR Spectroscopy

| | $^1$H chemical shift ($\delta$) | | |
|---|---|---|---|
| | (*S*)-MTPA ester | (*R*)-MTPA ester | $\Delta\delta$ |
| 1 | 4.97 | 4.98 | −0.01 |
| | 4.93 | 4.96 | −0.03 |
| 2 | 5.58 | 5.62 | −0.04 |
| 3 | 1.99 | 2.07 | −0.08 |
| 8 | 1.22 | 1.16 | +0.06 |
| 9 | 1.16 | 1.13 | +0.03 |
| 10 | 0.88 | 0.94 | −0.06 |

$^1$H NMR spectra were recorded at 500 MHz in $CDCl_3$ solution using TMS as an internal standard.

atom and two methyl groups linked to the methine group. The $^{13}$C NMR spectrum exhibited two signals ($\delta$ 74.3 and 70.3 ppm) linked to hydroxyl groups. In the characteristic HMBC spectrum, a correlation was observed for H-9 (1.26 ppm) with one methine group (47.5 ppm; C-6), quaternary carbon (74,3 ppm; C-7), and methylene group (70.3 ppm; C-8) and for H-1 (0.94 ppm) with three methine groups (42.1, 41.4, and 47.5 ppm; C-2, C-3, and C-6, respectively). A correlation spectroscopy (COSY) spectrum indicates that correlation cross-peaks were observed between H-2 (1.75−1.71 ppm) and H-6 (1.98−1.92 ppm). These data were indicated similar to that of the iridane structure. The specific rotation shows the (+) form. Therefore, it was concluded that the structure of **10** is (+)-iridan-7,8-diol.

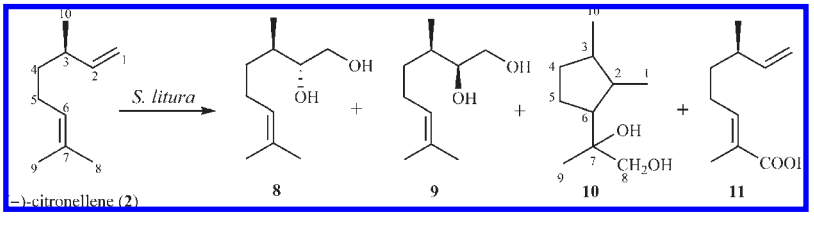
The spectral data of **11** was identical to **7**. The specific rotation showed that **11** ($[\alpha]^{24}_D$ −12.3 (*c* 0.5, $CHCl_3$)) was the (−) form. These spectral data suggested that metabolite **9** was (3*R*)-(6*E*)-(−)-3,7-dimethyl-1,6-octadien-8-oic acid.

## DISCUSSION

In the biotransforamtion of compounds **1** and **2** by *S. litura*, the larvae transformed compound **1** to compounds **3**−**7** and the larvae transformed compound **2** to compounds **8**−**11**. The main reaction positions of compounds **1** and **2** were the tendency to oxidize at the C-1 and C-2 positions (terminal double bond) and C-8 position (allylic methyl). Minor metabolites of compounds **1** and **2** were compounds **5** and **10**. These results indicate that there is little difference in the products and the proportion of products between compounds **1** and **2**. Furthermore, we revealed compounds **3**, **4**, **8**, and **9** as a mixture of diastereoisomers by a comparison of $^1$H NMR data in the literature (*15*−*17*). Consequently, compounds **3** and **4** were a 1:2 mixture of (2*S*,3*S*) and (2*R*,3*S*) forms; on the other hand, compounds **8** and **9** were a 2:1 mixture of (2*R*,3*R*) and (2*S*,3*R*) forms. In the past, biotransformation of (+)- and (−)-limonene ware reported (*18*). (+)- and (−)-Limonene were transformed to uroterpenol and perillic acid. Their substrates also showed a tendency to oxidize the terminal double bond and allyic methyl. That report is indicated that each limonene was preferentially transformed to the (*R*) form diol. In this study, the larvae did recognize the difference between (+) and (−) forms. In brief, the influence of the asymmetric C-3 carbon atom between (+) and (−) forms in the bodies of larvae was shown. In the biotransformation of compound **6**, the metabolite was **7**. Compound **6** was oxidized at the C-8 position by the larvae of *S. litura*. The result of the biotransformation of compounds **1** and **6** revealed that, in the biotransformation of compound **1** by the larvae of *S. litura*, the metabolites **6** and **7** were formed by one metabolic pathway (**1** → **6** → **7**) in the larvae of *S. litura* (**Scheme 1**). Although biotransformation of **2** was also progressive to carboxylic acid at the C-8 position (allylic methyl), the intermediary metabolite (8-hydroxy alcohol) was not isolated.

This is only one report on the biotransformation of compounds **1** and **2** by another organism. It seems natural to obtain different metabolites with different species of an organism. The oxidation

**Scheme 2.** Metabolites of (−)-Citronellene (**2**) by the Larvae of *S. litura*



at C-6 and C-7 positions is the main metabolic pathway in the biotransformation of compounds **1** and **2** by *Diplodia gossypina* ATCC 10936 (*1*, *19*). However, upon biotransformation by the larvae of *S. litura*, regioselective oxidation of C-1, C-2 (terminal double bond), and C-8 (allylic methyl) positions occurred in comparison to C-6 and C-7 positions in compounds **1** and **2**. Furthermore, it was revealed that stereoselective oxidation occurred when compared to compounds **1** and **2**. The C-6 position was oxidized on the (+)-(3*S*) form, whereas the C-2 and C-6 positions were cross-linked and the C-7 position was oxidized on the (−)-(3*R*) form.

## LITERATURE CITED


(1) Abraham, W. R.; Stumpf, B. Enzymic acyloin condensation of acyclic aldehydes. *Z. Naturforsch.* **1987**, *42c*, 559–566.

(2) Miyazawa, M.; Akazawa, S.; Sakai, H.; Nankai, H. Biotransformation of (−)-dihydromyrcenol and (−)-dihydromyrcenyl acetate using the plant parasitic fungus *Glomerella cingulate* as a biocatalyst. *J. Agric. Food Chem.* **2000**, *46*, 300–303.

(3) Miyazawa, M.; Kumagae, S.; Kameoka, H. Biotransformation of (+)-and (−)-menthol by the larvae of common cutworm (*Spodoptera litura*). *J. Agric. Food Chem.* **1999**, *47*, 3938–3940.

(4) Miyazawa, M.; Kumagae, S.; Kameoka, H. Biotransformation of (*R*)- and (*S*)-terpinen-4-ol by the larvae of common cutworm (*Spodoptera litura*). *J. Agric. Food Chem.* **2001**, *49*, 4312–4314.

(5) Miyazawa, M.; Takechi, H. Biotransformation of monoterpenoids by the larvae of common cutworm (*Spodoptera litura*). *Nat. Prod. Commun.* **2007**, *2*, 435–443.

(6) Miyazawa, M.; Ohsawa, M. Biotransformation of α-terpineneol by the larvae of common cutworm (*Spodoptera litura*). *J. Agric. Food Chem.* **2002**, *50*, 4916–4918.

(7) Miyazawa, M.; Miyamoto, Y. Biotransformation of (1*R*)-(+)- and (1*S*)-(−)-camphor by the larvae of common cutworm (*Spodoptera litura*). *J. Mol. Catal. B: Enzym.* **2004**, *27*, 83–89.

(8) Miyazawa, M.; Murata, T. Biotransformation of *β*-myrcene by the larvae of common cutworm (*Spodoptera litura*). *J. Agric. Food Chem.* **2000**, *48*, 123–125.

(9) Miyazawa, M.; Murata, T. Biotransformation of (−)-dihydromyrcenol and (−)-dihydromyrcenyl acetate by the larvae of common cutworm (*Spodoptera litura*). *J. Oleo Sci.* **2001**, *50*, 921–925.

(10) Miyazawa, M.; Wada, T.; Kameoka, H. Biotransformation of (+)-and (−)-limonene by the larvae of common cutworm (*Spodoptera litura*). *J. Agric. Food Chem.* **1998**, *48*, 4826–4829.

(11) Miyazawa, M.; Wada, T.; Kameoka, H. Biotransformation of α-terpinene in common cutworm larvae (*Spodoptera litura*). *J. Agric. Food Chem.* **2000**, *44*, 2889–2895.

(12) Miyazawa, M.; Wada, T. Biotransformation of *γ*-terpinene and (−)-α-phellandrene by the larvae of common cutworm (*Spodoptera litura*). *J. Agric. Food Chem.* **2000**, *48*, 2893–2895.

(13) Takechi, H.; Miyazawa, M. Biotransformation of geraniol by the larvae of common cutworm (*Spodoptera litura*). *J. Oleo Sci.* **2006**, *55*, 143–149.

(14) Yushima, K.; Kamano, S.; Tamaki, Y. *Rearing Methods of Insects*; Japan Plant Protection Association: Tokyo, Japan, 1991; p 214.

(15) Taber, D. F.; Houze, J. B. The 2-hydroxycitronellols, convenient chirons for natural products synthesis. *J. Org. Chem.* **1994**, *59*, 4004–4006.

(16) Sheppeck, J. F.; James, E.II; Liu, W.; Chamberlin, A. R. Total synthesis of the serine/threonine-specific protein phosphatase inhibitor tautomycin. *J. Org. Chem.* **1997**, *62*, 387–398.

(17) Banvell, M. G.; Bui, C. T.; Simpson, G. W. Diastereoselective synthesis of the tetrahydropyranoid core of the polyketide herbicide herboxidiene and model studies pertaining to attachment of the side chain. *J. Chem. Soc., Perkin Trans.* **1998**, *1*, 791–800.

(18) Miyazawa, M.; Wada, T.; Kameoka, H. Biotransformation of (+)-and (−)-limonene by the larvae of common cutworm (*Spodoptera litura*). *J. Agric. Food Chem.* **1998**, *46*, 300–303.

(19) Abraham, W. R.; Stumpf, B.; Kieslich, K. Chiral compounds with a 1,2-dihydroxy-2-methylpropyl structure. *Ger. Offen.* **1985**, 19.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