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ABSOLUTE CONFIGURATION OF SEX PHEROMONE FOR TEA TUSSOCK MOTH, *Euproctis pseudoconspersa* (STRAND) *VIA* SYNTHESIS OF (*R*)- AND (*S*)-10,14-DIMETHYL-1-PENTADECYL ISOBUTYRATES

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Abstract—(*R*)- and (*S*)-10,14-dimethyl-1-pentadecyl isobutyrates were synthesized from (*S*)- and (*R*)-citronellols, respectively. The *R* enantiomer was as active as the natural pheromone but the *S* enantiomer was less active in the electrophysiological analyses, which provided conclusive proof that the absolute configuration of the natural pheromone is *R*.

Key Words—Tea tussock moth, *Euproctis pseudoconspersa*, sex attractant pheromone, 10,14-dimethyl-1-pentadecyl isobutyrate, citronellol, dihydrocitronellol, chirality, absolute configuration.

INTRODUCTION

The tea tussock moth, *Euproctis pseudoconspersa* (Strand) (Lepidoptera: Lymantriidae), is a notorious pest of the family Theaceae, including *Camellia sinensis*, *Camellia japonica*, and *Camellia sasanqua*. The larvae have venomous spicules on their backs. The spicules are hazardous to human skin on contact (Ogata, 1958).

The major sex pheromone of *E. pseudoconspersa* has been identified as 10,14-dimethyl-1-pentadecyl isobutyrate (**1**) (Wakamura et al., 1994). The synthetic **1** (racemate) was found to show a potent attractant activity for the male, although the absolute configuration of C-10 remained to be determined.

Important advances in the asymmetric syntheses of insect pheromones (Mori, 1994) prompted us to synthesize *R*-**1** and *S*-**1** from (*S*)- and (*R*)-citronellols (*S*-**2** and *R*-**2**), respectively (Scheme 1). The biological activities of the

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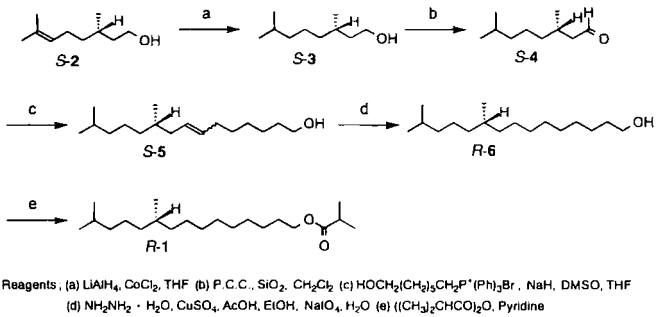


SCHEME 1. Synthetic pathway for *R*-**1**.

synthetic *R*-**1** and *S*-**1** were compared with that of the natural pheromone by a gas chromatograph equipped with an electroantennographic detector (Struble and Arn, 1984).

METHODS AND MATERIALS

General. Column chromatography was conducted employing a Wako C-100 or Wako C-200. NMR spectra were obtained on a JEOL EX90 or a JEOL GSX270 in $CDCl_3$ with TMS as an internal standard. IR spectra were recorded on a Shimadzu 8200 or a JASCO IR Report 100. GC-MS data were obtained from a Hewlett-Packard 5890 Series II gas chromatograph combined with an HP 5971A mass selective detector using DB-WAX (polyethylene glycol 20 M, 30 m × 0.25 mm ID × 0.25 mm film thickness, J & W Scientific). High-resolution mass spectra (HR-MS)(EI) were measured with a JEOL JMS-SX102A mass spectrometer. Optical rotations were determined on a JASCO DIP140 or a JASCO DIP181 digital polarimeter. CD spectra were recorded on a JASCO J-20 spectropolarimeter.

Commercially available (*S*)-citronellol (Aldrich 30,348-8) and (*R*)-citronellol (Aldrich 30,346-1) were used for the starting materials.

(S)-3,8-Dimethyl-1-octanol (S-3). *S*-**3** was synthesized from *S*-**2** in the same manner as previously reported by Mori et al. (1991) and Naoshima et al. (1991). A small amount of 1-butanol, probably derived from THF, was also obtained. We used the mixture without purification because the open column chromatography after PCC oxidation gives pure *S*-**4**.

(S)-3,8-Dimethyloctanal (S-4). Pyridinium chlorochromate (4.8 g) and SiO_2 (Wako C-100, 2.3 g) were dissolved in dry CH_2Cl_2 (15 ml) and cooled in an ice bath. *S*-**3** (500 mg) dissolved in CH_2Cl_2 (8 ml) was added dropwise to the solution. Two hours later, the mixture was directly added to a SiO_2 column and eluted by CH_2Cl_2. Further purification on the SiO_2 column gave *S*-**4** (280 mg).

(S)-10,14-Dimethyl-7-pentadecen-1-ol (S-5). *S*-**5** was synthesized in the same manner as used for *n*-alken-1-ol syntheses (Horiike et al., 1978). (7-Hydroxyheptyl)-triphenylphosphonium bromide (700 mg) in warm DMSO (2.2 ml) was added to a THF solution of methylsulfinylmethanide ion [prepared from sodium hydride (200 mg) and DMSO (1.8 ml) under nitrogen at 75°C] with cooling in an ice bath. The yellowish red solution of phosphorane was stirred at room temperature for 15 min, then *S*-**4** (180 mg) was added at 0°C. After stirring for 1 hr, the reaction mixture was poured into 20 ml of water and extracted three times with hexane. After removal of the solvent, subsequent open column chromatography gave *S*-**5** (52 mg).

Prolonged reaction time (5 hr) and increased phosphorane improved the yield. IR ν cm^{-1}: 3325 (br), 2925 (s), 2850 (s), 1465 (m), 1360 (m), 1055 (m). ^1H NMR (90 MHz): δ 5.4 (2 H, m), 3.63 (2 H, t, J = 7 Hz), 0.9–2.3 (2OH, m), 0.87 (9 H, d, J = 7 Hz). ^{13}C NMR (22.5 MHz): δ 130.43, 128.61, 62.98, 39.29, 36.90, 34.52, 33.41, 32.76, 29.68, 29.09, 27.95, 27.24, 25.65, 24.88, 22.70, 22.58, 19.60. MS: 254 (8%, M^+), 179 (1), 169 (6), 151 (12), 137 (4), 126 (46), 109 (33), 95 (57), 81 (55), 71 (89), 57 (100). HR-MS: $C_{17}H_{34}O$ (observed: 254.2611; calculated: 254.2610).

(R)-10,14-Dimethyl-1-pentadecanol (R-6). The solution of sodium metaperiodate (545 mg) in water (1 ml) was added dropwise during 1 hr to the ethanol solution (3 ml) of *S*-**5** (19 mg), hydrazine monohydrate (1 g), acetic acid (two drops), and sat. $CuSO_4$ (two drops). One week later, ethanol was removed in vacuo, and the resulting aqueous solution was extracted with hexane. The hexane solution was dried (Na_2SO_4) and concentrated in vacuo. The yield was 16 mg.

IR ν cm^{-1}: 3325 (br), 2915 (s), 2850 (s), 1460 (m), 1380 (m), 1055 (m). ^1H NMR (270 MHz): δ 3.62 (2 H, t, J = 7 Hz), 0.9–1.7 (24 H, m), 0.87 (6 H, d, J = 7 Hz), 0.82 (3 H, d, J = 7 Hz). ^{13}C NMR (67.5 MHz): δ 63.10, 39.36, 37.31, 37.09, 32.81, 32.75, 29.99, 29.62, 29.62, 29.44, 27.96, 27.07, 25.73, 24.79, 22.71, 22.62, 19.71. MS: 223 (1%, M^+-33), 210 (2), 195 (1), 182 (2), 168 (8), 153 (21), 140 (11), 126 (30), 111 (50), 97 (89), 83 (76), 71 (85), 69 (90), 57 (100), 55 (86). HR-MS: The dehydration peak was predominant. M^+-H_2O: $C_{17}H_{34}$ (observed: 238.2661; calculated: 238.2661).

(R)-10,14-Dimethyl-1-pentadecyl isobutyrate (R-1). *R*-**6** (20 mg) was dissolved in pyridine (0.4 ml) and isobutyric anhydride (0.4 ml). After 10 hr, the solution was poured into cooled 5% hydrochloric acid and extracted with hexane. After the usual work-up, the crude product was purified by open column chromatography (20.9 mg).

IR ν cm^{-1}: 2925 (s), 2850 (s), 1740 (s), 1470 (m), 1190 (m), 1155 (m) ^1H NMR (90 MHz): δ 4.06 (2 H, t, J = 7 Hz), 2.55 (1 H, sept, J = 7 Hz), 1.3–1.8 (24 H, m), 1.15 (6 H, d, J = 7 Hz), 0.87 (9 H, d, J = 7 Hz). ^{13}C NMR (22.5 MHz): δ 177.28, 64.41, 39.35, 37.29, 37.08, 34.04, 32.76, 29.98, 29.59, 29.59, 29.23, 28.64, 27.98, 27.06, 25.89, 24.79, 22.70, 22.61, 19.69,

19.00. 19.00 MS: 326 (4%, M^+), 283 (2), 239 (1), 210 (1), 183 (1), 168 (3), 153 (8), 126 (13), 111 (21), 97 (32), 89 (100), 88 (62), 83 (28), 71 (56), 69 (29), 57 (38), 55 (30). HR-MS: $C_{21}H_{42}O_2$ (observed: 326.3194; calculated: 326.3185). CD (*c*1, hexane): no measurable absorption was observed from 220 to 400 nm.

Reduction of 3,8-dimethyloctanal (4). *S*-**4** (30 mg) was dissolved in ether (1.5 ml) and cooled in an ice bath. Lithium aluminum hydride suspension (1.5 ml) in ether (1 mol/dm^3) was added to the solution. After 25 min, the reaction mixture was poured into cooled 3% hydrochloric acid. Then the solution was extracted with hexane two times. Work-up gave *S*-3 (quantitative). *S*-**3** (from *S*-**4**): $[\alpha]_D^{25}$ −5.7° (*c*0.72, MeOH). *R*-**3** (from *R*-**4**): $[\alpha]_D^{25}$ +5.6° (*c*2.0, MeOH); lit. (Mori et al., 1991) $[\alpha]_D^{20}$ +5.3° (neat, d = 0.827).

(S)-3,8-Dimethyl-1-octyl (R)-α-methoxy-α-trifluoromethylphenylacetate (S-7). *S*-**3** (1.6 mg, derived from *S*-**4**) was dissolved in 0.2 ml of dry pyridine. Then 15 μl of (*S*)-MTPA chloride was added. After 19 hr, the reaction mixture was poured into cooled 3% hydrochloric acid. Then the solution was extracted with hexane two times. Work-up gave crude *S*-**7** (7 mg). The ^{1}H NMR spectrum was measured without purification. The starting material *S*-**3** disappeared.

IR ν cm^{-1}: 2950 (s), 2925 (m), 1740 (s), 1270 (m), 1165 (s), 1120 (m), 1050 (m), 700 (m). ^{1}H NMR (270 MHz): δ 7.3–7.6 (5 H, m), 4.36 (2 H, dd, J = 8, 6 Hz), 3.55 (3 H, q, J = 0.5 Hz), 1.72 (1 H, m), 1.0–1.6 (9 H, m), 0.88 (3 H, d, J = 7 Hz), 0.85 (6 H, d, J = 7 Hz). MS: 344 (M^+-30, 1%), 189 (100), 158 (4), 141 (9), 139 (4), 127 (5), 119 (7), 105 (14), 99 (6), 91 (4), 85 (23), 71 (25), 69 (10), 57 (23), 55 (9).

(R)-3,8-Dimethyl-1-octyl (R)-α-methoxy-α-trifluoromethylphenylacetate (R-7). *R*-**7** was prepared in the same manner used for *S*-**7** synthesis. The starting material *R*-**3** also disappeared. IR ν cm^{-1}: 2950 (s), 2925 (m), 1740 (s), 1265 (m), 1160 (s), 1120 (m), 1050 (m), 700 (m). ^{1}H NMR (270 MHz): δ 7.3–7.6 (5 H, m), 4.36 (2 H, m), 3.57 (3 H, q, J = 0.5 Hz), 1.72 (1 H, m), 1.0–1.6 (9 H, m), 0.89 (3 H, d, J = 7 Hz), 0.86 (6 H, d, J = 7 Hz)., MS: 190 (M^+-184, 9%), 189 (100), 141 (9), 127 (5), 119 (7), 105 (14), 99 (6), 85 (23), 77 (6), 71 (23), 69 (8), 57 (22).

(S)-3,8-Dimethyl-6-octen-1-yl (R)-α-methoxy-α-trifluoromethylphenylacetate (S-8). *S*-**2** (2.0 mg), (*R*)-MTPA (11.0 mg), dicyclohexylcarbodiimide (15.0 mg), and pyridine (10 μl) were dissolved in 0.5 ml of dry dichloromethane. After 24 hr, the reaction mixture was directly added to the SiO_2 column and eluted by hexane–CH_2Cl_2. The yield was 4.0 mg. IR ν cm^{-1}: 2960 (s), 2920 (s), 1740 (s), 1445 (m), 1260 (m), 1180 (s), 1165 (s), 1120 (m), 1020 (m), 715 (m). ^{1}H NMR (270 MHz); δ 7.3–7.6: (5 H, m), 5.06 (1 H, br t, J = 7 Hz), 4.36 (2 H, dd, J = 7, 6 Hz), 3.55 (3 H, q, J = 0.5 Hz), 1.95 (2 H, m), 1.72 (1 H, m), 1.68 (3H, s), 1.60 (3H, s), 1.1–1.6 (4H, m), 0.90 (3 H, d, J = 7 Hz). MS: 203 (1%), 189 (78), 138 (M^+-MTPA, 51), 123 (34), 105 (30), 95 (46), 82 (40), 81 (57), 69 (100), 67 (21), 55 (32), 41 (54).

(R)-3,8-Dimethyl-6-octen-1-yl (R)-α-methoxy-α-trifluoromethylphenylacetate (R-8). *R*-**8** was prepared in the same manner used for *S*-**8** synthesis. The yield was 3.4 mg. IR ν cm^{-1}: 2960 (s), 2920 (s), 1740 (s), 1445 (m), 1265 (m), 1180 (s), 1165 (s), 1120 (m), 1020 (m), 715 (m). ^1H NMR (270 MHz): δ 7.3–7.6 (5 H, m), 5.06 (1H, br t, J = 7 Hz), 4.36 (2 H, m), 3.56 (3 H, q, J = 0.5 Hz), 1.95 (2 H, m), 1.72 (1 H, m), 1.68 (3 H, s), 1.60 (3 H, s), 1.1–1.6 (4 H, m), 0.90 (3 H, d, J = 7 Hz). MS: 203 (1%), 189 (73), 138 (M^+-MTPA, 49), 123 (33), 105 (29), 95 (46), 82 (40), 81 (58), 69 (100), 67 (22), 55 (32), 41 (56).

Gas Chromatograph–Electroantennographic Detection (GC-EAD). A Hewlett-Packard 5890 II gas chromatograph equipped with a cool on-column injector and flame ionization detector (FID) was operated using helium as carrier gas at a column head pressure of 55 kPa. Injections (0.6 μl) were made directly on a DB-1 fused silica capillary column (15 m × 0.25 mm ID, 0.25 μm film thickness; J & W Scientific, Folsom, California) at an initial column temperature of 50°C. After 1 min, oven temperature was programmed at 20°C/min to 190°C, then 10°C/min to 220°C, and held at the final temperature for 4 min.

The GC-EAD system was set up according to Struble and Arn (1984). Makeup gas (He, ca. 50 ml/min) was introduced at the end of the fused silica capillary column, and then the column flow was split into the FID and EAD lines at ca. 1 : 1 ratio.

RESULTS AND DISCUSSION

The terminal structure of **1** resembles the terpenoids such as the citronellols. Citronellol has been widely used as a chiral building block for the syntheses of optically active pheromone compounds (Mori et al., 1991; Naoshima et al., 1991).

(*S*)-Citronellol (*S*-**2**) was reduced with lithium aluminum hydride and cobalt(II) chloride to (*S*)-3,8-dimethyl-1-octanol (*S*-**3**) (Mori et al., 1991; Naoshima et al., 1991). This was oxidized with pyridinium chlorochromate to (*S*)-3,8-dimethyloctanal (*S*-**4**). Then *S*-**4** was coupled with the ylide (Horiike et al., 1978).

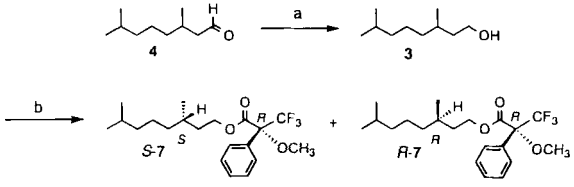
The alcohol *S*-**5** was reduced to *R*-**6** by diimide, which was generated in situ by the oxidation of hydrazine (Corey et al., 1961; Hoffman and Schlessinger, 1971; Wright et al., 1988). To avoid possible racemization (Mori et al., 1991; Naoshima et al., 1991), the catalytic hydrogenation was not adopted. Note that *R*-**6** was derived from *S*-**5**. Acylation of *R*-**6** by isobutyric anhydride in pyridine gave isobutyrate *R*-**1**. The physical properties of *R*-**1** were coincident with those of the natural pheromone and the racemic compound, which were previously reported (Wakamura et al., 1994).

S-**1**, the enantiomer of *R*-**1**, was also synthesized from (*R*)-citronellol

(*R*-**2**) in the same manner. Neither *R*-**1** nor *S*-**1** gave an $[\alpha]_D^{25}$ value (*c*1, CH_2Cl_2). Some predecessors had met similar phenomena, i.e., (*R*)- and (*S*)-15-methyltritriacontane, the sex stimulant pheromone of the stable fly *Stomoxys calcitrans* L., exhibited no measurable optical rotations at the sodium D-line (Naoshima and Mukaidani, 1987). Several other cases where the two enantiomers could not be distinguished by optical rotation were also listed in the literature. Racemic **1** showed a single GC peak on a chiral capillary column (Chirasil DEX CB, 0.25 mm ID × 25 m length, 0.25 μm film thickness) at 200°C when the carrier gas was helium at a column head pressure of 100 kPa. These circumstances compelled us to evaluate the enantiomeric purity at an earlier stage of the syntheses.

Both (*S*)- and (*R*)-3,8-dimethyloctanal (*S*-**4** and *R*-**4**) were reduced to *S*-**3** and *R*-**3** by lithium aluminum hydride in ether. *S*-**3** and *R*-**3** were then converted to the corresponding (*R*)-MTPA esters (Dale and Mosher 1973; Ohtani et al., 1991) (*R*-**7** and *S*-**7**, respectively) by treating with (*S*)-MTPA chloride in pyridine (Scheme 2). The C-1 methylene protons of *S*-**7** and *R*-**7** showed different coupling patterns. No isomer peak was observed in either sample (Figure 1), which indicated that the *S*-**4** and *R*-**4** employed for the Wittig reactions were enantiomerically pure. We therefore considered that the resulting compounds (*R*-**1** and *S*-**1**) were also optically pure because later reaction conditions suggested no isomerization reaction took place.

In the case of *S*-**7**, Figure 1 reveals that C-1 methylene protons form an A_2 pattern. On the other hand, *R*-**7**'s C-1 methylene protons form an AB pattern, and they couple with the diastereotopic C-2 methylene protons. Thus, C-1 methylene protons of *R*-**7** show a complicated coupling pattern. Most probably, the major cause of this difference is the magnetic anisotropy of the phenyl groups. We also synthesized *S*-**2** and *R*-**2** (*R*)-MTPA esters (*S*-**8** and *R*-**8**, respectively). The coupling patterns of the C-1 methylene signals of *S*-**8** and *R*-**8** (^{1}H NMR) were coincident with those of *S*-**7** and *R*-**7**, respectively. We think that we can determine the C-3 absolute configurations of some terpenoids possessing the 3-methyl-1-ol system by MTPA esterification.



SCHEME 2. Consideration of the optical purity of **4**.

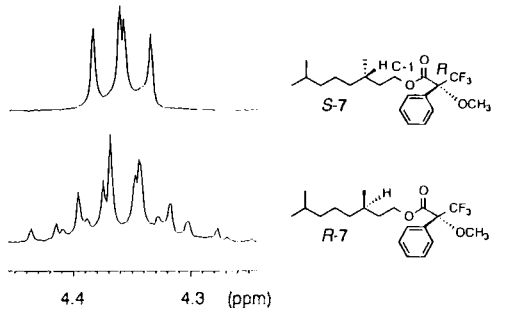


FIG. 1. ^{1}H NMR spectra of *S*-**7** and *R*-**7** (C-1 methylene region) (270 MHz, $CDCl_3$).

Electrophysiological activities of *R*-**1** and *S*-**1** were evaluated using a gas chromatograph equipped with an electroantennographic detector. The dose-response curve of *R*-**1** (3 pg–3 ng) based on the relative voltage generated by the male antenna of *E. pseudoconspersa* was well adjusted with that of the natural pheromone (Figure 2). *S*-**1** was apparently less active than *R*-**1**. We therefore concluded that the major sex pheromone component of *E. pseudoconspersa* is (*R*)-10,14-dimethyl-1-pentadecyl isobutyrate.

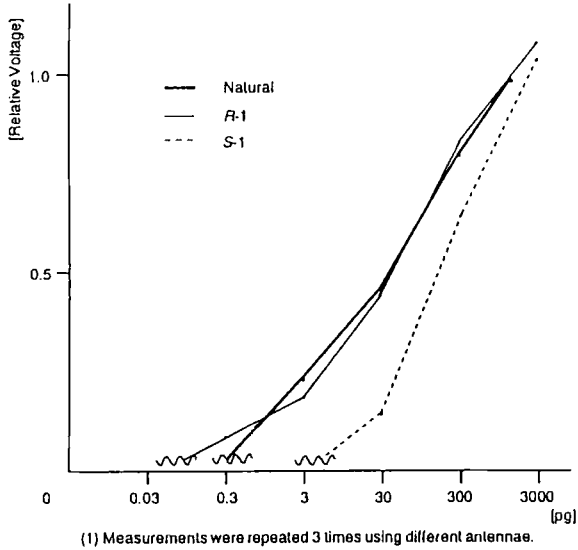


FIG. 2. Dose–response curves of GC-EAD experiment.

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