Journal of Chemical Ecology, Vol. 24, No. 8, 1998

RESPONSES OF TEA TUSSOCK MOTH, *Euproctis pseudoconspersa*, TO ITS PHEROMONE, (*R*)-10,14-DIMETHYLPENTADECYL ISOBUTYRATE, AND TO THE *S*-ENANTIOMER OF ITS PHEROMONE

CHENG-HUA ZHAO,[1] JOCELYN G. MILLAR,[2,*] KUN-HUI PAN,[3] and CAI-SHENG XU[3]

[1]*Institute of Zoology, Chinese Academy of Sciences Beijing 100080, China*

[2]*Department of Entomology, University of California Riverside California, 92521*

[3]*Yichun Station of Forest Diseases and Pest Control Yichun Jiangxi Province, 336000, China*

(Received July 10, 1997; accepted April 1, 1998)

Abstract—Field trials were conducted with each synthetic enantiomer (>98% ee) and blends of the two synthetic enantiomers of the female-produced sex pheromone (10,14-dimethylpentadecyl isobutyrate) of the tea tussock moth, *Euproctis pseudoconspersa*. Male moths were attracted to each enantiomer alone and to various blends of them. Short syntheses of both enantiomers of the pheromone from commercially available (*R*)- and (*S*)-citronellyl bromide and a method of checking the enantiomeric purity of the citronellyl bromide enantiomers are described.

Key Words—*Euproctis pseudoconspersa*, 10,14-dimethylpentadecyl isobutyrate, sex pheromone.

INTRODUCTION

The tea tussock moth, *Euproctis pseudoconspersa* (Strand) (Lepidoptera: Lymantridae), is a serious defoliating pest of tea, *Camellia sinensis*, in China and Japan, resulting in losses in both quality and quantity of tea produced. Furthermore, tea tussock moth larvae have urticating spines that cause severe

*To whom correspondence should be addressed.

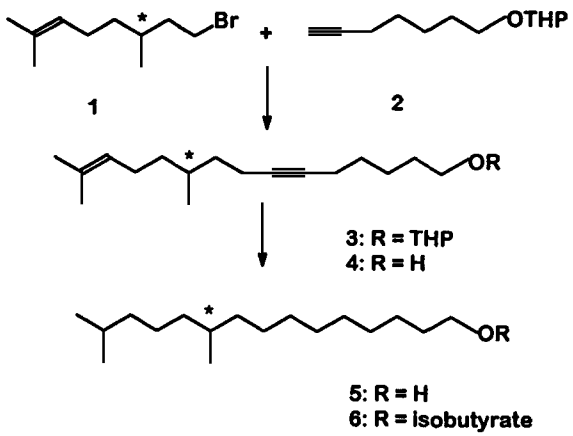
0098-0331/98/0800-1347/$15.00/0

skin irritation upon contact (Ogata, 1958). The major component of the female-produced sex attractant pheromone of the moth was recently identified by two research groups independently as 10,14-dimethylpentadecyl isobutyrate (Wakamura et al., 1994; Zhao et al., 1996). Both groups found that the racemic pheromone was attractive to male moths. The naturally produced pheromone was tentatively established as the *R* configuration about the C_{10} methyl group by comparison of electroantennogram (EAG) responses of male moth antennae to the synthetic enantiomers (Ichikawa et al., 1995). A more recent study reports that the *R*-enantiomer elicits stronger EAG responses from male moth antennae than the *S*-enantiomer or the racemic mixture (Wakamura et al., 1996a). In field trials, however, each enantiomer singly and in blends was equally attractive (Wakamura et al. 1996a). We report the syntheses of each of the enantiomers of the pheromone, a method for checking the enantiomeric purity of the chiral starting materials, and results of field trials in commercial tea plantations in Jiangxi province, China.

METHODS AND MATERIALS

Proton NMR spectra were prepared with a General Electric QE 300 instrument (300 MHz) in $CDCl_3$ solution. EI mass spectra (70 eV) were taken with a Hewlett-Packard (HP) 5970B mass selective detector interfaced to an HP 5890 GC (Avondale, Pennsylvania) fitted with a DB5-MS column (20 m × 0.2 mm ID, J&W Scientific, Folsom, California). Air- and/or water-sensitive reactions were carried out in oven-dried glassware under N_2 atmosphere. THF was purified by distillation from sodium-benzophenone ketyl under argon. Flash chromatography was carried out with 230–400 mesh silica gel (Aldrich Chemical, Milwaukee, Wisconsin).

Synthesis of 10,14-Dimethylpentadecyl Isobutyrate Enantiomers (Scheme 1). Butyllithium (8.8 ml of a 2.5 M solution in hexanes; 22 mmol) was added dropwise to a cooled (0°C) THF solution (25 ml) of the tetrahydropyranyl ether of 6-heptyn-1-ol, **2** (3.92 g, 20 mmol) (Zhao et al., 1996). The solution was stirred for 20 min, followed by the sequential addition of dry *N,N'*-dimethylpropyleneurea (DMPU) (10 ml) and (*R*)-(−)-citronellyl bromide, **1** [4.34 g, 20 mmol; $[\alpha]_D^{20} = -6.8°$ (neat), Aldrich Chemical]. The mixture was warmed to 20°C and stirred overnight, then poured into water, and the mixture was extracted three times with hexane. The combined hexane extracts were washed with water and brine, dried, and concentrated. The crude THP ether, **3**, was taken up in MeOH (25 ml), and *p*-toluenesulfonic acid (~1 g) was added in portions until the solution was acidic to moist litmus paper. The solution was stirred overnight, then 2.5 g of $NaHCO_3$ were added, and most of the MeOH was removed by rotary evaporation. The residue was partitioned between water and hexane, and



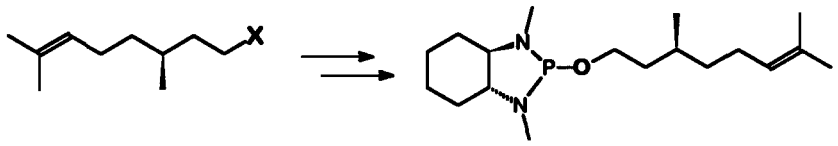
SCHEME 1.

the hexane layer was washed with brine, dried, concentrated, and flash chromatographed (20% EtOAc in hexane), yielding alcohol **4** (4.13 g, 80%). (*S*)-**4** was prepared in analogous fashion from (*S*)-(+)-citronellyl bromide [Aldrich Chemical; $[\alpha]_D^{20} = +6.8°$ (neat)] in 67% yield.

A slurry of 5% Pd on carbon (2 g) in hexane (50 ml) was flushed with Ar, then saturated with H_2. (*R*)-Alcohol **4** (2.5 g, 10 mmol), was added by syringe, and the mixture was stirred until the reaction was complete (8 hr), monitoring the reaction by GC. The mixture was then flushed thoroughly with Ar, filtered through a short plug of Cellite, and flash chromatographed (25% EtOAc in hexane), giving a quantitative yield of saturated (*S*)-alcohol **5**. NMR and mass spectra and GC retention time matched those of the previously synthesized racemic material (Zhao et al., 1996). (*R*)-**5** was similarly prepared from (*S*)-**4** in 83% yield.

Isobutyryl chloride (1 ml, 10 mmol) was added dropwise to a solution of (*S*)-alcohol **5** (1.7 g, 6.6 mmol) and pyridine (1.2 g, 15 mmol) in ether (100 ml) at 0°C. The mixture was stirred overnight, then filtered. The filtrate was washed thoroughly with 1 M $NaHCO_3$, 1 M HCl, and brine, then dried, concentrated, and Kugelrohr distilled (oven temperature 175°C, 0.7 mm Hg), yielding (*S*)-ester **6** (2.1 g, 98%). The mass, NMR, and IR spectra matched those of the racemic material (Zhao et al., 1996). The *R*-enantiomer of **6** was similarly prepared in quantitative yield.

Analysis of Enantiomeric Purity of Starting Material (Scheme 2). (*S*)-(+)-Citronellyl bromide **1** (90 mg, 0.4 mmol) was added to a solution of cesium acetate (800 mg, 4.1 mmol) in DMPU (2 ml), and the mixture was stirred at 60°C for 45 min. The mixture was cooled, poured into water, and extracted



1: X = Br
7: X = OAc
8: X = OH

9

SCHEME 2.

with hexane. The hexane extract was concentrated, giving crude acetate (*S*)-**7**. Ethanol (5 ml) and 2 M aq. NaOH (0.5 ml) were added, and the solution was stirred at room temperature for 4 hr. The solution was then diluted with water and extracted with hexane. The hexane extract was dried over anhydrous Na_2SO_4, concentrated, and Kugelrohr distilled (oven temperature 75°C, 0.5 mm Hg), yielding (*S*)-(−)-β-citronellol **8**. Retention time and mass spectrum matched those of an authentic sample of racemic β-citronellol (Aldrich Chemical).

An aliquot of distilled (*S*)-**8** (9 μl, 0.05 mmol) was added to an oven-dried 5-mm NMR tube, followed by 0.25 ml of dry deuterated benzene, and 0.5 ml of a 0.1 M solution of (3a*R*,7a*R*)-2-dimethylamino-1,3-dimethyl-octahydro-1H-1,3,2-benzodiazaphosphole (Fluka Chemical, Milwaukee, Wisconsin). The tube was sealed and allowed to stand at room temperature for two days, before being analyzed by phosphorus NMR at 202.47 MHz, using a General Electric GN-500 instrument. An analogous sample was prepared from racemic β-citronellol.

Field Trials. Field trials were carried out in commercial tea plantations in Jiangxi Province, People's Republic of China. In the first trial (June 6–12, 1995), pheromone solutions in CH_2Cl_2 were loaded onto rubber septum lures (11 mm, The West Co., Lititz, Pennsylvania; 1 mg dose/septum, five replicates) that were placed in Pherocon 1C sticky traps (Trece, Salinas, California). Traps were hung within the tea fields, suspended from wooden stakes at a height of ~1.5 m, at a spacing of at least 20 m between traps. Male moth captures were tabulated daily and traps were rerandomized every second day. In the second trial (June 6–20, 1996), 1-mg doses of pheromone on rubber septa were used as baits in water pan traps, as previously described (Zhao et al., 1996). Trap catch data were checked for normality and analyzed by analysis of variance, followed by Bonferroni's all pairwise multiple comparison procedure (SigmaStat, 1992).

RESULTS AND DISCUSSION

In the first field trial, each of the synthetic enantiomers alone or in various mixtures attracted equal numbers of moths (Table 1), corroborating the report

TABLE 1. MALE *E. pseudoconspersa* CAUGHT IN TRAPS BAITED WITH DIFFERENT RATIOS OF SYNTHETIC ENANTIOMERS OF 10,14-DIMETHYLPENTADECYL ISOBUTYRATE[a]

Ratio, R:S	Moths caught (Mean ± SE)
100:0	117.6 ± 19.0a
95:5	104.0 ± 11.0a
80:20	105.2 ± 14.4a
50:50	107.4 ± 9.5
20:80	80.6 ± 36.5a
5:95	68.2 ± 19.6a
0:100	69.8 ± 5.7a
Blank	6.2 ± 1.6b

[a]Mean catch per trap of the combined counts from five replicates counted seven times (i.e., treated statistically as five replicates). Total dose/lure: 1 mg. Numbers followed by the same letter are not significantly different (Bonferroni's multiple pairwise comparison procedure, $P = 0.05$).

of Wakamura et al. (1996a). Because it is unusual for pheromone enantiomers to be of similar attractiveness (Mori, 1996), a second test compared attractiveness of each enantiomer alone and that of the racemic mixture. The attractiveness of all three lures was again statistically indistinguishable, with the *R*, *S*, and racemic formulations attracting averages of 11.0 ± 2.85, 9.33 ± 2.32, and 6.67 ± 2.5 moths/trap respectively ($N = 6$, Student-Newman-Kuels test, $P > 0.05$).

Because the results of Wakamura et al. (1996a) were not known when these studies were executed, we were concerned that the unusual field-trapping results might be due to enantiomerically impure pheromone because starting materials [(*R*)- and (*S*)-citronellyl bromides **1**] of unknown chiral purity had been used in the synthesis (the supplier was not able to provide the enantiomeric purity). It was not possible to check the chiral purity of the synthetic pheromone enantiomers directly because the pheromone enantiomers have negligible optical rotations and are inseparable by gas chromatography (GC) on a chiral stationary phase (Ichikawa et al., 1995). Our synthetic sample of racemic **6** also gave a single peak on a chiral Cyclodex-B GC column, operated isothermally, so that the chiral purity of the synthetic pheromone enantiomers could not be checked directly.

However, the conditions used to generate the enantiomers of **6** from each citronellyl bromide enantiomer result in negligible loss of stereochemical integrity at the single chiral center in the molecule. Consequently, as a reasonable alternative to direct determination of the enantiomeric purity of the synthetic pheromone enantiomers, the enantiomeric purity of the starting material was checked instead. Because the citronellyl bromide enantiomers were also not

resolved on a Cyclodex-B GC column, this necessitated conversion of a sample of (*S*)-citronellyl bromide to a derivative whose enantiomeric purity could be readily determined. Thus, (*S*)-citronellyl bromide was converted to (*S*)-citronellol **8**, via the acetate **7**, then to the derivative **9** (Alexakis et al., 1992). A racemic sample of citronellol, derivatized under the same conditions, showed two ^{31}P NMR signals of equal size (137.33 and 136.70 ppm). Only a single peak was discernible with the compound derived from (*S*)-**8**, allowing us to conservatively estimate that the (*S*)-citronellyl bromide starting material had an enantiomeric excess of >98%. Because the commercial citronellyl bromide enantiomers had equal and opposite optical rotations, we conclude that the pheromones produced from them were of high enantiomeric purity, and that *E. pseudoconspera* male moths do not discriminate between the two enantiomers (Table 1).

Our field-trapping results corroborate the analogous report from Wakamura et al. (1996a). This research group had demonstrated in an earlier study that male antennae respond most strongly to stimulation by (*R*)-**6**, with a lesser response to the racemic mixture, and the least response to (*S*)-**6** (Ichikawa et al., 1995). Furthermore, EAG responses of male antennae exposed to either the insect-produced pheromone or (*R*)-**6** were indistinguishable (Wakamura et al. 1996a). Thus, the available evidence suggests that females produce (*R*)-**6**, or a nonracemic mixture containing a large preponderance of (*R*)-**6**, but for unknown reasons, males show no distinct preference for either enantiomer in field-trapping studies. Several examples of this unusual category of pheromone-mediated behavior have been documented in the Insecta (reviewed in Mori, 1996), but to our knowledge, this is the first instance of such a phenomenon in the Lepidoptera. In general, unnatural enantiomers, when presented to moths known to utilize chiral sex attractant pheromone components, either have no discernible biological activity or inhibit attraction of males (Mori, 1996).

For practical purposes, being able to use the cheaper and more readily available racemic material should expedite the commercialization of this pheromone for monitoring purposes. Furthermore, because the reproductive lifetime of female moths is short and highly synchronized in generations, it has been suggested that pheromone-based mating disruption may represent a feasible control strategy for this pest species (Wakamura et al., 1996b).

Acknowledgments—We thank J. Steven McElfresh for assistance with statistical analyses, and Dan Borchardt (Chemistry Department, University of California, Riverside) for ^{31}P NMR analyses. This study was supported by the National Natural Science Foundation of China.

REFERENCES

ALEXAKIS, A., MUTTI, S., and MANGENEY, P. 1992. A new reagent for the determination of the optical purity of primary, secondary, and tertiary chiral alcohols and of thiols. *J. Org. Chem.* 57:1224–1237.

ICHIKAWA, A., YASUDA, T., and WAKAMURA, S. 1995. Absolute configuration of sex pheromone for tea tussock moth, *Euproctis pseudoconspersa* (Strand) via synthesis of (*R*)- and (*S*)-10,14-dimethyl-1-pentadecyl isobutyrates. *J. Chem. Ecol.* 21:627–634.

MORI, K. 1996. Molecular asymmetry and pheromone science. *Biosci. Biotech. Biochem.* 60:1925–1932.

OGATA, K. 1958. Studies on the Far Eastern urticating moth, *Euproctis flava* Bremer, as a pest of medical importance. I. Morphological notes. *Jpn. J. Sanit. Zool.* 9:116–129.

SIGMASTAT. 1992. SigmaStat for Windows 1.0. Jandel Scientific, San Rafael, California.

WAKAMURA, S., YASUDA, T., ICHIKAWA, A., FUKUMOTO, T., and MOCHIZUKI, F. 1994. Sex attractant pheromone of the tea tussock moth *Euproctis pseudoconspersa* (Strand) (Lepidoptera: Lymantridae). Identification and field attraction. *Appl. Entomol. Zool.* 29:403–411.

WAKAMURA, S., ICHIKAWA, A., YASUDA, T., ARAKAKI, N., and FUKUMOTO, T. 1996a. EAG and field responses of the male tea tussock moth *Euproctis pseudoconspersa* (Strand) (Lepidoptera: Lymantridae) to (*R*)- and (*S*)-enantiomers and racemic mixture of 10,14-dimethylpentadecyl isobutyrates. *Appl. Entomol. Zool.* 31:623–625.

WAKAMURA, S., YASUDA, T., and MOCHIZUKI, F. 1996b. Mating behavior of the tea tussock moth *Euproctis pseudoconspersa* (Strand) (Lepidoptera: Lymantridae). *Appl. Entomol. Zool.* 31:619–621.

ZHAO, C.-H., MILLAR, J. G., WEN, Z., WANG, S., WANG, X., and ZHU, Y. 1996. Isolation, identification, and synthesis of the female sex pheromone of the tea tussock moth, *Euproctis pseudoconspersa* (Lepidoptera: Lymantridae). *Entomologia Sinica* 3:58–69.