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3,13-DIMETHYLHEPTADECANE: MAJOR SEX PHEROMONE COMPONENT OF THE WESTERN FALSE HEMLOCK LOOPER, *Nepytia freemani* MUNROE (LEPIDOPTERA: GEOMETRIDAE)

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Abstract—3,13-Dimethylheptadecane (3,13-dime-17Hy) is the major sex pheromone component of the western false hemlock looper (WFHL), *Nepytia freemani* Munroe. It was identified in extracts of female pheromone glands by coupled gas chromatographic–electroantennographic detection (GC-EAD) and coupled GC–mass spectroscopy (GC-MS). Traps baited with 100 μg of 3,13-dime-17Hy attracted large numbers of male WFHL. Of five additional candidate pheromone dimethylated hydrocarbons, only 3,13-dimethylhexadecane attracted male WFHL. However, neither 3,13-dime-16Hy nor the other four compounds enhanced attraction to 3,13-dime-17Hy when tested in binary or ternary combination at respective ratios of 100:10, 100:1, or 100:1:1. Identification of the complete WFHL sex pheromone requires structural elucidation of all 12 EAD-active components in gland extracts, determination of their chirality, and field testing of antennally active isomers in appropriate combinations and ratios. Stereoisomeric 3,13-dime-17Hy as trap bait may already be used to monitor WFHL populations.

Key Words—Lepidoptera, Geometridae, sex pheromone, 3,13-dimethylhep-

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tadecane, 3,13-dimethylhexadecane, 3,13-dimethyloctadecane, 5,13-dimethylheptadecane, 3,11-dimethylhexadecane, 3,11-dimethylpentadecane.

INTRODUCTION

The western false hemlock looper (WFHL), *Nepytia freemani* Munroe, occurs in the northwestern United States and southwestern Alberta and British Columbia (Ferris and Woensdregt, 1983), feeding on a variety of coniferous trees including Douglas fir, *Pseudotsuga menziesii* (Mirb) Franco; white fir, *Abies concolor* (Gord. & Glend.) Lindl.; western larch, *Larix occidentalis* Nutt.; western hemlock, *Tsuga heterophylla* (Raf.) Sarg.; and Engelmann spruce, *Picea engelmannii* Parry (Furniss and Carolin, 1977). In British Columbia, populations of the WFHL increased to very high levels four times between 1947 and 1984 (Harris et al., 1985), but epidemics occurred only in the drier part of the Douglas fir range (Shepherd, 1977). Repeated defoliation of Douglas fir from 1947 to 1949 in the Windermere Valley resulted in top killing and scattered tree mortality over several thousand hectares and prompted an application of DDT over 4400 ha to protect the threatened Christmas tree industry (Ferris and Woensdregt, 1983). Annual appraisal of WFHL larval populations by Forestry Canada's Forest Insect and Disease Survey (FIDS) allows prediction of population level and potential damage in the following year, but the use of synthetic sex pheromone in survey traps could greatly facilitate and improve the monitoring of WFHL populations. The presence of a sex pheromone has been demonstrated in the WFHL (Shepherd, 1979). We report the identification and field testing of sex pheromone components of the WFHL.

METHODS AND MATERIALS

Laboratory Analyses

Fourth- and fifth-instar WFHL larvae were field collected near Kamloops, British Columbia, in June 1991 and reared to the adult stage in the laboratory at a photoperiod of 14:10 (L:D). Four to 5 hr into the scotophase (Shepherd, 1979), pheromone glands of 2- to 3-day-old virgin females were removed and extracted for 5 min in hexane. Extracts were subjected to gas chromatographic–electroantennographic analysis (GC-EAD) (Arn et al., 1975) on two capillary columns (Hewlett Packard 5890A; DB-210, DB-1, each 30 m × 0.25 mm ID). Coupled GC–mass spectroscopy (GC-MS) (Hewlett Packard 5985B) in full-scan and selected ion monitoring mode (SIM) was conducted to identify pheromone components or confirm their presence in gland extracts. For GC-MS-SIM, full-scan electron impact spectra of synthetic candidate compounds were obtained to select diagnostic ions. In sequence, 200 pg of synthetic compounds, hexane,

and a concentrated pheromone gland extract were then chromatographed, each time scanning for the diagnostic ions. Synthetic candidate pheromone components were further subjected to GC-EAD analyses to compare their EAD activity with those of female-produced compounds.

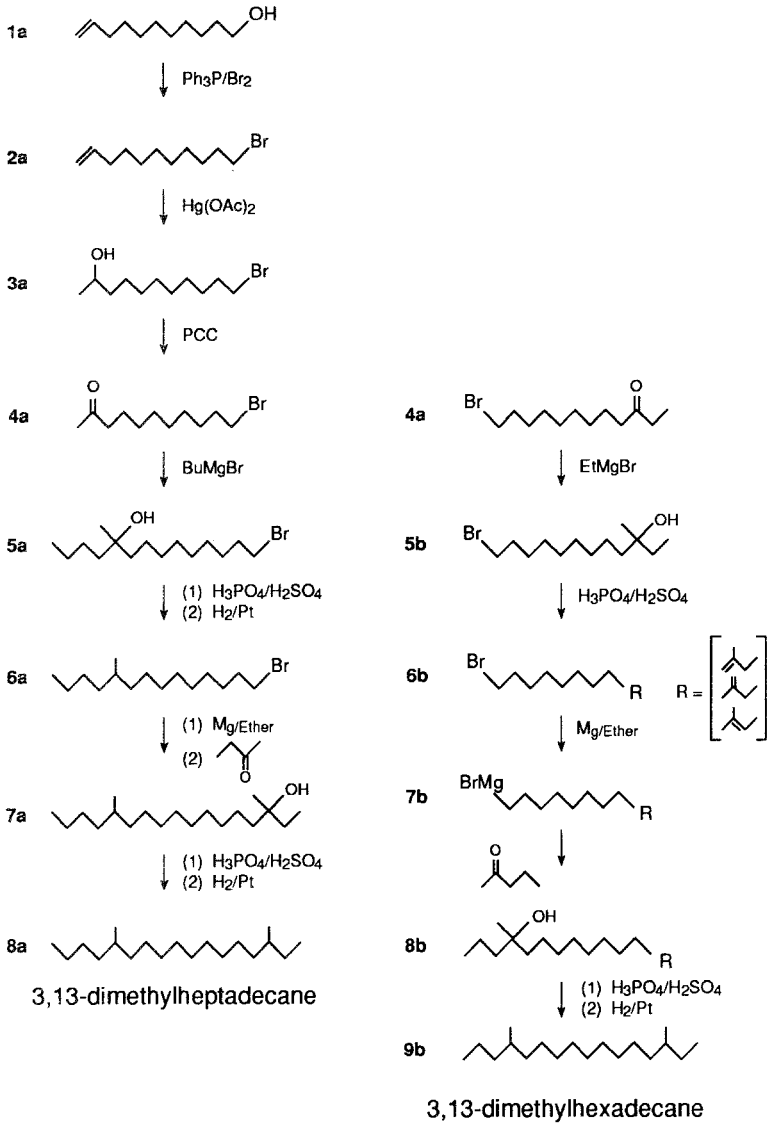
Synthesis of Dimethylated Hydrocarbons

3,13-Dimethylheptadecane (3,13-Dime-17H:H). 10-Undecen-1-ol (Scheme 1, **1a**)) (Aldrich, Milwaukee, Wisconsin) was treated with triphenylphosphine dibromide in dichloromethane containing 2 equivalents of pyridine. The resulting bromoundecene **2a** was reacted with mercuric acetate in aqueous THF for 0.5 hr followed by aqueous sodium borohydride to yield 11-bromo-2-undecanol **3a.** Treatment of **3a** with pyridinium chlorochromate (PCC) produced the corresponding ketone **4a,** which was reacted with butylmagnesium bromide to yield 14-bromo-5-methyl-5-tetradecanol **5a.** Dehydration at 25°C with a mixture of phosphoric and sulfuric acids (10:1) resulted in bromoalkenes. Hydrogenation over 5% platinum on asbestos resulted in **6a,** which was converted in diethyl ether (Et_2O) to the Grignard reagent and reacted with 2-butanone to yield **7a.** Dehydration of **7a,** followed by hydrogenation as above, produced **8a** with 5% overall yield. EI mass spectrum *m/z*: 268 (M^+) 1%, 239 ($M-C_2H_5^+$) 5%, 211 ($M-C_4H_9^+$) 11%, 85 ($C_6H_{13}^+$) 100%, 84 ($C_6H_{12}^+$) 54%, 57 ($C_4H_9^+$) 96%, 56 ($C_4H_8^+$) 39%, 55 ($C_4H_7^+$) 42%.

3,13-Dimethylhexadecane (3,13-Dime-16:H). The previously prepared ketone **4a** was reacted with excess ethylmagnesium bromide to give 12-bromo-3-methyl-3-dodecanol **5b.** Dehydration of **5b** with a mixture of phosphoric and sulfuric acids (10:1) resulted in bromoalkenes **6b,** which were converted to Grignard reagent **7b** in Et_2O, and reacted with 2-pentanone to yield the dimethylated unsaturated alcohols **8b.** Dehydration and hydrogenation of **8b** yielded **9b** with 9% overall yield. EI spectrum *m/z* of **9b:** 254 (M^+) 1%, 225 ($M-C_2H_5^+$) 4%, 211 ($M-C_3H_7^+$) 4%, 71 ($C_5H_{11}^+$) 77%, 70 ($C_5H_{10}^+$) 42%, 57 ($C_4H_9^+$) 100%, 56 ($C_4H_{10}^+$) 25%, 43 ($C_3H_7^+$) 88%.

Field Bioassay

In August–September 1991, field experiments were conducted 30 km west of Kamloops, British Columbia, in a widely spaced mature Douglas fir stand with noticeable, recent defoliation. Experiments were set up in randomized complete blocks, with traps and blocks at approximately 40-m intervals. Traps were suspended 1.5–2 m above the ground and baited with rubber septa (Thomas Scientific, Swedesboro, New Jersey) impregnated with candidate pheromone components in HPLC grade hexane. "Guard" traps baited with 100 μg of 3,13-dime-17:H were placed at the end of trap lines to avoid bias in catches of experimental traps located at the upwind end of each trapping line.



SCHEME 1.

Delta 2-liter milk cartons served as traps. The inner 855 cm^2 surface was covered with adhesive Tangle-Trap (Tanglefoot Company, Grand Rapids, Michigan) to retain moths approaching the chemical-impregnated septum pinned to the middle of the trap. Traps were recorded and advanced one position daily, and traps containing more than 20 moths were replaced, reusing the same lure.

The first experiment tested the following six candidate pheromone components alone at 100 μg each: 3,11-dimethylpentadecane (3,11-dime-15Hy), 3,11-dimethylhexadecane (3,13-dime-16Hy), 3,13-dimethylhexadecane (3,13-dime-16Hy), 5,13-dimethylheptadecane (5,13-dime-17Hy), 3,13-dimethylheptadecane (3,13-dime-17Hy) and 3,13-dimethyloctadecane (3,13-dime-18Hy). The second and third experiment tested the major sex pheromone component, 3,13-dime-17Hy, alone (100 μg) and in binary combinations with the other five candidate components at ratios of 100:10 and 100:1. A final experiment employed Unitraps (Phero Tech Inc., Delta, British Columbia V4G 1E9) and tested 3,13-dime-17Hy alone and in binary and ternary combination with the other candidate pheromone components at respective ratios of 100:1 and 100:1:1.

RESULTS

Laboratory Analyses

GC-EAD analyses of female gland extracts revealed 12 EAD-active compounds (Figure 1) with retention indices similar to nona-, octa-, hepta-, and hexadecane. The structure of the major EAD-active and FID-detectable compound (X in Figure 1) was derived from a mass spectrum containing 54 female equivalents (FEQ). The fragmentation pattern indicated 3,13-dime-17Hy (Figure 2). Identical mass spectral and retention characteristics of authentic 3,13-dime-17Hy with those of the female-produced compound confirmed this structural assignment.

GC-EAD analysis of pheromone gland extracts on DB-210 and DB-1 columns revealed a similar antennal response pattern, but GC-EAD response VIII disappeared upon hydrogenation, suggesting that all compounds but VIII are saturated hydrocarbons. Based upon previous experience with mono- and dimethylated hydrocarbons (Gries et al., 1991a) and with model compounds, which indicated how retention indices are affected by methyl branches in relation to both their position in and the chain length of the molecule, candidate pheromone components were synthesized. 3,11-Dime-15Hy, 3,11-dime-16Hy, 3,13-dime-16Hy, 5,13-dime-17Hy, and 3,13-dime-18Hy coincided with GC-EAD responses I, III, IV, VII, and XII (Figure 1), respectively. All synthetic compounds elicited good antennal responses. EAD activity of 5,13-dime-17Hy was as strong as that of 3,13-dime-17Hy, the major sex pheromone component.

GC-MS analyses of authentic standards and 200 FEQ of gland extract and scanning for ions diagnostic of methyl branch positions resulted in exact retention time and excellent ion ratio matches of synthetic 3,13-dime-16Hy and 3,13-dime-18Hy versus female-produced compounds: synthetic 3,13-dime-16Hy: *m/z* 70 (67), 211 (33), gland extract: *m/z* 70 (64), 211 (36); synthetic 3,13-dime-

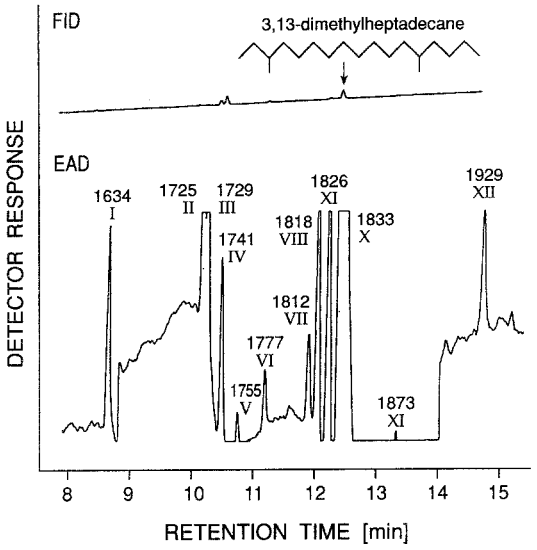


FIG. 1. Detector responses to one female equivalent of pheromone extract. Chromatography: DB-210 column, 1 min at 70°C, 20°C/min to 130°C, 2°C to 220°C. The antennal recording was carried out with a male *N. freemani* antenna. Antennal responses are superscripted by their retention indexes. I: 3,11-dime-15Hy, IV: 3,13-dime-16Hy, X: 3,13-dime-17Hy, XII: 3,13-dime-18Hy; III and VII were tentatively identified as 3,11-dime-16Hy and 5,13-dime-17Hy, respectively. The structural identity of EAD-active compounds II, III, IV, VII, IX, XI is not yet known.

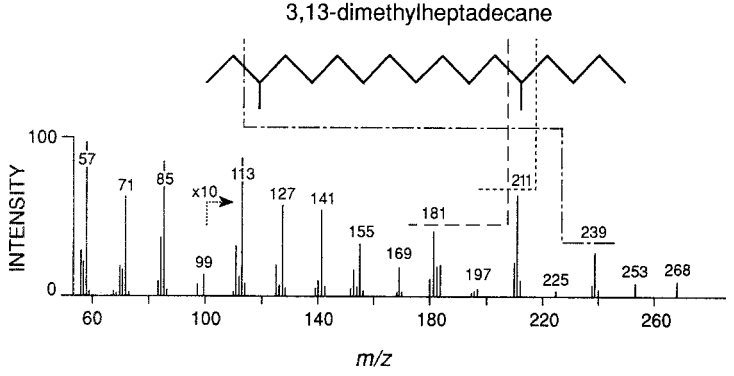


FIG. 2. Mass spectrum of 3,13-dimethylheptadecane present in female pheromone gland extracts.

18Hy: *m/z* 91(81), 211 (19), gland extract: *m/z* 91 (81), 211 (18). 3,11-Dime-15Hy was barely detectable in gland extracts; synthetic 3,11-dime-15Hy: *m/z* 183 (63), 211 (36), gland extract: *m/z* 183 (58), 211 (41). 3,11-Dime-16Hy and 5,13-dime-17Hy were not detectable in female extracts by GC-MS in SIM mode.

Field Trapping

Of the six candidate pheromone components tested alone, only 3,13-dime-17Hy and 3,13-dime-16Hy attracted male moths (Figure 3). 3,13-Dime-17Hy was significantly most attractive and is the major sex pheromone component in WFHL. All six candidate pheromone components combined at 100 μg each completely inhibited response (Figure 3). Binary and ternary combinations of 3,13-dime-17Hy with the other components at respective ratios of 100:10, 100:1 and 100:1:1 did not enhance attraction to 3,13-dime-17Hy alone.

DISCUSSION

Dimethylated hydrocarbons as lepidopteran sex pheromone components have only been recently identified. 5,9-Dimethylheptadecane (Francke, 1987; Francke et al., 1987) and 5,9-dimethyloctadecane (Riba et al., 1990) are major

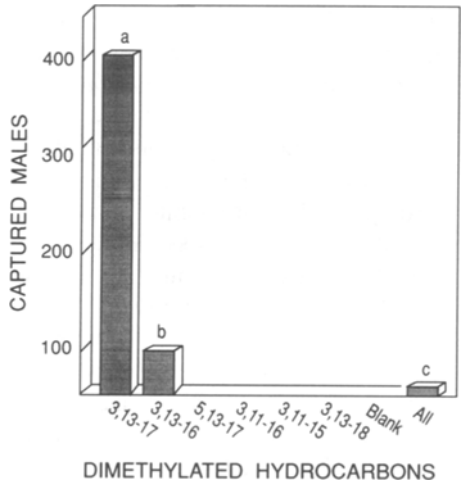


FIG. 3. Total catches of male *Nepytia freemani* in sticky traps baited with candidate pheromone components alone (100 μg each) and in a mixture of six components at 100 μg each, August 24–27, 1991, Savona, British Columbia. $N = 28$. Bars superscripted by the same letter are not significantly different (ANOVA followed by Duncan's multiple range test, $P < 0.05$; treatments that did not attract any moths were excluded from the analysis).

and minor sex pheromone components, respectively, of the mountain-ash bentwing, *Leucoptera malifoliella* (Costa), formerly *L. scitella* (Zeller) (Lepidoptera: Lyonetiidae). 5,9-Dimethylpentadecane is a very attractive sex pheromone component of the coffee leaf miner, *Leucoptera coffeella* (Francke et al., 1988), and 5,11-dimethylheptadecane and 2,5-dimethylheptadecane comprise the sex pheromone of the eastern hemlock looper, *Lambdina fiscellaria fiscellaria* (Guen.) (Gries et al., 1991a,b). The same two dimethylheptadecanes and 7-methylheptadecane are sex pheromone components of the western hemlock looper, *L. f. lugubrosa* (Hulst) (Gries et al., 1993). In this paper we report 3,13-dime-17Hy as major sex pheromone component of the WFHL. It is a new dimethylated hydrocarbon to be identified as a sex pheromone component in the Lepidoptera.

Accumulation and concentration of 54 female equivalents of pheromone gland extracts was sufficient to obtain a mass spectrum of the major EAD-active compound (Figure 2). As mass spectra of mono- and dimethylated hydrocarbons have diagnostic ions indicative of methyl branch positions (Pomonis et al., 1980), the compound was readily identified as 3,13-dime-17Hy. In field trapping experiments, 3,13-dime-17Hy alone attracted a large number of male looper (Figure 3). 3,13-Dime-16Hy by itself was also attractive, but in binary combination with 3,13-dime-17Hy did not enhance attraction. With its homologous molecule structure, it may have only mimicked behavioral activity of the major sex pheromone component. Field attraction to pheromone homologs has also been reported in the gypsy moth, *Lymantria dispar* (L.), (Sarmiento et al., 1972) and in the geometrid moth *Semiothisa signaria dispunctata* (Walker) (Millar et al., 1987).

None of the other candidate pheromone components was attractive by itself or in combination with 3,13-dime-17Hy at the two ratios tested. All six components combined at 100 μg each even inhibited response by WFHL males. As 3,13-dime-17Hy in gland extracts was exceedingly more abundant than other candidate pheromone components, equal amounts of compounds in the six-component blend were likely unnatural and possibly also inhibitory.

Lack of synergism between pheromone components and inhibition of response to the 6-component blend may have also been caused by the presence of unnatural stereoisomers in synthetic compounds. Each of the field tested dimethylated hydrocarbons occurred as a mixture of all possible stereoisomers. As shown in *Leucoptera scitella* (Zeller) (Tóth et al., 1989) and *Lambdina fiscellaria* (Li et al., 1993), male WFHL are likely to be attracted to only one stereoisomer of the respective dimethylated hydrocarbons in pheromone gland extracts. Although the presence of synthetic unnatural stereoisomers did not interfere with attraction of male *L. scitella* and *L. fiscellaria*, unnatural stereoisomers may have inhibited response by male WFHL.

Identification of the complete WFHL sex pheromone requires structural

elucidation of all 12 EAD-active components in gland extracts, determination of their chirality (if optically active), and field testing of antennally active isomers in appropriate combinations and ratios. Stereoisomeric 3,13-dime-17Hy as trap bait may be used to monitor WFHL populations.

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