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IDENTIFICATION OF MALE-SPECIFIC VOLATILES FROM NEARCTIC AND NEOTROPICAL STINK BUGS (HETEROPTERA: PENTATOMIDAE)

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Abstract—Males of the Central American stink bug species, *Euschistus obscurus*, produce an attractant pheromone composed of a blend of compounds characteristic of North American *Euschistus* spp. and the South American soybean pest, *E. heros*. The range of *E. obscurus* extends into the southern United States, the species is easy to rear, and males produce an exceptionally large quantity of pheromone (>0.5 μg/day/male). These factors made *E. obscurus* useful for characterizing the novel pheromone components of *E. heros* without importing this pest species into the United States. *Euschistus obscurus* males produce methyl (2*E*,4*Z*)-decadienoate (61%) in abundance, which is characteristic of North American species, and methyl 2,6,10-trimethyltridecanoate (27%), the main male-specific ester of *E. heros*. The chirality of *Euschistus* spp. methyl-branched esters, and field activity of synthetic formulations, remain to be determined.

Key Words—Heteroptera, Pentatomidae, pheromone, attractant, *Euschistus*, soybean, methyl 2,6,10-trimethyltridecanoate.

INTRODUCTION

Methyl (2*E*,4*Z*)-decadienoate is the major male-specific volatile of five Nearctic stink bugs (Heteroptera: Pentatomidae): *Euschistus conspersus*, *E. tristigmus*,

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E. servus, *E. politus*, and *E. ictericus* (Aldrich et al., 1991). Females, males, and nymphs of the first four of these species were significantly attracted to this ester in the field. Tests in Maryland also demonstrated that parasitic tachinid flies use the unsaturated methyl-ester as a host-finding kairomone (Aldrich et al., 1991). In a sixth species, *E. obscurus*, whose northern range extends into Texas and Florida (Froeschner, 1988), methyl (2*E*,4*Z*)-decadienoate was reported to be a relatively minor male-specific component, with the major component being tentatively identified as methyl 2,6-dimethyltetradecanoate (Aldrich et al., 1991).

Establishment of a prolific laboratory colony of *E. obscurus* has enabled us to reinvestigate the tentative identification of methyl 2,6-dimethyltetradecanoate from *E. obscurus*, which was based on analysis of a single field-collected male. We recently found that the main volatile from males of the South American soybean pest, *E. heros*, is identical to the ester tentatively identified in *E. obscurus* (ignoring chirality), giving added significance to structural verification of the novel *E. obscurus* pheromone component.

We correct here our earlier misidentification of the major male-specific volatile from *E. obscurus* (Aldrich et al., 1991) and provide more detailed information on the presence of other male-specific volatiles for this species and for *E. heros*.

METHODS AND MATERIALS

Insects. Euschistus heros used in the study were obtained from a colony started from adults collected near the Centro Nacional de Recuros Geneticos e Biotechnologic, Brasilia D.F., Brazil, and *E. obscurus* was obtained from a laboratory colony of Dr. Walker Jones (USDA-ARS, Weslaco, Texas). *Euschistus heros* was reared in Brazil on fresh green beans, raw peanuts, and water at 26 ± 1°C with a 16:8-hr light–dark photoperiod. *Euschistus obscurus* was reared in the Beltsville laboratory under similar conditions except that sunflower seeds were used instead of peanuts. The sunflower seeds were glued onto sheets of brown wrapping paper with wallpaper paste, and cut into 10-cm squares that were discarded after depletion by the insects.

Extractions. Airborne extracts were prepared from *Euschistus* spp. in the respective laboratories by confining 20–50 insects in a glass column (ca. 1 liter), drawing air for 24 hr by vacuum (100 ml/min) through ca. 30 mg of activated charcoal inside a Swinney Luer-lock filter holder (13 mm; Thomas Scientific, Philadelphia, Pennsylvania), and extracting the filter with 100–200 μl of CH_2Cl_2 or heptane (Aldrich et al., 1989, 1991).

Chemical Analyses. Brazilian samples were analyzed initially by gas chromatography (GC) on a bonded methyl silicone column (0.25 μm film, 30 m ×

0.25 mm ID; DB-1, J&W Scientific, Folsom, California) in a Varian 3700 GC with hydrogen as carrier (40 cm/sec), a temperature program from 45°C for 2 min to 230°C at 15°/min, a flame ionization detector (FID), and a Shimadzu C-R3A recorder. Samples of *E. obscurus* volatiles, and heptane extracts of *E. heros* brought to the United States, were analyzed on a DB-1 column (0.25 μm film, 30 m × 0.25 mm ID) in a Varian 3500 GC with helium as carrier (50 cm/sec), a temperature program from 50°C for 2 min to 235°C at 15°/min, and an FID. Data were recorded using the Varian GC Star Workstation software on a Gateway 2000 386/25 computer. GC traces presented in figures begin at 2.5 min (at left) so as to exclude the solvent peak and normalize peaks to the most abundant natural product. Inverted GC traces in figures were created using Corel Draw version 3.0 software.

Electron impact mass spectra (MS) were obtained using either a Finnigan 4510 GC-MS equipped with an INCOS Data System, at 70 eV, and a 30-m DB-1 column, programmed from 60°C for 2 min to 250°C at 5°/min, or a Hewlett Packard 5971 GC-MS instrument at 70 eV, with a HP-5 column (0.11 μm film; 25 m × 0.2 mm ID), programmed from 50°C for 2 min to 250°C at 15°/min.

Hydrogenation/Hydrogenolysis Reaction. High-temperature treatment of primary alcohols with lithium aluminum hydride plus platinum on alumina has been successfully applied to produce a mixture of the corresponding hydrocarbon and chain-shortened hydrocarbon (Bierl-Leonhardt and DeVilbiss, 1983; Aldrich et al., 1986). To test whether this reaction would be suitable for structure determination of the acid moieties of methyl esters, the procedure was applied to a 5-μl solution of methyl dodecanoate (2.7 μg/μl hexane). Dodecane and undecane were obtained (ca. 4 : 1 respectively), with complete disappearance of the parent ester. Therefore, the reaction was carried out using an aeration sample of 30 male *E. obscurus*, comparable to that shown in Figure 1 below, as follows. $LiAlH_4$ and 5% Pt on Al_2O_3 (Aesar, Seabrook, New Hampshire) (14 mg + 16 mg, respectively) were pulverized together, and 3 mg of the powder was transferred into a melting point capillary tube (caution: ignition hazard). Fifty microliters of the aeration extract was concentrated in a conical vial, then pentane was used to transfer the residue to the capillary containing the catalytic mixture. The solvent was evaporated with a slow stream of argon through a syringe needle, and the tube was then flame-sealed. After heating for 40 min at 250°C, the tube was allowed to cool to room temperature, opened, and the products were extracted with a few small portions of hexane (total ≤ 20 μl). This solution was filtered through a few grains of silica gel using an additional 5–10 μl of hexane, and the eluate was concentrated to 10–15 μl for GC and GC-MS analyses.

Standards. Methyl (2*E*,4*Z*)-decadienoate and ethyl (2*E*,4*Z*)-decadienoate

(pear ester) were obtained from Bedoukian Research Inc., Danbury, Connecticut. The pear ester was used as an internal standard in some aeration extracts.

2,6-Dimethyltetradecanoic acid was prepared by successive malonic ester condensations. The anion of diethyl methylmalonate was alkylated with 1-bromooctane in refluxing ethanol, then saponified with KOH–ethanol. After removal of ethanol, the salt was acidified (HCl) and the precipitated malonic acid recrystallized from hexane (two crops, 77%, mp 92–97°C). Decarboxylation (oil bath, 175°C) and distillation gave 2-methyldecanoic acid (bp 161–163°C/23 mm) in quantitative yield. The acid was reduced with $LiAlH_4$ in refluxing ether to give the alcohol (bp 129–132°C/23 mm, 87%), followed by conversion to the bromide with Ph_3PBr_2 in CH_2Cl_2 at 0–10°C. The bromide was separated by partition between hexane and 90% dimethyl sulfoxide, the hexane solution was passed through silica gel and the product distilled (bp 129–132°C/24 mm, 74%). A similar reaction of this bromide with the anion of diethyl malonate gave the chain-extended malonic acid (two crops from hexane, mp 51–54°C, 64%). Decarboxylation (oil bath, 180°C) and distillation (135–140°C/0.5 mm) gave a colorless oil that was crystallized from pentane to give colorless platelets (mp 19.5–21.5, 50% from the bromide). Reduction with $LiAlH_4$ in ether gave crude 4-methyldodecanol (two runs, combined yield of 56%, 99% pure by GC). Conversion to the bromide (bp 155–157°C/22 mm, 88.5%), reaction with the anion of methyl malonic ester as above, and saponification gave methyl 4-methyldodecyl malonic acid as a colorless oil (70%). The acid was purified through the bis(dicyclohexylamine) salt, mp 154–156°C dec. from acetone, with 71% recovery. Decarboxylation and distillation gave 2,6-dimethyltetradecanoic acid as a colorless oil (bp 208–211°C/22 mm) in 81% yield. Gas chromatography of the methyl ester (diazomethane) showed a purity of 98.9%, and mass spectra were consistent with the expected structure.

2,6,10-Trimethyldodecane was prepared by hydrogenation (Pd/C, 1 atm, ethanol) of farnesol (Bedoukian Research Inc.) followed by hydrogenation/hydrogenolysis of hexahydrofarnesol as described above.

2,6,10-Trimethyltridecane was synthesized by an organocopper coupling reaction (Posner, 1975) as follows. A solution of 472 mg of copper iodide in 10 ml of tetrahydrofuran (THF) was treated with 3 ml of 1.4 M methyllithium under argon for several min at −40°C, then the CuI/MeLi mixture was warmed slightly (−30°C), and ca. 75 mg (0.25 mmol) of hexahydrofarnesyl bromide (1-bromo-4,8,11-trimethyldodecane; prepared by reaction of hexahydrofarnesol and triphenylphosphine dibromide) in 0.5 ml THF was added. After stirring for 30 min at −30°C, the mixture was allowed to slowly warm to 15°C, then was partitioned between aqueous NH_4Cl and pentane. The pentane solution was rinsed with 2 N HCl, H_2O, and aqueous $NaHCO_3$, then was dried, and concentrated to provide a nearly quantitative yield of the title hydrocarbon.

RESULTS

Four compounds account for nearly 92% (by GC) of the total volatiles in aeration extracts ($N = 7$) of male *E. obscurus* that were uncontaminated by metathoracic scent gland secretion ($X \pm$ SEM; R_t = retention time): compound I (60.97% ± 1.45; 9.92 min), II (1.13% ± 0.12; 10.22 min), III (2.59% ± 0.14; 12.36 min), and IV (27.10% ± 1.89; 13.04 min) (Figure 1A). Gas chromatograms of aerations of *E. obscurus* females (not shown) either totally lacked compounds I–IV, or contained only metathoracic scent gland components if bugs were disturbed during loading or died during the aeration period (Figure 1A).

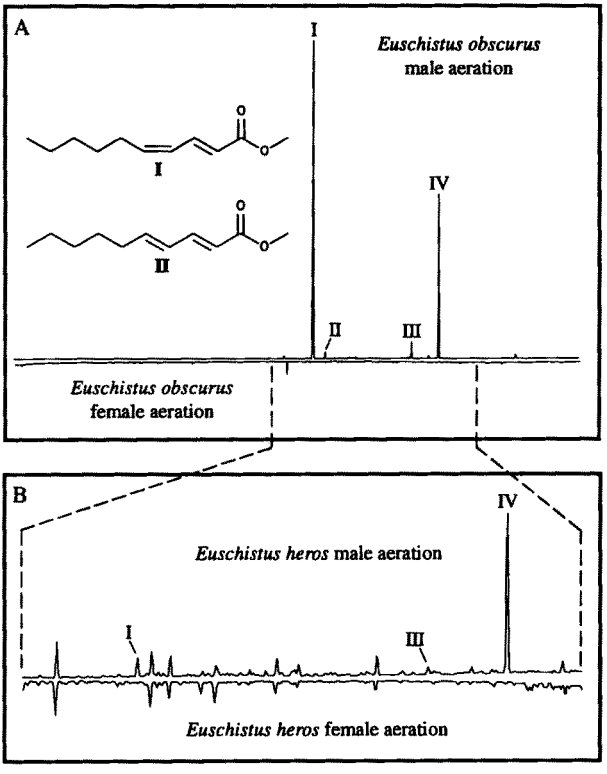

FIG. 1. (A) Typical gas chromatograms for aeration extracts of 40 sexually mature *Euschistus obscurus* males (top), and females (bottom). (B) Chromatograms of aeration extracts of 40 mature male (top), and female (bottom) *Euschistus heros*, expanded to show the region containing male-specific esters and attenuated ca. 10× that of the chromatograms shown for *E. obscurus*.

Aeration extracts of *E. heros* were prepared in Brazil using hexane that later proved to contain a substantial level of impurities. However, GC traces of the R_t = 9–14 min range for aeration extracts of *E. heros* males (Figure 1B) contained a major component having an R_t and EI-MS matching those of compound IV of *E. obscurus*, and minor components matching compounds I and III of *E. obscurus*. Compounds I, III, and IV were absent from comparably prepared aeration extracts of *E. heros* females (Figure 1B). Compound IV is released at a much lower rate by *E. heros* males (0.04 μg/male/day) than by *E. obscurus* males (0.48 μg/male/day).

Mass spectral (Baeckstrom et al., 1988) and gas chromatographic data verified the earlier report (Aldrich et al., 1991) of methyl (2*E*,4*Z*)-decadienoate (I) and methyl (2*E*,4*E*)-decadienoate (II) from male *E. obscurus* (Figure 1A). However, synthetic methyl 2,6-dimethyltetradecanoate (R_t = 13.68) did not coelute with compound IV, despite the close similarities of the MS of the natural product and synthetic standard (Figure 2A and B). The MS of III suggests that this compound is a chain-shortened analog of IV (Figure 2C).

Hydrogenation/hydrogenolysis of a pooled sample of four aeration extracts of *E. obscurus* males (N = 160) yielded ca. 1:5 ratio of nonane and decane, substantiating the presence of methyl decadienoates, plus a set of compounds eluting in the range expected for C_{14-16} hydrocarbons (Figure 3A). Synthetic 2,6,10-trimethyltridecane, and the compound derived from the natural product eluting at 10.84 min by GC-MS, coeluted on both the 30-m DB-1 and HP-5 GC columns. The identity of the 10.84-min unknown hydrocarbon as 2,6,10-trimethyltridecane was confirmed by mass spectrometry (Figure 3B and C). The 10.30-min compound derived from the aeration sample matched the MS of 4,8-dimethyltridecane (the expected chain-shortened hydrocarbon, M^+ = 212) retrieved from the computerized library of spectra. Thus, compound IV is methyl 2,6,10-trimethyltridecanoate.

Similarly, synthetic 2,6,10-trimethyldodecane, and the compound derived from the natural product eluting at 10.03 min by GC-MS, coeluted on both columns and produced virtually identical mass spectra (Figure 3D and E). Thus, compound III is methyl 2,6,10-trimethyldodecanoate.

DISCUSSION

Methyl (2*E*,4*Z*)-decadienoate and methyl 2,6,10-trimethyltridecanoate account for 88% of the total male-specific volatiles of *Euschistus obscurus*. Several minor methyl-esters are also released by males, including methyl 2,6,10-trimethyldodecanoate and methyl (2*E*,4*E*)-decadienoate. Determination that the noval volatiles from *E. obscurus* males are trimethyl-branched compounds corrects the earlier tentative report of dimethyl branching for these methyl-esters

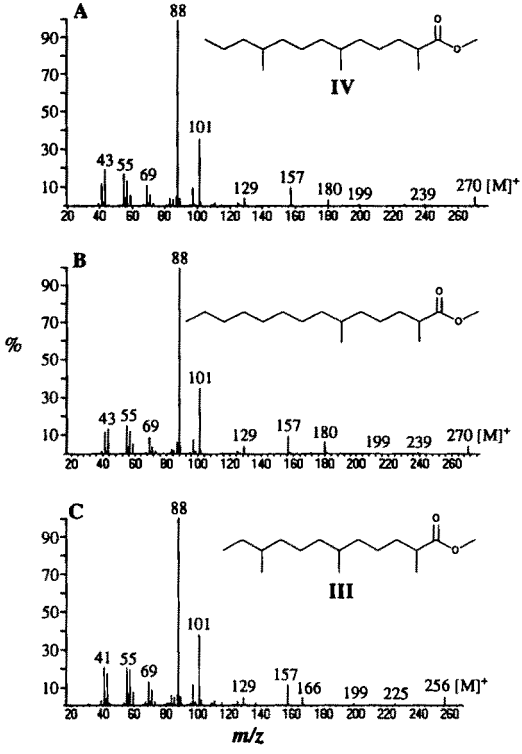

FIG. 2. Electron impact mass spectra of *Euschistus obscurus* (A) male-specific compound IV, (B) a standard of the compound tentatively identified earlier as the structure for IV (methyl 2,6-dimethyltetradecanoate), and (C) male-specific compound III.

(Aldrich et al., 1991). Live *E. obscurus* males and aeration extracts of males were attractive to conspecific females in laboratory bioassays (Borges and Aldrich, 1994).

Comparison of aerations from males of the Neotropical species, *E. heros*, to those of *E. obscurus*, indicates that only methyl 2,6,10-trimethyltridecanoate is a major component of the suspected pheromone for the South American species. In addition, sexually active *E. heros* males apparently release only about a tenth as much pheromone per day as do *E. obscurus* males. Thus, research on the relatively innocuous *E. obscurus* offered a means to characterize the main male-specific volatile from *E. heros* without importing this soybean pest into the United States (Borges and Aldrich, 1994). The chirality of methyl 2,6,10-trimethyltridecanoate is currently unknown, but synthesis of stereoisomers for

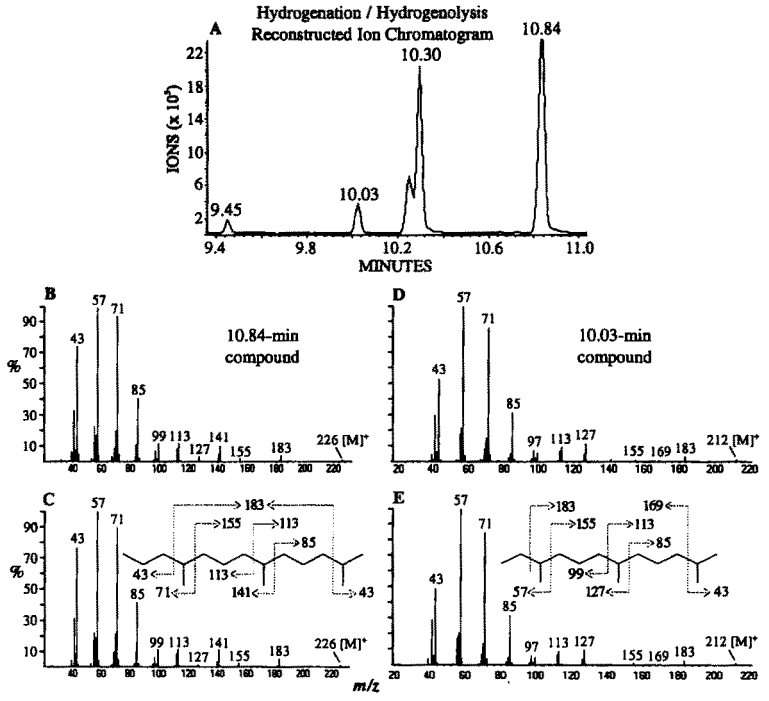

FIG. 3. (A) Reconstructed ion chromatogram for the region showing the hydrogenation/hydrogenolysis reaction products from *Euschistus obscurus* male-specific compounds III (R_t = 9.45 and 10.03 min) and IV (R_t = 10.30 and 10.84 min). Electron impact mass spectra of (B) the 10.84-min compound derived from natural product IV, (C) a standard of 2,6,10-trimethyltridecane, (D) the 10.03-min compound derived from natural product III, and (E) a standard of 2,6,10-trimethyldodecane.

further bioassay experiments with the South American pest species is underway (K. Mori, personal communication).

Prior to the present chemical analysis of a Neotropical *Euschistus* sp., it was suggested that the unusual chemistry of *E. obscurus* males may provide a clue as to the pheromonal pattern of South American *Euschistus* spp. (Aldrich et al., 1991). This speculation was supported by the lack of response of two Neotropical species (*E. taurulus* and *E. acutus*) to methyl (2*E*,4*Z*)-decadienoate in the field (Aldrich et al., 1991). The discovery that *E. heros* males produce predominantly methyl 2,6,10-trimethyltetradecanoate substantiates our earlier speculation, notwithstanding the correction herein for *E. obscurus* methyl-branched esters. Analyses of more Neotropical and Central American Nearctic *Euschistus* spp. are needed to determine if the most recent generic revisions are

consistent with the pheromonal chemistry of the group (Rolston, 1974, 1984). From a practical standpoint, we hope that identification of the suspected pheromone components of *E. heros* will lead to the use of semiochemicals for management of this pest.

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