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SYNTHESIS AND BIOLOGICAL ACTIVITY OF METHYL 2,6,10-TRIMETHYLDODECANOATE AND METHYL 2,6,10-TRIMETHYLTRIDECANOATE: MALE-PRODUCED SEXUAL PHEROMONES OF STINK BUGS *Euschistus heros* AND *Piezodorus guildinii*

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Abstract—Methyl 2,6,10-trimethyltridecanoate **(1)** and methyl 2,6,10-trimethyldodecanoate **(2)** have been identified as male-produced sex pheromones of the Brazilian soybean stink bugs *Euschistus heros* (F.) and *Piezodorus guildinii* (Westwood). In order to establish a defined attractive blend for both species, compounds **1** and **2** were synthesized as mixtures of stereoisomers to be employed in behavior bioassays. (±)-Citronellol **(3)** was utilized as starting material, and the syntheses was carried out in six steps with good overall yield. When tested alone, synthetic compounds **1** and **2** proved to be active in a two-choice olfactometer; however, a 20 : 1 mixture of **1** and **2** was much more attractive to *E. heros* females. A similar blend had been found among the headspace volatiles of males.

Key Words—Methyl 2,6,10-trimethyltridecanoate, methyl 2,6,10-trimethyldodecanoate, *Euschistus heros, Piezodorus guildinii*, pheromone, synthesis, Heteroptera, Pentatomidae, biological activity, stink bugs.

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INTRODUCTION

Soybean, *Glycine max* (L.) Merryl, is intensely attacked by complexes of stink bugs around the world. In Brazil, the most important members are *Nezara viridula* (L.), *Euschistus heros* (F.), and *Piezodorus guildinii* (Westwood) (Panizzi and Rossi, 1991), which lead to the use of more than four million liters of chemical insecticides annually (Correa-Ferreira and Moscardi, 1996). Therefore, efforts have been made to develop alternative methods in pest management, such as application of pheromones.

The first attractant pheromone identified for a member of this complex was that of the southern green stink bug, *N. viridula* (Baker et al., 1987). The chemical communication system of *E. heros* was elucidated by Aldrich et al. (1994), and evidence was presented that methyl 2,6,10-trimethyltridecanoate **(1)** is a component of the male-produced pheromone of the species (Borges et al., 1998a). Recently, we identified methyl 2,6,10-trimethyltridecanoate **(1)** and methyl 2,6,10-trimethyldodecanoate **(2)** as two male-specific compounds of *P. guildinii*, and reported the sharing of pheromone components between *P. guildinii* and *E. heros* (Borges et al., 1999).

To date, no bioassays have been conducted to check the biological activity of mixtures of stereoisomers of methyl 2,6,10-trimethyldodecanoate **(2)**, nor has the reaction of the stink bugs to different blends of compounds **1** and **2** been described. It was previously observed that the activity of pure isomers of **1** in *E. heros* does not significantly differ from that of the racemic mixture (Costa et al., 1999).

Here we describe a straightforward synthesis of compounds **1** and **2** as mixtures of stereoisomers and the biological activity of the synthetic compounds against females of *E. heros*. The material will also be used to monitor the response of *P. guildinii* for the first time in the field.

METHODS AND MATERIALS

Analytical Procedures. The IR spectra refer to films and were measured on a Bomem M-102 spectrometer. The ^{1}H NMR spectra were recorded with TMS as an internal standard at 400 MHz on a Bruker ARX 400 spectrometer. The ^{13}C NMR spectra were recorded with TMS as an internal standard at 100 MHz on a Bruker ARX 400. GC-MS. Analysis was carried out on a Shimadzu QP-5000 GC-MS spectrometer in a split injector mode. A DB-5 capillary column (30 m $\times$ 0.53 mm $\times$ 0.25 μm) used in GC and GC-MS analogs was operated at 70°C for 1 min, increased to 270°C at a rate of 70°C/min, and held at this temperature for 10 min.

Synthesis. All reagents and solvents used in the syntheses were of the high-

est commercially available standard. Chromatographic purification were carried out on silica gel 60, Merck, 230–400 mesh.

*(3*R/S*)-3,7-Dimethyl-6-octenyl-4-methyl-l-benzenesulfonate **(4)***. (*R/S*)-Citronellol **(3)** (2.0 g, 12.8 mmol) was dissolved in chloroform (10 ml) and cooled in an ice bath (0°C). Pyridine (2.0 ml, 25.6 mmol) was added, followed by addition of *p*-toluenesulfonylchloride (3.65 g, 19.2 mmol) in small portions with constant stirring. The reaction was completed after 3.5 hr. Ether (50 ml) and water (10 ml) were added, and the organic layer was washed successively with 10% HCl, sat. $NaHCO_3$ and water and then dried ($MgSO_4$). The solvent was removed under reduced pressure, and the crude tosylate was column chromatographed (hexane–ethyl acetate 8 : 2) on silica gel yielding 3.0 g (9.66 mmol, 75%) of (*R/S*)-**4**. IR (ν_{max} film cm^{-1}): 3032, 2930, 1597, 1360, 1179, 948; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.81 (d, J = 6.4 Hz, 3H); 1.10–1.30 (m, 2H); 1.40–1.55 (m, 2H); 1.57 (s, 3H); 1.67 (d, J = 1.2 Hz, 3H); 1.82–1.96 (m, 2H); 2.45 (s, 3H); 4.01–4.10 (m, 2H); 5.02 (tq, J = 1.2, 7.2 Hz, 1H); 7.34 (d, J = 8 Hz, 2H); 7.79 (d, J = 8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 17.5; 18.9; 21.5; 25.1; 25.6; 28.7; 35.5; 36.5; 68.9; 124.2; 127.7; 129.7; 131.3; 133.0; 144.6.

*(2*R/S*)-2Methyl-l-bromobutane **(5a)***. To a stirred solution of Ph_3P (38.51 g, 147 mmol) in CH_2Cl_2 (300 ml) at 0°C, Br_2 (23.46 g, 147 mmol, 7.52 ml) was added dropwise until the reaction mixture turned orange. A few crystals of Ph_3P were added until the mixture was decolorized again. The alcohol **5** (12.96 g, 147 mmol) was added dropwise, and the solution was stirred at room temperature for 4 hr. Subsequently, 500 ml of sat. $NaHCO_3$ was added cautiously, and the organic layer was diluted with 800 ml pentane. The layers were separated, and the aqueous layer was extracted with pentane (2 × 150 ml). The combined organic layers were dried ($MgSO_4$), the solvent was removed cautiously by distillation, and 150 ml of pentane was added. The flask was kept in a freezer for 3 hr, and the mixture was separated from precipitated Ph_3PO by rapid filtration through a Buchner funnel. Pentane was removed, and the residue was distilled to give 10.90 g (72 mmol, 49%) of **5a**, bp 95°C/506 mbar. ^1H NMR (400 MHz, $CDCl_3$) δ: 0.91 (t, J = 7.6 Hz, 3H); 1.01 (d, J = 6.6 Hz, 3H); 1.22–1.57 (m, 2H); 1.73 (oct, J = 6.6 Hz, 1H); 3.34 (dd, J = 9.6, J = 6.6 Hz, 1H); 3.40 (dd, J = 9.6, J = 6.6 Hz, 1H).

*(2*R/S*)-2-Methyl-l-bromopentane **(6a)***. As described above, the alcohol **6** (10.0 g, 97.9 mmol) was converted into the bromide **6a** (9.71 g, 70.9 mmol, 73%; bp 122°C/540 mbar) by using 25.67 g (97.9 mmol) PPh_3 and 15.64 g (97.9 mmol, 5.01 ml) Br_2 in 200 ml CH_2Cl_2. ^1H NMR (400 MHz, $CDCl_3$) δ: 0.91 (t, J = 7.4 Hz, 3H); 1.01 (d, J = 6.6 Hz, 3H); 1.16–1.48 (m, 4H); 1.81 (oct, J = 6.6 Hz, 1H); 3.32 (dd, J = 9.7, J = 6.6 Hz, 1H); 3.40 (dd, J = 9.7, J = 6.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.14; 18.75; 20.02; 34.95; 37.10; 41.61.

*6*R/S*,10*R/S*)-2,6,10-Trimethyl-2-dodecene **(7)***. The Grignard reagent was prepared from bromide **5a** (4.40 g, 32.0 mmol) and magnesium (0.78 g, 32.0

mmol) in dry THF (12 ml). This slurry was added dropwise to a solution of **4** (2.0 g, 6.4 mmol) in dry THF (12 ml) with stirring and cooling below −60°C. A solution of Li_2CuCl_4 in THF (0.1 M, 0.37 ml) was added to the mixture, and the temperature was gradually raised to room temp. After stirring overnight, the mixture was poured into ice and ammonium chloride solution and subsequently extracted with ether. The extract was washed with saturated $NaHCO_3$ solution and brine, dried with $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel (hexane–ether 9.5 : 0.5) to give the alkene **7** in 85% yield (1.15 g, 5.47 mmol). IR (ν_{max} film cm^{-1}): 2920, 1600, 1455, 1376, 1069; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.85 (d, J = 6.4 Hz, 3H); 0.85 (t, J = 6.4 Hz, 3H); 0.86 (d, J = 6.8 Hz, 3H); 1.00–1.16 (m, 4H); 1.26–1.44 (m, 8H); 1.60 (s, 3H); 1.68 (s, 3H); 1.88–2.04 (m, 2H); 5.11 (bt, J = 6.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: [11.4, 11.43]; 17.63; [19.23, 19.28]; [19.61, 19.67]; 24.46; 25.62; 25.74; [29.50, 29.58]; [32.44, 32.47]; 34.44; [36.95, 36.99]; [37.14, 37.22]; [37.31, 37.34]; 125.14; 130.94; GC-MS (70 eV) m/z %: 210 (M^+, 16.17), 140 (5.68), 125 (16.63), 111 (25.59), 97 (16.46), 83 (35.42), 70 (100), 56 (92.00).

*(6*R/S*,10*R/S*)-2,6,10-Trimethyl-2-tridecene* ***(7a)***. As described above, compound **4** (2.0 g, 6.4 mmol) was converted into the alkene **7a** (1.13 g, 5.04 mmol, 79%) by using 4.78 g of bromide **6a** (31.7 mmol). ^1H NMR (400 MHz, $CDCl_3$) δ: 0.82–0.89 (m, 9H); 1.00–1.17 (m, 4H); 1.20–1.40 (m, 10H); 1.60 (bs, 3H); 1.68 (bs, 3H); 1.88–2.05 (m, 2H); 5.10 (bt, J = 6.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$ δ: 14.43; 17.63; [19.61, 19.65]; [19.66, 19.73]; 20.18; 24.43; [25.62, 25.73]; [32.44, 32.47]; [32.50, 32.54]; [34.38, 34.44]; [37.15, 37.23]; [37.31, 37.34, 37.39, 37.45]; [39.35, 39.43]; [39.51, 39.58]; 125.14; 130.94.

*(6*R/S*,10*R/S*)-2,6,10-Trimethyl-(*E*)-2-dodecen-1-ol* ***(8)***. To a solution of SeO_2 (27.5 mg, 0.25 mmol) and salicylic acid (0.17 g, 1.23 mmol) in CH_2Cl_2 (7.5 ml) at 0°C, *t*-BuOOH (80%, 5.50 ml, 47.6 mmol) was added in one pot. Compounds **7** (2.5 g, 11.9 mmol) in CH_2Cl_2 (7.5 ml) was added over 30 min. The solution was stirred at room temperature for 24 hr, diluted in benzene (10.0 ml), and concentrated in vacuo. Ether was added (25.0 ml), and the organic layer was washed with a 10% solution of KOH and again concentrated. The residue was dissolved in cold acetic acid (5.0 ml) and kept in an ice bath during the addition of dimethylsulfide (5.5 ml). The reaction was stirred for 5 hr at room temperature, and a 20% aqueous solution of K_2CO_3 was added. The organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, and concentrated furnishing the crude product as a yellow oil. This was dissolved in cold ethanol (10.0 ml), and $NaBH_4$ (0.45 g, 11.9 mmol) was added over 30 min, and the solution stirred for 15 min. After addition of a 1 N solution of HCl (15.0 ml) and water (15.0 ml), the mixture was extracted with ether (3 × 60 ml). The organic layer was washed with sat. $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by column chromatography on silica get (hexane–ethyl ether 7 : 3), and the alcohol **8** was obtained in

37% yield (1.0 g, 4.42 mmol). IR (ν_{max} film cm^{-1}): 3332, 2960, 1459, 1371, 1008; ^1H NMR (400 MHz, $CDCl_3$) δ: 0.83–0.89 (m, 9H); 1.07–1.20 (m, 4H); 1.24–1.46 (m, 8H); 1.61 (bs, 1H); 1.67 (bs, 3H); 1.98–207 (m, 2H); 4.00 (s, 2H); 5.4 (bt, J = 7.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 11.40; 13.62; [19.17, 19.22]; [19.52, 19.57]; 24.44; 25.18; [29.47, 29.57]; [32.47, 32.50]; 34.43; [36.74, 36.8]; [36.94, 36.98]; [37.26; 37.30]; 69.16, 126.92; 134.38; GC-MS (70 eV) m/z %: 208 (M-18, 0.6), 140 (0.8), 123 (7.2), 109 (18.0), 83 (13.8), 71 (57.2), 57 (50.7), 43 (100), 41 (60.8).

*(6*R/S*,10*R/S*)-2,6,10-Trimethyl-(*E*)-2-tridecen-1-ol* ***(8a)***. As described above, compound **7a** (2.66 g, 11.9 mmol) was converted into alcohol **8a** (1.30 g, 5.41 mmol, 45%). ^1H NMR (400 MHz, $CDCl_3$) δ: 0.80–0.90 (m, 9H); 1.03–1.12 (m, 4H); 1.15–1.46 (m, 11H); 1.67 (bs, 3H); 1.96–2.10 (m, 2H); 3.99 (s, 2H); 5.41 (bt, J = 7.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.67; 14.42; [19.57, 19.63]; [19.65, 19.72]; 20.16; 24.41; 25.19; [32.50, 32.51]; [36.74, 36.82]; [37.26, 37.44]; [39.40, 39.49]; 69.12, 126.94; 134.37.

*(2*R/S*,6*R/S*,10*R/S*)-2,6,10-Trimethyldodecan-1-ol* ***(9)***. Compound **8** (1.0 g, 4.4 mmol) in 15 ml of hexane was hydrogenated over PtO_2 (5.0 mg) at room temperature and 25 psi in a Parr apparatus for 4.0 hr. The mixture was filtered through Celite, and the filtrate was evaporated at reduced pressure to afford 0.9 g (3.9 mmol) of alcohol **9**, 88% yield. ^1H NMR (400 MHz, $CDCl_3$) δ: 0.84–0.91 (m, 9H); 0.92 (d, J = 6.8 Hz, 3H), 1.10–1.17 (m, 4H); 1.24–1.42 (m, 12H); 1.60–1.67 (m, 2H); 3.42 (dd, J = 10.4, J = 6.4 Hz, 1H); 3.50–3.54 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 11.4; 16.6; [19.2, 19.3]; 19.7; 24.4; 24.5; [29.4, 29.6]; 32.7; 33.4; 34.4; 35.8; 37.0; 37.3; 37.5; 68.5.

*(2*R/S*,6*R/S*,10*R/S*)-2,6,10-Trimethyltridecan-1-ol* ***(9a)***. As described above, compound **8a** (1.1 g, 4.6 mmol) was converted into alcohol **9a** (1.1 g, 4.5 mmol, 99%). ^1H NMR (400 MHz, $CDCl_3$) δ: 0.80–0.90 (m, 9H); 0.93 (d, J = 7.2 Hz, 3H), 1.00–1.15 (m, 4H); 1.20–1.42 (m, 14H); 1.58–1.68 (m, 2H); 3.42 (dd, J = 10.4, J = 6.6 Hz, 1H); 3.48–3.54 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.42; [16.57, 16.64]; [19.65, 19.70]; [19.72, 19.78]; 20.16; [24.40, 24.43]; [24.45, 24.48]; [32.49, 32.52]; 32.77; [33.45, 33.50]; 35.81; [37.29, 37.34]; 37.39; [37.44, 37.50]; [39.41, 39.49]; [68.41, 68.47].

*(2*R/S*,6*R/S*,10*R/S*)-Methyl-2,6,10-trimethyldodecanoate* ***(2)***. Jones CrO_3 (~20.0 ml) was added to a solution of the alcohol **9** (0.65 g, 2.85 mmol) in acetone (85.0 ml), and the mixture was stirred for 30 min at −5°C. The excess CrO_3 was destroyed with *i*-PrOH, and the mixture was concentrated in vacuo, diluted with water, and extracted with ether. The ether extract was washed with H_2O and sat. NaCl solution, dried with $MgSO_4$, and concentrated. The residue obtained was diluted in ether (20 ml) and treated with ethereal CH_2N_2 at 0°C, until the reaction mixture turned yellow. The solution was stirred for 30 min, concentrated, and the residual oil was chromatographed on silica gel (hexane–ethyl acetate 9.5 : 0.5), yielding 0.55 g of **2** (2.14 mmol, 75%). ^1H NMR (400 MHz, $CDCl_3$) δ: 0.83–0.87

(m, 9H); 1.01–1.10 (m, 3H); 1.14 (d, J = 6.8 Hz, 3H); 1.18–1.45 (m, 11H); 2.45 (sext, J = 6.8 Hz, 3H); 3.67 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: [11.42, 11.43]; [17.03, 17.14]; [19.21, 19.27]; [19.60, 19.65]; 24.47; 24.71; [29.47, 29.58]; 32.65; [34.12, 34.16]; 34.43; 36.84; [36.93, 36.97]; [37.31, 37.35, 37.36, 37.39]; [39.47, 39.50]; 51.44; 177.43; GC-MS (70 eV) m/z %: 256 (M^+, 0.6); 232 (0.6); 203 (1.3); 152 (0.5); 119 (9.6); 101 (29.8); 88 (100); 69 (18.3); 55 (29.8).

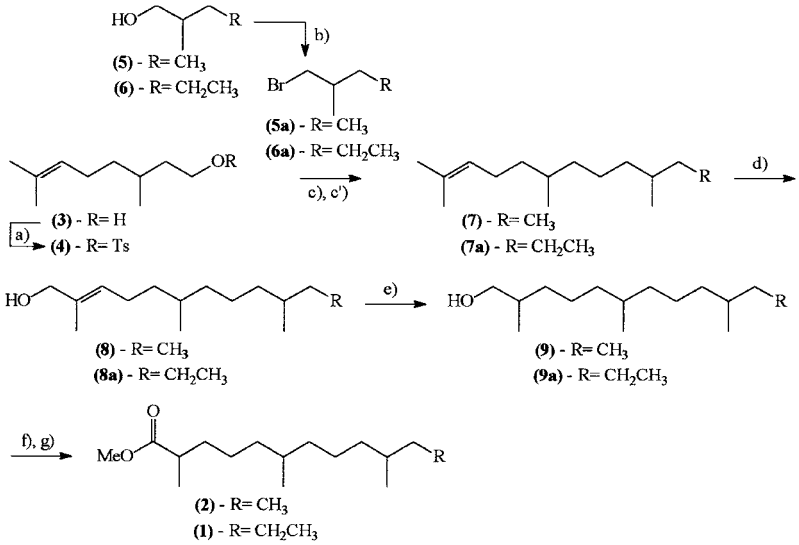
*(2*R/S*,6*R/S*,10*R/S*)-Methyl-2,6,10-trimethyltridecanoate (1).* As described above, compounds **9a** (0.68 g, 2.81 mmol) was converted into **1** (0.62 g, 2.30 mmol, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.80–0.90 (m, 9H); 1.00–1.11 (m, 3H); 1.14 (d, J = 7.2 Hz, 3H); 1.19–1.45 (m, 13H); 2.44 (sext, J = 7.2 Hz, 3H); 3.67 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.43; [17.03, 17.13]; [19.60, 19.64]; [19.66, 19.71]; 20.15; 24.44; 24.71; [32.49, 32.51]; 32.63; [34.12, 34.16]; 36.84; 36.93; [37.31, 37.37]; 37.42; 39.47; 51.45; 177.44; GC-MS (70 eV) m/z %: 270 (M^+, 0.8); 227 (0.4); 199 (0.5); 180 (2.2); 157 (6.0); 101 (33.4); 88 (100); 69 (26.0); 55 (52.0).

Insects. Euschistus heros were collected near Brasilia and raised continuously on soybean seeds (*Glycine max*), green beans (*Phaseolus vulgaris*), peanuts (*Arachis hypogaea*), and sunflower seeds (*Helianthus annuus*) at 26 ± 2°C, 70% relative humidity, and a 14L : 10D photo period.

Bioassay. The two-choice olfactometer was constructed from one large (27.5 × 22.0 cm) and two small (13.5 × 7.5 cm) clear plastic containers and two glass tubes (3.1 cm ID), each 20 cm long. The large container was the release chamber, while one of the small chambers was used for the treatment and the other for the control. An air current through the small chambers was produced and regulated by a single-phase fan controlled by double "variac" voltage regulators. The air current speed at the center of the olfactometer was 17.0 cm/sec. The upwind end of the treatment and the control chambers and the downwind end of the release chamber were pierced with small holes (Borges, 1995). Treatment and control chemicals were each applied to pieces of filter paper (4 × 1 cm) that were placed inside the treatment chamber. The activity of synthetic pheromones was monitored by using 10 *E. heros* females per experiment that were observed for 20 min. Samples were used at 10 μg (Borges et al., 1998a) per treatment (dissolved in hexane), and *n*-hexane was applied as a control. Bioassays were performed for three days during the periods of 08:00–12:00 and 14:00–18:00 hr. Numbers of replications varied between three and six. Behavior data were statistically analyzed with SigmaStat software (Kuo et al., 1992), and differences between means were tested for significance by *t* test.

RESULTS AND DISCUSSION

Mori and Murata (1994a) synthesized (±)-**1** by employing the Julia cyclopropane cleavage as the key reaction. Later, the same authors described the syn-



SCHEME 1. Synthesis of methyl 2,6,10-trimethyltridecanoate **(1)** and methyl 2,6,10-trimethyldodecanoate **(2)**. Reagents: (a) TsCl, Py, 75%; (b) Ph_3P/Br_2, CH_2Cl_2, 49/73%; (c) **(5a)**/Mg, Et_2O, Li_2CuCl_4, 85%; (c′) **(6a)**/Mg, Et_2O, Li_2CuCl_4, 79%; (d) 1: SeO_2/*t*-BuOOH, 2: $NaBH_4$/MeOH, 37/45%; (e) H_2/Pt_2O, 88/99%; (f) [O] Jones/acetone; and (g) CH_2N_2, Et_2O, 75/82%.

thesis of all eight stereoisomers of this compound (Mori and Murata, 1994b). We previously described the synthesis of the (2*R*,6*S*,10*S*) and (2*S*,6*S*,10*S*) iomers of methyl 2,6,10-trimethyldodecanoate **(2)** over 15 steps (Ferreira and Zarbin, 1996). We have now developed a synthesis of a mixture of stereoisomers of pheromones **1** and **2** in a total yield of 8% and 16%, respectively, over six steps. The examples given here show that these pheromones can be obtained by a flexible and operationally simple strategy.

Our route to compounds **1** and **2** is shown in Scheme 1. Citronellol **3** was transformed into the known tosylate **4** (Kabalka et al., 1986) in 75% yield. Hydrocarbons **7** and **7a** were obtained by coupling tosylate **4** with a Grignard reagent prepared from bromides **5a** and **6a**, derived from 2-methyl-1-butanol **(5)** and 2-methyl-1-pentanol **(6)**, respectively (Ph_3P and Br_2, 49% and 73%), in 85% and 79% yield (Fouquet and Schlosser, 1974; Zarbin et al., 1998). Oxidation of **7** and **7a** was carried out by employing selenium dioxide (SeO_2) and *t*-butyl hydroperoxide (*t*-BuOOH) in CH_2Cl_2 (Tanis, 1988). To reduce the corresponding aldehydes formed during the reactions, the crude residues were treated with $NaBH_4$ in methanol, affording the allylic alcohols **8** or **8a** in 37% and 45% yield, respectively. Hydrogenation over PtO_2 catalyst in hexane afforded the saturated

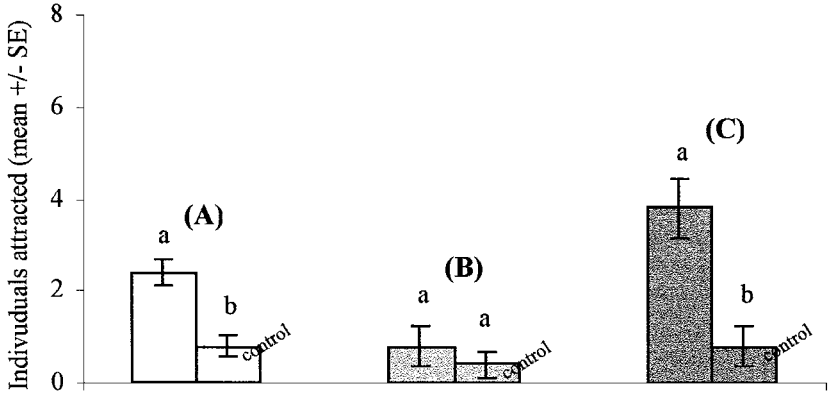


FIG. 1. Number (mean ± SE) of females *Euschistus heros* responding, in an olfactometer bioassay, to different treatments. (A) Methyl 2,6,10-trimethyltridecanoate **(1)** vs. control ($t = 5.060$, $P < 0.001$); (B) methyl 2,6,10-trimethyldodecanoate **(2)** vs. control ($t = 0.894$, $P = 0.397$); (C) methyl 2,6,10-trimethyltridecanoate **(1)** plus methyl 2,6,10-trimethyldodecanoate **(2)**, in a 20 : 1 ratio, vs. control ($t = 4.330$, $P = 0.003$). Means followed by the same letters are not significantly different, according to *t* test.

alcohols **9** and **9a** in 88% and 99%, respectively (Leal et al., 1995). Finally, the Jones oxidation furnished the carboxylic acids, which were directly treated with diazomethane to give the desired pheromones, methyl 2,6,10-trimethyltridecanoate **(1)** and methyl 2,6,10-trimethyldodecanoate **(2)** as mixtures of stereoisomers in 75% and 82% yields, respectively (Ferreira and Zarbin, 1996). Mass spectra and retention times of the synthetic products were identical with those of the natural pheromones (Borges et al., 1999).

During laboratory bioassays, compounds **1** and **2** per se showed significant behavior-releasing activities against *E. heros* females, such as elevating the antennae, wing fanning, searching, walking towards the odor source and touching it, when compared with the solvent control. However, a 20 : 1 mixture of **1** and **2**, which was previously found in a similar blend in the headspace of males (Aldrich et al., 1994), showed a much higher micro-behavior-releasing capacity. On one hand, the total number of *E. heros* females showing any micro-behavior reactions increased significantly. On the other, the reaction of *E. heros* females against this mixture, expressed by walking towards the pheromone loaden filter paper and touching it, was more specific and similar to the reaction against a natural odor source. The significant difference of this latter behavior-releasing capacity of pure compounds **1** and **2**, compared with a 20 : 1 mixture of **1** and **2**, is demonstrated in Figure 1 (treatment A, $t = 5.060$, $P < 0.001$; treatment B, $t = 0.894$, $P = 0.397$; treatment C, $t = 4.330$, $P = 0.003$; *t*-test).

In laboratory bioassays, methyl 2,6,10-trimethyltridecanoate **(1)** induced the same level of behavior response in females of *E. heros* as males (Borges et al., 1998a). During field tests, however, by using a similar pheromone blend, *P. guildinii* was caught in baited traps in significantly larger numbers than *E. heros* (Borges et al., 1998b). These observations support the idea that both compounds **1** and **2** are important to attract females of *E. heros*, and suggest that methyl 2,6,10-trimethyldodecanoate **(2)** is a decisive component of the distinct synergistic communication system of this species. Specific studies on the chemical ecology of *P. guildinii* remain to be carried out to better understand its behavior. A complete set of data may enable the formulation of defined synthetic blends attractive for *P. guildinii* and *E. heros*, which would be an extremely useful tool in integrated pest management (IPM).

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