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The Male-produced Sex Pheromone of the True Bug, *Phthia picta*, is an Unusual Hydrocarbon

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Abstract *Phthia picta* is part of a complex of true bugs (Heteroptera) in Brazil that attack tomatoes, being particularly damaging because nymphs and adults feed on both leaves and fruit. Gas chromatography (GC) of aeration extracts of adult males vs. females revealed the presence of a male-specific compound. GC-electroantennographic detector experiments indicated that the antennae of females are highly sensitive to this male-specific compound. GC-mass spectrometry and GC-FTIR analyses suggested a methyl branched hydrocarbon structure for this compound. After synthesis of three different proposed structures, the natural product was indentified as 5,9,17-trimethylhenicosane, which was strongly attractive to females in Y-tube olfactometer bioassays. Analysis of dissected body parts of adults revealed that the pheromone is produced in the lateral accessory glands of the metathoracic scent gland of males only.

Keywords *Phthia picta* · Coreidae · 5,9,17-Trimethylhenicosane · Metathoracic scent gland · Heteroptera

Introduction

Tomatoes (*Lycopersicon esculentum* Mill) are an important food commodity in Brazil and elsewhere for which quality is fundamental. *Phthia picta* (Heteroptera: Coreidae) is a particularly damaging tomato pest because nymphs and adults feed on both leaves and fruit. The piercing-sucking mode of feeding exhibited by true bugs directly damages fruit, and opens fruit to attack by other insects, fungi, and microorganisms, hastening deterioration and causing great economic losses (Silva and Carvalho, 2001; Silva et al., 2001).

Phthia picta occurs in California, Texas, Florida, Mexico, the Caribbean, and South America, including most regions of Brazil. The bug feeds on wild solanaceous and cucurbit host plants, and cultivated tomatoes are the preferred crop host. Nymphs and adults were described by Serantes (1973), and Silva et al. (2001) described some morphologic characteristics of the egg, the five nymphal instars, and adults. *Phthia picta* adults are elongated (males: 14.2 mm, and females: 15.5 mm long) black insects with yellow or orange pronotal margins and a traverse stripe.

In this work, we identified and synthesized the male-produced sex pheromone of *P. picta*, and tested its attractiveness to adults in an olfactometer. In addition, we determined the site of pheromone accumulation. Identification of this pheromone may eventually be useful for integrated pest management in tomatoes, one of the crops most heavily treated with pesticides in the country, and the discovery reveals a new and novel type of pheromone molecule for the Heteroptera.

Methods and Materials

Insects Phthia picta adults and nymphs were collected in commercial plantations of tomato in Uraí, Paraná, Brazil. Insects were sexed and maintained separately under controlled conditions (25 °C, 70 % relative humidity and a

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14:10 hL:D), and fed exclusively on tomatoes (Silva and Carvalho, 2001).

Collection of Volatiles Groups of 8males and females, separated by sex, were placed in glass aeration chambers (35 cm high × 3.5 cm outside diam). Emitted volatiles were trapped on 0.8 g of Super Q (Alltech, Deerfield, IL, USA) columns daily for 24 h over 20 consecutive days as previously reported (Zarbin et al., 2003a). A humidified and charcoal filtered air flow (1 $L.min^{-1}$) was maintained through the apparatus. Volatiles were eluted from Super Q with 600 μl of double-distilled hexane, and adsorbent traps were changed after each 10 collections. The daily extracts were not combined, and each was concentrated to 100 μl under an argon stream prior to analyses (Zarbin et al., 1999; Fonseca et al., 2010).

Extracts were analyzed by gas chromatography–mass spectrometry (GC–MS) using a Shimadzu GC (model 2010) coupled to a Shimadzu QP 2010 Ultra MS operated in the electron impact ionization mode (70 eV). The GC was operated in the splitless mode, and was equipped with a DB-5 (0.25, 0.25 mm × 30 m, J&W Scientific, Folsom, CA, USA) capillary column. The column oven was maintained at 50 °C for 3 min, increased to 250 °C at 7 °C/min, and then kept at this temperature for 5 min.

Kovats indices (Kovats, 1965) of analytes were calculated with reference to *n*-alkanes standards for determination of relative retention indexes.

Gas Chromatography-Infrared Spectroscopy (GC-FTIR) Infrared spectra were recorded using a DiscovIR-GC Spectra Analysis GC coupled to a Shimadzu GC (model 2010). The GC was operated in the splitless mode, and was equipped with a DB-5 capillary column and conditions as above, with helium as carrier gas. A liquid nitrogen-cooled photoconductive mercury-cadmium-telluride (MCT) detector was used with FT-IR resolution of 8 cm^{-1}.

GC-Electroantennogram Recordings (GC-EAD) A Shimadzu 2010 GC was coupled to a Syntech EC-03-300 model electroantenographic detector system (Hilversum, The Netherlands). GC-EAD experiments were performed with antennae from males and females (Cortés et al., 2010). A GC equipped with an RTX-5 column (0.25 × 0.25 mm × 30 m; J&W Scientific) with a splitless injector was used in these analyses. The oven was programmed as above, except starting at 100 °C. The EAD branch passed through a heated conduit (250 °C) with an humidified air flow (300 $ml.min^{-1}$) directed over the insect antennal preparation. The antenna was fixed between two stainless steel electrodes (Syntech probe) using conductive gel (Signa gel, Parker Labs, NJ, USA). Signals were registered and analyzed with Syntech GC-EAD32 software (version 4.6).

Extraction of Metathoracic Gland Secretion An adult *P. picta* was pinned dorsal-side-up through the prothorax in a Petri dish, and submerged in tap water. The dissection process (using small surgical scissors and sharpened forceps) consisted of removing the wings, cutting the lateral margins of the abdomen anteriorly up to the metathorax, and transversely cutting the anterior margin of the scutellum. The tergal cuticle was pulled back, and the viscera were removed. The scent gland complex, located in the ventral metathoracic region, could then be reached and removed by cutting laterally through the meso- and metathorax, turning the preparation over, and cutting transversely between the meta- and prothorax. The gland reservoir and the lateral accessory glands (Aldrich et al., 1978; Favaro et al., 2011) were removed separately, dried with tissue paper, immersed in 20 μl of analytical grade hexane, and each extract was stored at−20 °C until analysis. Three metathoracic scent glands (MSG) extracts were prepared for each sex.

Olfactometer Bioassay Responses of *P. picta* to volatiles from each sex were tested in a binary choice Y-tube olfactometer using humidified, charcoal filtered air at a rate of 4 L/min (Zarbin et al., 2007). Hydrochloric acid and ammonium hydroxide were mixed to visualize the plume distribution inside the system (Boyer et al., 1997). The olfactometer consisted of a Y-shaped glass tube 4 cm diam with a 40-cm long main tube and two 20-cm long arms. Odor sources consisted of 2×2 cm pieces of filter paper loaded with 20 μl of a 1000-ppm hexane solution of the standard synthetic molecule or hexane (control), placed in the base of the olfactometer arms. One male or female was introduced into the base of the main tube of the olfactometer, and behavior was observed for 20 min. Insects that walked upwind and made direct contact with the filter paper containing the odor source within 20 min were recorded as a positive response. An insect that did not walk upwind to either odor source within 20 min was recorded as no response. Insects that did not make a choice were excluded from statistical analysis. We tested 50 individuals, and each individual was tested once only, representing one replicate experiment. The Y-tube was cleaned with alcohol after 4 insects were tested, left to dry for 5 min, and the positions of the olfactometer arms were inverted between odor sources to avoid any positional bias. Statistics for the bioassays was performed with binomial test in BioEstat 3.0 software.

Synthesis High-grade reagents and solvents were used in the syntheses. Crude products were purified by flash chromatography on silica gel (230–400 mesh). ^{1}H- and ^{13}C-NMR spectra were recorded using a Bruker ARX-200 spectrometer (200 and 50 MHz, respectively) in a $CDCl_3$ solution. Chemical shifts are expressed in ppm relative to $CDCl_3$ (7.27 and 77.23 ppm for ^{1}H and ^{13}C NMR, respectively).

The infrared (IR) spectra were measured as films using a Bomem B100 spectrometer. GC-MS data for synthetic intermediates were obtained as described above.

Syntheses of 5,9,12,16-Tetramethyleicosane (7)

2-Cyclopropylhexan-2-ol **(2)** A solution of the Grignard reagent was prepared by slow, continuous addition of 1-bromobutane (10 mmol, 1.1 ml) to a stirred suspension in dry diethyl ether (5 ml) of magnesium turnings (11 mmol, 0.266 g) previously activated with iodine. To this a solution of cyclopropylmethylketone **1** (12 mmol, 1.2 ml) in dry diethyl ether (5 ml) was added gradually, and the mixture was stirred overnight at room temperature. The reaction was quenched by the addition of ice and saturated NH_4Cl solution. The ether layer was separated, and the aqueous layer was extracted with diethyl ether. The combined ether solutions were washed with brine, dried over anhydrous Na_2SO_4, and concentrated by rotary evaporation under reduced pressure. The crude product was purified by column chromatography on silica gel (hexane – ethyl acetate 8:2) yielding 1.36 g (95 %). IR υ_{max}, cm^{-1}: 3460, 2932, 1465, 1390, 1114, 1014. ^1H NMR (200 MHz, $CDCl_3$): δ 0.35 (m, 4H), 0.92 (m, 3H), 1.09 (s, 3H), 1.36 (m, 4H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 0.05, 0.32, 13.80, 20.73, 23.00, 25.33, 25.87, 42.72, 70.96. MS: *m/z* (%): 127 (5), 114 (5), 109 (2), 101 (6), 95 (2), 91 (1), 85 (100), 81 (2), 77 (1), 71 (15), 67 (10), 58 (5), 57 (10), 43 (100), 41 (10).

1-Bromo-4-methyloct-3-ene **(3)** In a portion, 48 % hydrobromic acid (4 ml) was added to stirred and ice-cooled **2** (5.8 mmol, 1 g), and the mixture was stirred for 15 min at 0 °C. Distilled water was added, the organic layer was separated, and the aqueous layer was extracted with hexane. The combined organic solutions were washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane) yielding 1.4 g (83 %). IR υ_{max}, cm^{-1}: 2920, 2859, 1663, 1451, 1238, 840, 642. ^1H NMR (200 MHz, $CDCl_3$): δ 0.90 (t, 3H), 1.34 (m, 4H), 1.62 (s, 3H), 1.99 (t, 2H), 2.57 (q, 2H), 3.34 (t, 2H), 5.12 (ddq, 1H, *J* 1.2, 7.0, 7.6). ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.97, 16.21, 22.37, 30.06, 31.66, 32.94, 39.29, 120.69, 121.18, 139.09. MS: *m/z* (%): 204 (10), 162 (35), 149 (1), 135 (1), 125 (1), 121 (1), 111 (1), 95 (15), 83 (70), 79 (20), 69 (100), 55 (100), 51 (5), 41 (65).

5,9,12,16-Tetramethylicosa-5,15-diene-9,12-diol **(5)** As described above, compound **3** (20 mmol, 4.1 g) was converted into **5** (2.93 g, 80 % yield) using magnesium turnings (24 mmol, 0.58 g). To this, a solution of hexane-2,5-dione **(4)** (5 mmol, 0.58 g) in dry diethyl ether (2 ml) was gradually added . IR υ_{max}, cm^{-1}: 3270, 3054, 2963, 2926, 2855, 1671, 1451, 1380, 1085. ^1H NMR (200 MHz, $CDCl_3$): δ 0.89 (m, 6H), 1.19 (s, 6H), 1.32 (m, 8H), 1.52 (m, 8H), 1.64 (m, 6H), 2.00 (m, 8H), 5.13 (ddq, 2H, *J* 1.2, 7.0, 7.6). ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.99, 15.79, 22.68, 26.90, 30.24, 35.59, 39.45, 41.87, 72.67, 124.01, 124.74, 135.94. MS: *m/z* (%): 346 (10), 331 (10), 281 (30), 253 (10), 223 (20), 207 (60), 164 (100), 135 (15), 123 (20), 107 (75), 95 (40), 69 (25), 57 (5), 44 (100).

5,9,12,16-Tetramethyleicosane-9,12-diol **(6)** Compound **5** (2.72 mmol, 1.0 g) in hexane (10 mL) was hydrogenated over palladium on charcoal (10 % Pd, 5.0 mg) at room temperature and 25 psi in a Parr apparatus for 3.0 h. The mixture was filtered, and the filtrate was evaporated at reduced pressure to afford 0.9 g (2.45 mmol) of **6**, in 90 % yield. IR υ_{max}, cm^{-1}: 3276, 2963, 2926, 2861, 1460, 1386. ^1H NMR (200 MHz, $CDCl_3$): δ 0.9 (m, 12H), 1.12 (m, 3H), 1.20 (s, 6H), 1.30 (m, 15H), 1.43 (m, 4H), 1.55(s, 14H), 1.88 (m, 3 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.14, 19.63, 21.30, 22.81, 26.78, 29.25, 32.58, 35.36, 36.56, 37.43, 42.36, 72.56. MS: *m/z* (%): 337 (6), 319 (1), 281 (1), 249 (1), 243 (1), 236 (1), 225 (100), 171 (12), 150 (9), 137 (11), 123 (17), 109 (26), 97 (36), 95 (26), 83 (21), 69 (36), 55 (21), 43 (33).

5,9,12,16-Tetramethyleicosane **(7)** Compound **6** (0.88 g, 2.37 mmol) and *p*-toluenesulfonic acid (*p*-TSA) (5 mg) in benzene (10 ml) were heated to reflux for 3.0 h. The mixture was cooled to room temperature, and extracted with distilled water. The organic phase were washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. This was hydrogenated without purification, as described above, for the synthesis of **6**. The mixture was filtered, and the filtrate was evaporated at reduced pressure. The crude product was purified by column chromatography on silica gel (hexane) yielding 0.2 g (25 %) in two stages. IR υ_{max}, cm^{-1}: 2957, 2928, 2861, 1467, 1374, 726. ^1H NMR (200 MHz, $CDCl_3$): δ 0.86 (m, 18H), 1.07 (m, 4H), 1.26 (m, 28H). ^{13}C NMR (50MHz, $CDCl_3$): δ 14.14, 19.70, 23.05, 24.47, 29.36, 32.75, 33.06, 33.10, 34.35, 34.43, 36.77, 36.85, 37.30, 37.43. MS: *m/z* (%): 338 (1), 323 (1), 309 (1), 295 (1), 281 (2), 253 (2), 239 (1), 225 (2), 211 (13), 183 (9), 169 (9), 155 (26), 141 (19), 127 (24), 113 (32), 99 (47), 85 (91), 71 (90), 57 (100), 43 (47).

Syntheses of 5,9-Dimethyldocosane (11)

5,9-Dimethyldocos-5-en-9-ol **(9)** As described above for the synthesis of **2**, bromide **3** (5.85 mmol, 1.2 g) was converted into **9** (1.24 g, 66 % yield) using magnesium turnings

(6 mmol, 0.145 g). To this, a solution of 2-pentadecanone **(8)** (5.83 mmol, 1.32 g) in dry diethyl ether (3 ml) was gradually added . IR υ_{max}, cm^{-1}: 3287, 3043, 2963, 2932, 2861, 1671, 1460, 1375, 1090. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.91 (m, 6H), 1.19 (s, 3H), 1.28 (s, 26H), 1.48 (m, 4H), 1.63 (s, 2H), 1.69 (m, 1H), 2.03 (m, 4H), 5.15 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.97, 14.08, 15.85, 22.33, 22.56, 22.66, 23.38, 23.94, 26.85, 29.33, 29.64, 30.16, 30.24, 31.90, 41.56, 41.86, 41.93, 72.83, 124.07, 124.80, 135.59, 135.85. MS: *m/z* (%): 334 (6), 277 (6), 249 (7), 207 (8), 165 (5), 151 (58), 137 (5), 123 (11), 109 (34), 95 (100), 81 (47), 69 (68), 55 (39), 43 (28).

5,9-Dimethyldocos-9-ol **(10)** Applying the same method to **6**, compound **9** (1.1 g, 3.4 mmol) was converted into **10** (1.07 g, 3.28 mmol, 97 % yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (m, 9H), 1.15 (s, 3H), 1.26 (s, 30H), 1.40 (m, 6H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.11, 19.66, 21.00, 21.30, 22.66, 22.99, 23.89, 26.96, 29.33, 29.64, 30.23, 31.90, 32.69, 36.72, 37.62, 41.88, 42.16, 72.82. MS: *m/z* (%): 336 (2), 227 (76), 207 (6), 171 (56), 153 (56), 139 (8), 125 (18), 110 (62), 97 (100), 83 (177), 69 (81), 55 (70), 43 (71).

5,9-Dimethyldocosane **(11)** As described above for the synthesis of **7**, compound **10** (0.535 g, 1.64 mmol) was converted into **11** (0.1616 g, 0.52 mmol, 32 % yield). IR υ_{max}, cm^{-1}: 2958, 2917, 1469, 1378, 728. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.85 (m, 12H), 1.06 (m, 2H), 1.26 (s, 32H), 1.60 (s, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.11, 19.70, 19.75, 22.70, 23.05, 24.45, 27.08, 29.35, 30.03, 31.92, 32.74, 36.75, 36.83, 37.06, 37.13, 37.37, 37.42. MS: *m/z* (%): 338 (1), 323 (1), 309 (1), 295 (1), 281 (7), 253 (3), 239 (1), 225 (2), 210 (7), 183 (5), 169 (5), 155 (20), 141 (10), 127 (15), 113 (23), 99 (39), 85 (85), 71 (81), 57 (100), 43 (51).

Syntheses of 5,9,17-Trimethylhenicosane (17)

2-Cyclopropyl-6-methyldec-5-en-2-ol (12) A Grignard reagent solution was prepared by addition of a solution of **3** (2.0 g, 9.75 mmol) in dry diethyl ether (3 ml) to a stirred suspension of magnesium (0.243 g, 10 mmol) in dry diethyl ether (2 ml). To this, a solution of **1** (1.15 ml, 12 mmol) in diethyl ether (5 ml) was gradually added, and the mixture was stirred overnight at room temperature. The reaction was quenched by addition of ice and saturated NH_4Cl solution. The ether layer was separated, and the aqueous layer was extracted with diethyl ether. The combined ether solutions were washed with brine, dried over anhydrous Na_2SO_4, and concentrated by rotary evaporation under reduced pressure. The crude product was purified by column chromatography on silica gel (hexane – ethyl acetate 8:2) yielding 1.36 g (85 %)). IR υ_{max}, cm^{-1}: 3469, 3087, 2917, 1672, 1452, 1372, 1101, 1061, 901, 820. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.35 (m, 4H), 0.9 (m, 4H), 1.11 (s, 3H), 1.13 (s, 1H), 1.31 (m, 4H), 1.57 (m, 2H), 1.61 (s, 2H), 1.96 (q, *J* 6.61Hz, 2H,), 2.12 (q, *J* 8.03Hz, 2H), 5.15 (tq, *J* 7.06 Hz *J* 1.34 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 0.40, 0.51, 14.01, 16.17, 20.84, 22.28, 22.67, 25.87, 30.54, 39.53, 43.12, 71.51, 123.98, 124.94, 135.84, 135.95. MS: *m/z* (%): 192 (8), 177 (3), 163 (5), 149 (5), 135 (65), 125 (4), 124 (13), 107 (82), 165 (6), 95 (64), 85 (53), 79 (43), 69 (77), 55 (63), 43 (100).

1-Bromo-4,8-dimethyldodeca-3,7-diene (13) In a 48 % portion, hydrobromic acid (3 ml) was added to stirred and ice-cooled **12** (6.0 mmol, 1.26 g), and the mixture was stirred for 20 min at 0 °C. Distilled water was added, the organic layer was separated, and the aqueous layer was extracted with hexane. The combined organic solutions were washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane) yielding 1.0 g (80 %). IR υ_{max}, cm^{-1}: 2963, 2932, 2855, 1671, 1445, 1375, 1267, 1207, 1122, 852. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (t, *J* 6.96Hz, 3H), 1.31 (m, 4H), 1.61 (d, *J* 8.80Hz, 3H), 1.69 (dq, *J* 10.16Hz *J* 1.18Hz, 3H), 2.03 (m, 6H), 2.56 (q, *J* 7.32Hz, 2H), 3.34 (t, *J* 7.32Hz, 2H), 5.11 (m, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.02, 15.88, 16.26, 22.39, 23.40, 26.36, 30.24, 31.77, 32.82, 39.33, 39.71, 39.96, 120.86, 121.57, 123.63, 124.35, 135.54, 138.65. MS: *m/z* (%): 271 (1), 259 (1), 215 (2), 187 (7), 165 (3), 151 (1), 137 (1), 123 (2), 111 (16), 95 (7), 81 (9), 69 (100), 55 (43), 41 (16).

5,9-Dimethyltrideca-4,8-dienal (14) The Grignard reagent was prepared from bromide 13 (1.0 g, 3.65 mmol) and magnesium (0.088 g, 3.65 mmol) in dry diethyl ether (2 ml). To this, was added slowly a solution of *N,N*-dimethylformamide (DMF) (0.3 ml, 4 mmol) in diethyl ether (5 ml) at 0 °C over a period of 5 min. An instant exothermic reaction takes place with the formation of a sticky white precipitate. The mixture then was brought to room temperature and stirred for 1 h. Subsequently, the reaction mixture was carefully quenched with hydrochloric acid solution (1 mol/L) until the solution became acidic. The product was extracted with diethyl ether. The extract was washed with saturated $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane–ethyl acetate 8:2) to give **14** in 52 % yield. IR υ_{max}, cm^{-1}: 2932, 2845, 2707, 1729, 1654, 1441, 1390. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.91 (m, 3H), 1.31 (m, 4H), 1.60 (d, *J* 8.80Hz, 3H), 1.70 (dq, *J* 10.16Hz *J* 1.18Hz, 3H), 2.03 (m, 6H), 2.36 (q, *J* 7.32Hz, 2H), 2.45 (t, *J* 7.32Hz, 2H), 5.11 (m, 2H), 9.81 (t, *J* 1.64, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.32, 15.80, 21.64, 30.05, 39.35, 39.35, 44.00, 122.08, 122.74, 123.93, 124.30, 135.40, 135.72, 136.99, 137.26, 202.63. MS: *m/z* (%): 222 (2), 204 (1), 189 (1),

178 (3), 165 (3), 147 (3), 137 (9), 119 (3), 111 (11), 93 (12), 81 (7), 69 (100), 55 (52), 53 (5), 41 (18).

5,13,17-Trimethylhenicosa-5,12,16-trien-9-ol (15) As described above, compound **3** (3.8 mmol, 0.78 g) was converted into **15** (0.37 g, 56 % yield) using magnesium turnings (3.8 mmol, 0.092 g). To this, a solution of **14** (1.9 mmol, 0.422 g) in dry diethyl ether (3 mL) was gradually added. IR υ_{max}, cm^{-1}: 3264, 2963, 2932, 2855, 1671, 1457, 1380, 1079, 923. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (m, 6H), 1.32 (m, 8H), 1.50 (m, 4H), 1.60 (m, 6H), 1.67 (m, 3H), 2.02 (m, 12H), 3.6 (m, 1H), 5.12 (m, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.00, 15.87, 15.97, 22.35, 22.68, 23.40, 24.30, 26.56, 30.16, 31.57, 37.40, 37.63, 39.38, 39.76, 40.07, 71.64, 123.79, 124.05, 124.53, 124.82, 135.32, 135.63, 135.78, 135.99, 136.22. MS: *m/z* (%): 300 (1), 287 (1), 263 (2), 245 (4), 219 (3), 203 (2), 178 (4), 163 (9), 149 (10), 135 (13), 121 (16), 109 (20), 95 (43), 81 (43), 69 (100), 55 (65), 41 (25).

5,13,17-Trimethylhenicosan-9-ol (16) In the same manner as described above for **6**, compound **15** (0.37 g, 1.07 mmol) was converted into **16** (0.37 g, 1.04 mmol, 97 % yield). IR υ_{max}, cm^{-1}: 3276, 2963, 2926, 2861, 1460, 1386. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.88 (m, 16H), 1.12 (m, 6H), 1.30 (m, 18H), 1.43 (m, 10H), 3.62 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.22, 19.70, 23.10, 24.54, 29.28, 32.78, 36.73, 36.84, 37.08, 37.34, 37.84, 72.06. MS: *m/z* (%): 336 (4), 308 (1), 294 (1), 278 (2), 252 (1), 238 (1), 227 (18), 208 (6), 168 (6), 157 (26), 139 (15), 125 (23), 111 (44), 97 (69), 83 (100), 69 (76), 57 (76), 43 (63).

5,9,17−Trimethylhenicosane (17) As described above for **7**, compound **16** (0.37 g, 1.04 mmol) was converted into **17** (0.14 g, 0.44 mmol, 42 % yield). IR υ_{max}, cm^{-1}: 2961, 2928, 2857, 1469, 1378. ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.89 (m, 16H), 1.10 (m, 4H), 1.30 (m, 30H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.16, 19.75, 23.08, 24.46, 27.03, 29.30, 29.78, 30.10, 32.71, 36.80, 37.16, 37.47. MS: *m/z* (%): 338 (1), 323 (1), 309 (1), 295 (1), 281 (6), 253 (3), 239 (1), 225 (2), 211 (7), 183 (6), 169 (7), 155 (17), 141 (11), 126 (4), 113 (21), 99 (34), 85 (81), 71 (73), 57 (100), 43 (71).

Chemical Standards 1-Hexenal and 1-hexanol were purchased from Acros Organics (Geel, Turnhout, Belgium). Hexanoic acid, hexyl acetate, octyl acetate, and tridecane were purchased from Aldrich Chemical Company (Milwaukee, WI, USA).

Results and Discussion

The chromatographic profile of extracts from males and females *P. picta* showed one male-specific compound, with

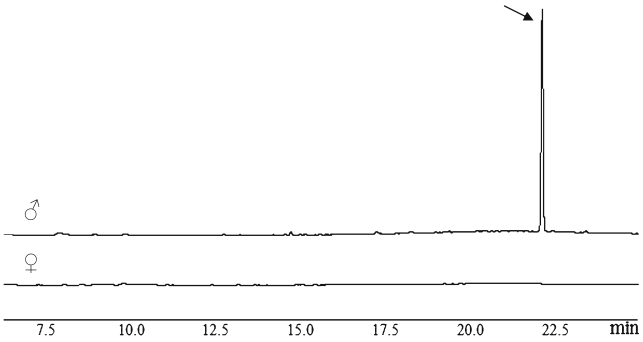


Fig. 1 Gas chromatographic analysis of volatiles obtained from male and female *Phthia picta* adults (the arrow marks the male-specific compound)

retention time (Rt) of 21.94 min, and Kovats Index (KI) of 2232 on an RTX-5 column (2203 on an RTX-Wax column and 2238 on a DB-1 column) (Fig. 1) (Kovats, 1965). Gas chromatography-electroantennogram detection analysis of the male extract showed that only the antennae of female *P. picta* responded strongly to this male-specific compound (Fig. 2).

The FTIR spectra of the male-specific compound exhibited bands in the region of asymmetrical axial deformation of methyl groups at 2960 cm^{-1}, a band of symmetrical axial deformation of methyl groups overlapping the CH_2 band of asymmetrical axial deformation at 2928 cm^{-1}, and symmetrical axial deformation at 2858 cm^{-1}. Symmetrical bending vibration of methyl groups also was observed at 1379 cm^{-1} and asymmetrical bending vibration at 1468 cm^{-1}. These data suggested that the male-specific compound could be a saturated methyl-branched hydrocarbon (Fig. 3a) (Silverstein et al., 2005).

The mass spectrum of the male-specific compound showed a molecular ion at *m/z* 338 (Fig. 3b), suggesting a $C_{24}H_{50}$ molecular formula for the hydrocarbon. The absence of linearity for the C_{14} units confirmed the existence of methyl branches in the molecule, in agreement with FTIR spectral data (Silverstein et al., 2005). The high relative

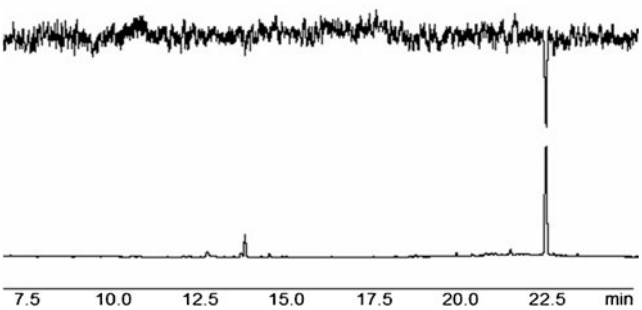


Fig. 2 Coupled gas chromatograph-electroantennogram detection analysis of male *Phthia picta* antenna of a female to the aeration extract from a *P. picta* male; the flame ionization detector response is below

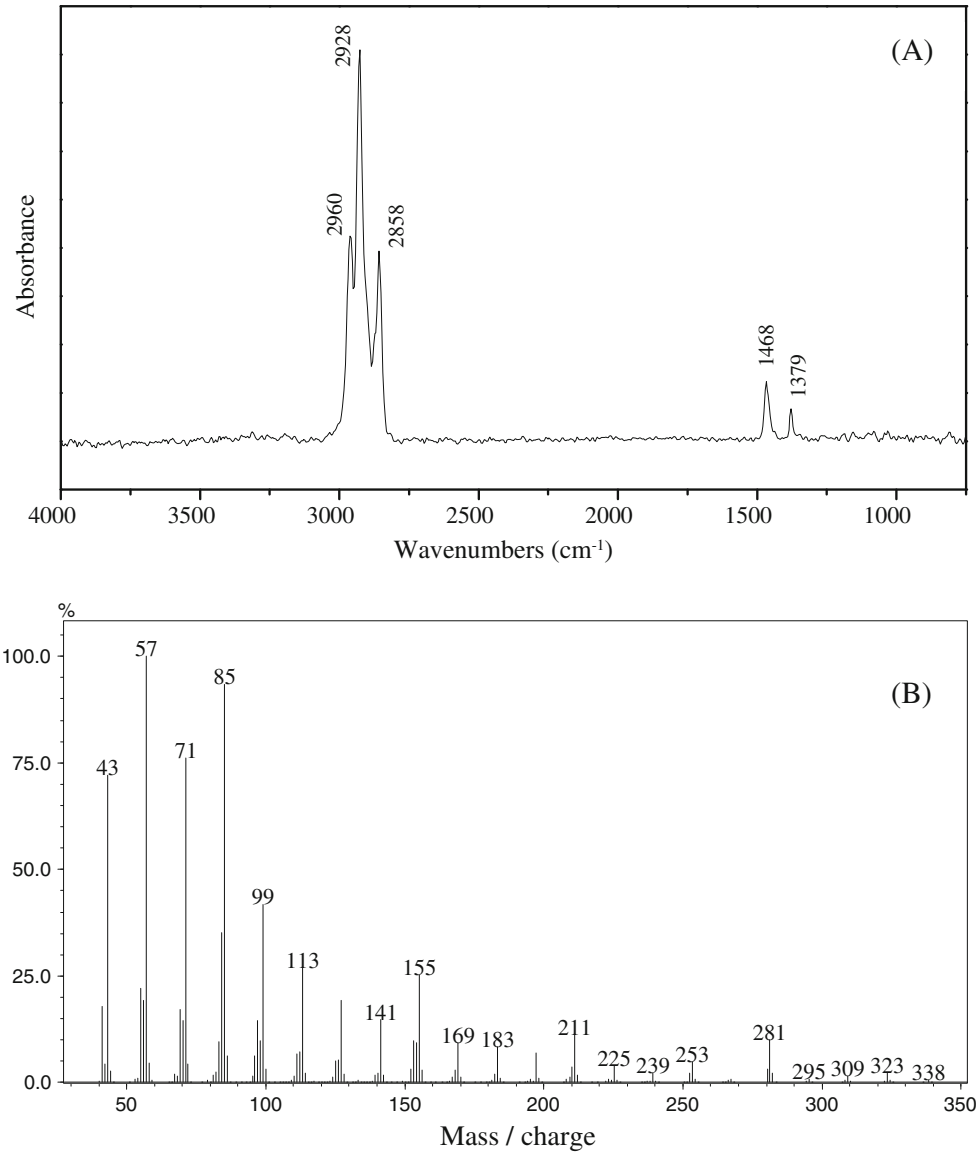
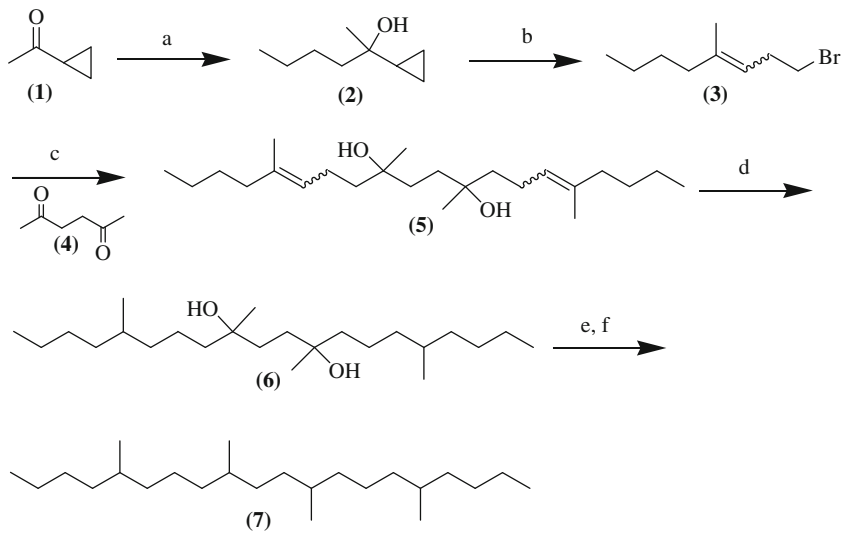


Fig. 3 **a** Coupled gas chromatography-infrared spectroscopy analysis of the male-specific *Phthia picta* compound. **b** Electron impact ionization mass spectrum of the male-specific compound of *P. picta*



Scheme 1 Synthesis of 5,9,12,16-tetramethyleicosane. (**a**) *n*-BuBr, Mg^0, Et_2O, 95 %, (**b**) HBr 48 %, 0 °C, 83 %, (**c**) Mg^0, Et_2O, hexan-2,5-dione **(4)**, 80 %, (**d**) H_2/PdC, hexane, 90 %, (**e**) *p*-TSA, benzene, reflux, (**f**) H_2/PdC, hexane, 25 % for both steps

abundance of *m/z* 85 ion, associated with the *m/z* 57 ion, suggested the presence of a methyl group at C_5; the high relative abundance of *m/z* 155 ion suggested a second methyl group at C_9; the *m/z* 211 ion suggested the presence of a third methyl group at C_{12}; and the *m/z* 281 ion suggested the presence of a fourth methyl branch at C_{16}. Based on these data, 5,9,12,16-tetramethyleicosane was our initial candidate structure for the natural product.

5,9,12,16-Tetramethyleicosane was synthesized as a mixture of all stereoisomers as showed in Scheme 1. The Grignard reaction of buthylmagnesium bromide with cyclopropylmethylketone **(1)** yield the alcohol **2** in 95 % yield (Biernacki

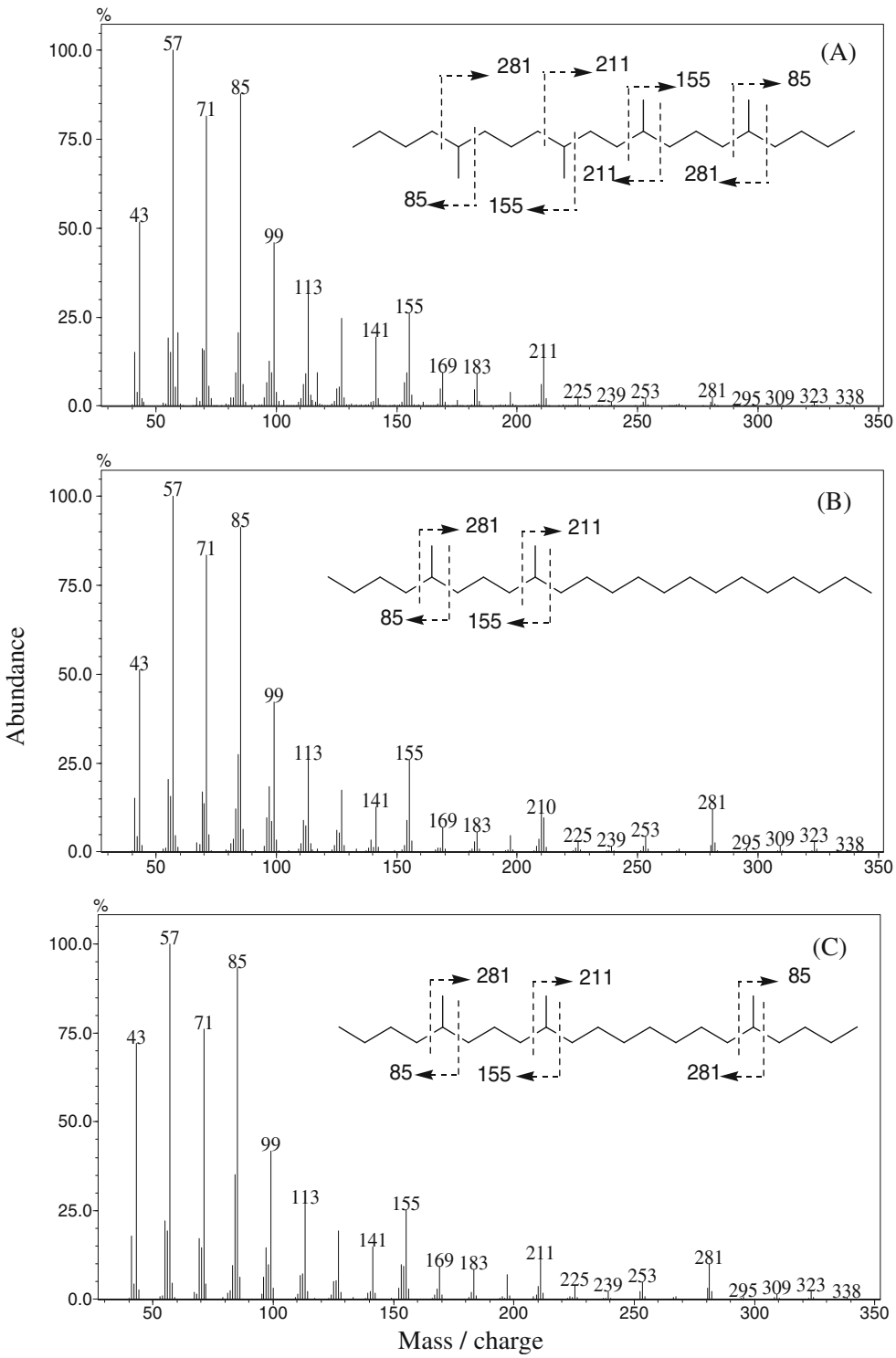
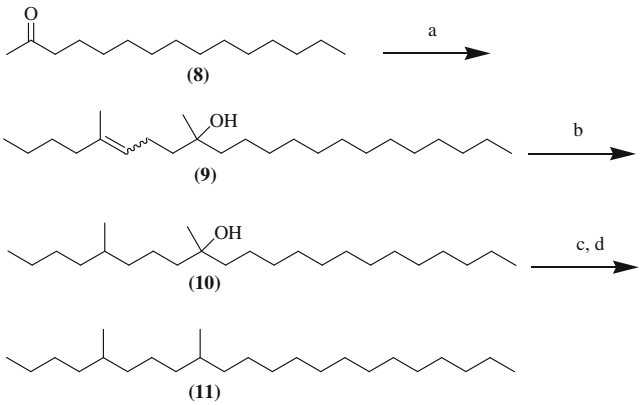


Fig. 4 Mass spectra and the fragmentations of synthetic compounds: (**a**) 5,9,12,16-tetramethyleicosane; (**b**) 5,9-dimethyldocosane; (**c**) 5,9,17-trimethylhenicosane



Scheme 2 Synthesis of 5,9-dimethyldocosane. (**a**) **3**/Mg^0, Et_2O, 66 %, (**b**) H_2/PdC, hexane, 97 % (**c**) *p*-TSA, benzene, reflux, (**d**) H_2/PdC, hexane, 32 % for both steps

and Gdula, 1979; Mori and Murata, 1994; Zarbin et al., 1998), which was treated with 48 % bromic acid that, through Julia's cylcopropane cleavage, led to bromide **3** in 83 % yield. The Grignard reagent prepared from **3** was allowed to react with hexan-2,5-dione **(4)**, previously prepared by Jones oxidation of the commercially available hexan-2,5-diol in 98 % yield (Ferreira and Zarbin, 1996; Kim et al., 2011), providing the key intermediate **5**, which was submitted to catalytic hydrogenation with Pd/C in hexane affording the saturated alcohol **6** (Zarbin et al., 2000). Finally, diol **6** was dehydrated with *p*-TSA in benzene (Utermoehlen et al., 1987), obtaining the corresponding alkene that was directly hydrogenated over Pd/C leading to 5,9,12,16-tetramethyleicosane **7** in 14 % overall yield after six steps (Scheme 1).

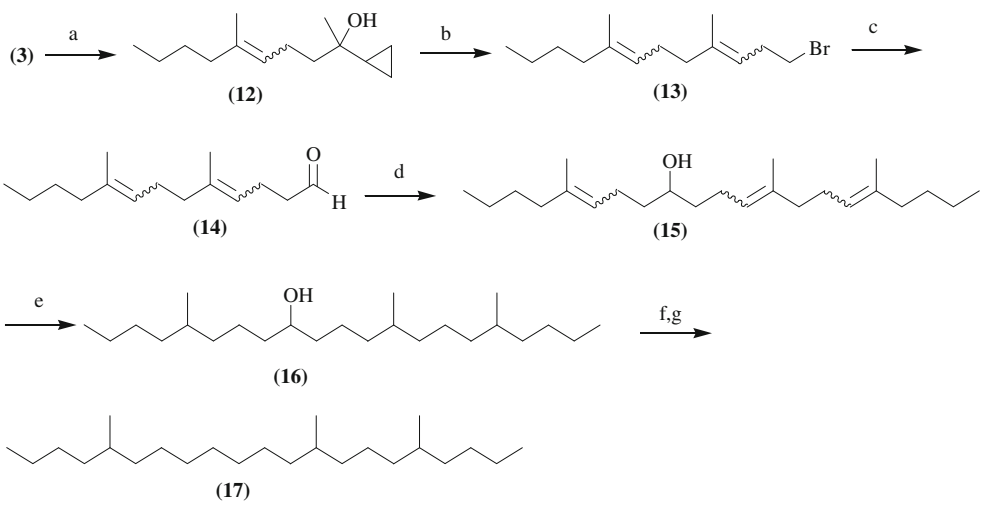
Synthetic **7** produced a broad GC peak, and a mass spectrum similar to that of the natural product (Fig. 4a). However, its Rt was almost one minute earlier on the RTX-5 column, with different Kovats indexes (2157 on the RTX-5 column, 2103 on the RTX-WAX column, and 2161 on the DB-1 column).

Due to the fact that the synthetic compound with four methyl branches exhibited lower Kovats indexes than those of the natural product, we suspected that 5,9-dimethyldocosane could be the true structure. The methyl branches at C_5 could be associated to the fragments *m/z* 85 and *m/z* 281, while the methyl branch at C_9 would be in accordance with *m/z* 155 and *m/z* 211 fragments.

5,9-Dimethyldocosane was synthesized as a mixture of all four stereoisomers using a similar methodology as deployed for Scheme 1 (Scheme 2). The Grignard reagent prepared from bromide **3** obtained in the first synthesis was added to commercial pentadecan-2-one (**8**), leading to the alcohol **9** in 66 % yield, which was catalytically hydrogenated with Pd/C to give saturated alcohol **10** in 97 % yield. Dehydration (Zarbin et al., 2003b) followed by hydrogenation afforded the final compound, 5,9-dimethyldocosane (**11**), in 21 % overall yield in four steps.

The mass spectrum of **11** (Fig. 4b) also was similar to the natural product, however, its Rt was approximately one minute longer than that of the natural product on the RTX-5 column (KI 2279 on the RTX-5 column, 2353 on the RTX-WAX column, and 2283 on the DB-1 column).

The Kovats indexes obtained from the previous synthesized molecules suggested that the natural product should contain three methyl branches in a C_{21} carbon chain. The accumulated data collected strongly supported methyl branches at positions C_5 and C_9. So, the remaining candidate structure with strong diagnostic ions at *m/z* 85, 155, 211, and 281 seemed to be a molecule with the third methyl branch at position C_{17}. A third methyl branch at C_{12} also could produce ions at *m/z* 155 that would be compatible with mass spectrum. However, in this case, it would be expected to see an even mass ion from this fragment with single methyl group (*m/z* 154/155), similar to the one found for the 5,9-dimethyldocosane **11** (*m/z* 210/211, see Fig. 4b). Therefore, we synthesized 5,9,17-trimethylhenicosane as showed in Scheme 3.



Scheme 3 Synthesis of 5,9,17-trimethylhenicosane. (**a**) Mg^0, Et_2O, **1,** 85 %, (**b**) HBr 48 %, 80 % (**c**) Mg^0, Et_2O, DMF, 52 %, (**d**) **3**/Mg^0, Et_2O, 56 %, (**e**) H_2/PdC, hexane, 97 % (**f**) *p*-TSA, benzene, reflux, (**g**) H_2/PdC, hexane, 42 % for both steps

Again, a sequence of Julia's rearrangements was used as the key reaction (Mori and Murata, 1994). The Grignard reagent prepared from previously synthesized bromide **3** was added to cyclopropylmethylketone (**1**) leading to the alcohol **12** in 85 % yield. This alcohol was treated with 48 % bromic acid, obtaining the new bromide **13** in 80 % yield. The Grignard reagent prepared from **13** was added to N,N-dimethylformamide (DMF) in ether to give, upon acidic work-up, the corresponding aldehyde **14** in 52 % yield (Olah et al., 1984; Xiao et al., 2010). A Grignard reagent prepared from bromide **3** was added to aldehyde **14**, leading to the alcohol **15** in 56 % yield, which was hydrogenated, and then suffered dehydration and hydrogenation (Utermoehlen et al., 1987) affording the desired 5,9,17-trimethylhenicosane (**17**) in 12 % overall yield after seven steps.

5,9,17-Trimethylhenicosane did, indeed, produce and an identical mass spectrum (Fig. 4c), retention times, and Kovats indexes on the three GC columns employed, as did the natural product. These data confirm that the male-produced compound from *Phthia picta* is 5,9,17-trimethylhenicosane.

The behavioral responses of *P. picta* males and females to synthetic 5,9,17-trimethylhenicosane were evaluated in a Y-tube olfactometer. In the bioassay, approximately 80 % of the females were attracted to the synthetic compound ($P<0.001$). In contrast, when males were tested, attraction was not different from the control ($P=0.206$) (Fig. 5). The fact that only females were attracted to the treatment suggests that the 5,9,17-trimethylhenicosane is a sex pheromone produced by *P. picta* males. This is the first time that a hydrocarbon has been identified as a pheromone in Coreidae bugs.

Besides sex pheromone, true bugs produce large quantities of strong-smelling and irritating defensive chemicals that are released when these insects are disturbed (Aldrich et al., 1982; Aldrich, 1988; Aldrich et al., 1993; Durak and Kalender, 2007). Odorous compounds are produced by both adults and immatures that defend against predation (Staddon, 1979). These chemicals also may have a role as alarm pheromones (Gunawardena and Bandumathie, 1993; Leal et al., 1994). The defensive/alarm compounds of adult bugs are produced in metathoracic scent glands (MSG) usually composed of a reservoir and a pair of lateral glands that each connect to the reservoir through a duct (Staddon, 1979, 1986; Aldrich, 1988; Durak and Kalender, 2007; Durak, 2008).

The metathoracic scent gland of true bugs in the infraorder Pentatomomorpha (Tian et al., 2011; Weirauch and Schuh, 2011) is a compartmentalized gland that includes a pair of lateral accessory glands and a median reservoir (Aldrich et al., 1978; Aldrich, 1988). This gland produces defensive secretions characteristic of heteropterans. However, in certain Lygaeidae (Aldrich et al. 1997, 1999) and Alydidae (e.g., Aldrich et al., 2000), attractant pheromones are released from the lateral accessory glands of males or females, thus by-passing the reservoir. Therefore, our analyses of *P. picta* included sampling by dissecting the lateral accessory glands and reservoirs of adult males and females separately, as well as aerations of the sexes.

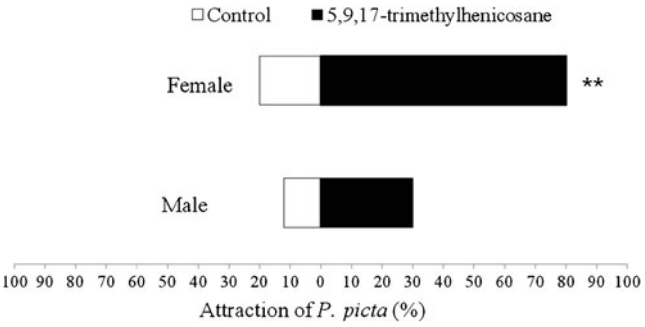


Fig. 5 Responses of *Phthia picta* males and females to synthetic 5,9,17-trimethylhenicosane in Y-olfactometer test (** statically significant; $P<0.001$, binomial test)

Dissections of the MSG reservoir and lateral glands separately for *P. picta* males and females, followed by GC-MS analysis of the extracts, showed different results. The MSG reservoirs of males and females each contained the typical coreid defensive compounds; i.e., 1-hexanal, 1-hexanol, hexanoic acid, hexyl acetate, octyl acetate, and tridecane (Fig. 6C) (Aldrich et al., 1993; Leal et al., 1994). These secretory components exhibited mass spectra that matched the spectra of the aforementioned components in the NIST library, and full identification was confirmed by GC coinjection with authentic standards. However, the contents of lateral gland showed a pronounced difference between the sexes. While almost no components were detected in the lateral glands of females, the corresponding extract from males contained one compound in high concentration, whose Kovats indexes and mass spectra matched perfectly

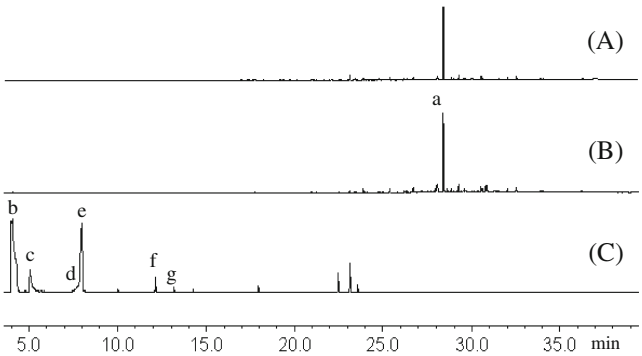


Fig. 6 Gas chromatographic analysis of compounds obtained from *Phthia picta* adult males: (**A**) volatiles obtained by aeration; (**B**) extract of lateral gland of the MTG; (**C**) extract of MSG reservoir compounds: (a) 5,9,17-trimethylhenicosane, (b) 1-hexanal; (c) 1-hexanol; (d) hexanoic acid; (e) hexyl acetate; (f) octyl acetate; and (g) tridecane. Remaining peaks are unidentified

with the male-specific compound detected by aeration (Fig. 6A and B).

In summary, we identified the male-produced sex pheromone of *P. picta* as 5,9,17-trimethylhenicosane, and we also described the contents of the reservoir of metathoracic gland from males and females. In addition, we determined that the sex pheromone is produced in the lateral glands of the MSG of males in this species. The results herein described could become useful for managing this elusive and unpredictable pest.

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