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SEX PHEROMONE OF TOMATO PEST *Scrobipalpuloides absoluta* (LEPIDOPTERA: GELECHIIDAE)

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Abstract—The sex attractant of *Scrobipalpuloides absoluta* females is a 90:10 mixture of (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrien-1-yl acetate and (3*E*,8*Z*)-3,8-tetradecadien-1-yl acetate. Tetradecadienyl acetates bearing 8*Z*,11*Z*; 3*E*,8*Z*; and 3*E*,11*Z* double bonds were synthesized by stereospecific procedures; the mass spectral and gas chromatographic properties of the 3*E*,8*Z* isomer were found to be congruent with those of the tetradecadienyl acetate from *S. absoluta*. In wind tunnel bioassays, a 10:1 mixture of synthetic (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrien-1-yl acetate and (3*E*,8*Z*)-3,8-tetradecadien-1-yl acetate was highly attractive to *S. absoluta* males. Interestingly, the presence of (8*Z*,11*Z*)-8,11-tetradecadien-1-yl acetate (10%) inhibited the response to (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrien-1-yl acetate completely.

Key Words—Lepidoptera, Gelechiidae, *Scrobipalpuloides absoluta*, tomato, pest, pheromone.

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INTRODUCTION

We found recently that the female sex pheromone of *Scrobipalpuloides absoluta* (Lepidoptera: Gelechiidae), a devastating tomato pest in South America, consists of two components. The major component, which represents about 90% of the volatile material found in the sex gland of calling females, was identified as (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrien-1-yl acetate (**1**, Figure 1) (Attygalle et al., 1995; 1996). The minor constituent (10%), was characterized partially by GC-MS as a tetradecadienyl acetate. However, the minute amount of natural material available precluded a full structural assignment by conventional methods.

In order to identify the diene acetate, we decided to synthesize the three possible dienes (**2–4**, Figure 1) with double-bond positions and configurations corresponding to those of the major pheromone component, (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrien-1-yl acetate, and to compare the properties of the synthetic samples with those of the natural material. Results of these experiments, and subsequent bioassays, established the pheromone as a mixture of (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrien-1-yl acetate (**1**) and (3*E*,8*Z*)-3,8-tetradecadien-1-yl acetate (**3**).

METHODS AND MATERIALS

NMR spectra were recorded on a Unity-200 (^{1}H, 200 MHz, Varian), a Unity-400 (^{1}H NMR, 400 MHz; ^{13}C NMR, 100.6 MHz, Varian), and a Unity-

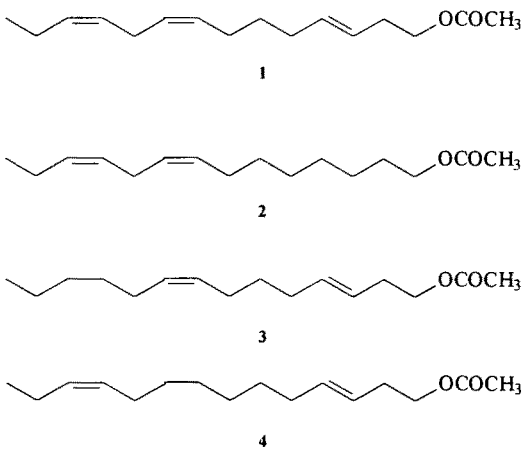


FIG. 1. Structures of compounds **1–4.**

500 instrument (^{1}H NMR, 500 MHz; ^{13}C NMR, 125.7 MHz, Varian) as $CDCl_3$ solutions at room temperature. Chemical shifts, given in parts per million, are expressed as δ values measured from the residual $CHCl_3$ signal (7.26 ppm). Vapor-phase infrared spectra were recorded using a Hewlett-Packard (HP) 5965A IRD coupled to a HP 5890 GC. Electron impact (70 eV) spectra were obtained using a HP 5890 GC coupled to an ion trap detector (ITD 800, Finnigan) or a mass selective detector (HP 5970 MSD). Fast atom bombardment (FAB, in a glycerin matrix) mass spectra were obtained using a ZAB-Q (VG) instrument. For GC analysis a Supelco fused-silica capillary column (30 m × 0.25 mm) coated with SE-54 (0.25 μm) fitted in a HP 5890 GC equipped with a flame-ionization (FID) detector was used. Synthetic samples (hexane, ca. 1 mg/ml) were injected in split mode using a temperature program of 60°C for 4 min, 10°C/min to 270°C, and held for 20 min. Column chromatography was run on silica gel (Merck, H 60) and reactions were monitored by TLC on Baker-flex Silica gel IB2-F plates (J. T. Baker) and on Silufol TLC plates (Kavalier, Sázava, Czech Republic). Silver-nitrate column chromatography was done on silica gel impregnated with $AgNO_3$ (20% of $AgNO_3$ on silica gel Merck H 60).

Biological Material

Pheromone extracts were prepared as described previously (Attygalle et al., 1996).

Chemicals

Butyllithium (1.6 M and 2.5 M solutions in hexane), 1-(tetrahydropyran-2-yloxy)-3-butyne [2-(3-butynyloxy)tetrahydro-2*H*-pyran], $LiAlH_4$, $NaBH_4$, borane–methylsulfide complex, Cu(I)Br·Me_2S complex, and *p*-toluenesulfonyl chloride were from Aldrich Chemical Co. (Milwaukee, Wisconsin), and bromine, ethylenediamine, nickel(II) acetate, and triphenylphosphine from Fluka Chemical Co. (Ronkonkoma, New York); they were used as purchased. 1-(Tetrahydropyran-2-yloxy)-4-pentyne (**5**) (Robertson, 1960) and 1-bromo-6-(tetrahydropyran-2-yloxy)-hexane (**20**) (Kang et al., 1985; Miyashita et al., 1977) were prepared according to published procedures. 1,3-Dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU, Aldrich Chemicals) was dried over molecular sieves.

(8Z,11Z)-8,11-Tetradecadienyl Acetate (2)

This compound was synthesized according to Scheme 1. Compounds **5–9** were synthesized according to procedures described previously for the synthesis of (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrien-1-yl acetate (**1**) (Attygalle et al., 1995; 1996).

Magnesium turnings (30 mg, 1.28 mmol) were warmed, stirred for 1 hr under dry argon, and covered with dry THF (100 μl). Ten drops of a solution of (4Z,7Z)-1-bromo-4,7-decadiene (**9**) (200 mg, 0.925 mmol) in dry THF (3 ml) were added, and the mixture was brought to reflux. After the reaction started, the remainder of the bromide solution was added dropwise at 0°C over a period of 1.5 hr, and the mixture was stirred for 2 hr at room temperature.

Dry THF (3 ml) was treated with trimethylsilyl iodide (260 mg, 185 μl, 1.30 mmol) at room temperature and stirred for 15 min to form 4-trimethylsilyloxy iodide (**11**) (Jung and Lyster, 1977). The reaction mixture was treated with a solution of $CuCN \cdot (LiCl)_2$ in THF (1 M, 100 μl), cooled to −40°C, and the solution of Grignard reagent **10** described in the preceding paragraph was then added over a period of 30 min (Tamura and Kochi, 1971; Lipshutz et al., 1984). The mixture was stirred at −40 to −20°C for 1 hr, followed by an additional 1 hr at room temperature. The reaction was quenched with NH_4Cl solution and ammonia mixture (2 : 1 v/v), and the product was extracted with hexane–ether (1 : 1) mixture (R_f = 0.60, 19 : 1 hexane–ethyl acetate, v/v). The crude product was dissolved in THF (6 ml), and deprotected with tetrabutylammonium fluoride solution in THF (1 M, 2 ml). The alcohol formed was acetylated with Ac_2O–pyridine mixture (1 : 10 v/v). Flash chromatographic purification afforded (8Z,11Z)-8,11-tetradecadien-1-yl acetate **2** (80 mg) in 34% yield based on the starting bromide **9.**

^{1}H NMR (400 MHz) δ: 5.36 (m, 4H, CH=CH-8, 9, 11, 12), 4.05 (t, J = 6.7, 7.0 Hz, 2H, CH_2-1), 2.77 (dd, J = 6.4, 6.4 Hz, 2H, =CH-CH_2-CH=), 2.06 (m, 4H, CH_2-7, 13), 2.04 (s, 3H, -$COCH_3$), 1.60 (tt, J = 7.3, 7.3, 7.6, 7.6 Hz, 2H, CH_2-6), 1.4-1.2 (m, 8H, CH_2-2, -3, -4, -5) 0.97 (t, J = 7.3, 7.6 Hz, 3H, CH_3-14). ^{13}C NMR (100.6 MHz) δ: 171.2 (C=O), 131.7 (=CH-12), 130.0 (=CH-11), 128.0 (=CH-9), 127.3 (=CH-8), 64.6 (CH_2-1), 29.5 (CH_2-2), 29.1 (CH_2-3, 4), 28.6 (CH_2-5), 27.2 (CH_2-6), 25.9 (CH_2-7), 25.5 (CH_2-10), 21.0 (CH_3C=O), 20.5 (CH_2-13), 14.3 (CH_3). MS [EI MSD, m/z (%)] 252 (M^+, 2), 209 (M^+ − 43, 1), 192 (M^+ − 60, 23), 163 (4), 149 (8), 135 (14), 121 (38), 110 (19), 107 (19), 95 (39), 93 (40), 82 (26), 81 (57), 79 (66), 67 (100), 55 (38), 43 (93), 41 (50). IR (gas phase, cm^{-1}) 3017 (*cis*=C−H str), 2936 (CH_2), 1761 (C=O), 1233, 1040 (−O−).

(3E,8Z)-3,8-Tetradecadienyl Acetate (3)

1-(Tetrahydropyran-2-yloxy)-4-decyne (13). A solution of 1-(tetrahydropyran-2-yloxy)-4-pentyne (**5**) (0.66 g, 3.90 mmol) in THF (3 ml) was metallated with *n*-butyllithium solution (2.5 M) in hexane (1.87 ml, 1.2 eq.), and a solution of *n*-pentyl bromide (0.725 ml, 1.5 eq.) in DMPU (2 ml) was added. This procedure gave 0.54 g (58% yield) of compound **13.**

^{1}H NMR (200 MHz) δ: 4.60 (m, 1H, CH-2′), 3.65 (m, 4H, CH_2-1, 6′),

2.26 (tt, J = 7.0, 7.0, 2.0, 2.0 Hz, 2H, CH_2-3), 2.13 (tt, J = 7.0, 7.0, 2.5, 2.5 Hz, 2H, CH_2-6), 1.77 (tt, J = 6.8, 6.8, 7.0, 7.0 Hz, 2H, CH_2-2), 1.33 (m, 6H, CH_2-7, 8, 9), 1.52 (m, 6H, CH_2-3′, 4′, 5′), 0.89 (t, J = 7.0 Hz, 3H, CH_3). MS [FAB, m/z(%)] 237 ($M^+ - 1$), 85 (100). IR (CCl_4, cm^{-1}) 2229, 1322, 1331, 1201, 1138, 1121, 1034.

(4Z)-1-(Tetrahydropyran-2-yloxy)-4-decene (14). 1-(Tetrahydropyran-2-yloxy)-4-decyne (**13**) (0.5 g, 2.1 mmol) was hydrogenated in ethanol (20 ml) over *P*2-Ni, formed from nickel acetate (75 mg) deactivated with ethylenediamine (0.2 ml) for 4 hr (Brown and Ahuja, 1973). After chromatography on silica gel, the product (**14**) (0.43 g) was obtained in 85% yield. GC (R_t): starting material **13,** 22.18 min, 0.4%; desired product **14,** 21.59 min, 97.9%; the 4*E* isomer of **14,** 21.75 min, 1.7%.

^{1}H NMR (200 MHz) δ: 5.37 (m, 2H, CH=CH-4, 5), 4.58 (m, 1H, CH-2′), 3.60 (m, 4H, CH_2-1, 6′); 2.12 (m, 2H, CH_2-6); 2.04 (m, 2H, CH_2-3); 1.16 (tt, 2H, J = 6.8, 6.8, 7.0, 7.0 Hz, CH_2-2); 1.4–1.9 (m, 6H, CH_2-3′, 4′, 5′); 1.29 (m, 6H, CH_2-7, 8, 9); 0.88 (t, 3H, J = 7.0 Hz, CH_3). IR (CCl_4, cm^{-1}) 3007, 1653, 1201, 1138, 1120, 1034. MS [FAB, m/z (%)] 239 ($M^+ - 1$), 85 (100).

(4Z)-1-Bromodec-4-ene (15). To an ice-cool solution of triphenylphosphine (0.72 g, 2.74 mmol) and bromine (0.41 g, 2.6 mmol) in CH_2Cl_2, a solution of (4*Z*)-1-(tetrahydropyran-2-yloxy)-4-decene (**14**) in CH_2Cl_2 (0.41 g, 1.71 mmol) was added dropwise (Bestmann and Gunawardena, 1992). The usual work-up (Attygalle et al., 1996) gave the product **15** in 90% yield (0.33 g).

^{1}H NMR (200 MHz) δ: 5.42 (dtt, J = 11.0, 7.0, 7.0, 1.0, 1.0 Hz, 2H, CH=CH-4, 5), 3.41 (t, J = 6.6, 6.6 Hz, 2H, CH_2-1), 2.20 (dt, J = 7.0, 7.0, 7.0 Hz, 2H, CH_2-3), 2.04 (dt, J = 6.5, 6.5, 6.5 Hz, 2H, CH_2-6), 1.91 (tt, J = 6.5, 6.5, 6.5 Hz, 2H, CH_2-2), 1.30 (m, 6H, CH_2-7, 8, 9), 0.89 (t, J = 7.0 Hz, 3H, CH_3). MS [EI MSD, m/z (%)] 220 (M^+, 8), 218 (M^+, 8), 164 (12), 162 (12), 150 (23), 148 (23), 135 (8), 109 (12), 97 (44), 95 (14), 83 (55), 82 (14), 81 (37), 79 (15), 70 (21), 69 (100), 67 (39), 56 (29), 55 (93), 54 (20), 43 (21), 42 (20), 41 (92), 39 (30). IR (CCl_4, cm^{-1}) 3008 (*cis* CH=CH), 1246 (RCH_2CH_2Br), 649, 566 (C-Br).

(8Z)-1-(Tetrahydropyran-2-yloxy)-8-tetradecen-3-yne (16). 1-(Tetrahydropyran-2-yloxy)-3-butyne (0.30 g, 1.94 mmol) was metallated at 0°C with *n*-butyllithium (2.0 M) hexane solution (1.0 ml, 1.0 eq.). The lithium acetylide that formed was alkylated with the bromide **15** (0.20 g, 0.92 mmol), using 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidine (DMPU) as the base. This reaction afforded 214 mg of a mixture containing the desired product **16,** 1-(tetrahydropyran-2-yloxy)-3-butyne (one of the starting materials; 29%), and a nonpolar impurity identified as (8*Z*)-1,8-tetradecadien-4-yne (**19**) (28%). The impurities were removed from compound **16** (52% yield based on **15**) by repeated column chromatography.

^{1}H NMR (200 MHz) (**16**) δ: 5.35 (dtt, J = 11.0, 7.0, 7.0, 1.0, 1.0 Hz, 2H, CH=CH-8, 9), 4.64 (m, 1H, CH-2′), 3.58 (m, 4H, CH_2-1, 6′), 2.46 (tt, J = 7.5, 7.5, 2.5, 2.5 Hz, 2H, CH_2-2), 2.14 (tt, J = 7.0, 7.0, 2.5, 2.5 Hz, 2H, CH_2-5), 2.10 (m, 2H, CH_2-7), 2.04 (dt, J = 6.5, 6.5, 7.0 Hz, CH_2-10), 1.29 (m, 6H, CH_2-11, 12, 13), 1.9–1.4 (m, 8H, CH_2-6, 3′, 4′, 5′), 0.89 (t, J = 7.0 Hz, 3H, CH_3). MS [EI MSD, m/z (%)] (**16**) 133 (12), 107 (10), 85 (100), 67 (15), 55 (8). MS [FAB, m/z] 293.2 (M^+). IR (CCl_4, cm^{-1}) (**16**) 3007 (*cis*-CH=CH), 2232 (acetylenic), 1403, 1201, 1184, 1137, 1122, 1034 (CH_n—O—).

^{1}H NMR (200 MHz) [(8*Z*)-1,8-tetradecadien-3-yne(**19**)] δ: 5.78 (ddt, J = 22.5, 10.5, 2.2, 2.2 Hz, 1H, =CH-2), 5.54 (dd, J = 22.5, 2.7 Hz, 1H, $=CH_2$-1a), 5.36 (dd, J = 10.5, 2.2 MHz, 1H, CH_2-1b), 5.36 (dtt, J = 11.0, 7.0, 7.0, 1.0, 1.0 Hz, 2H, CH=CH-8, 9), 2.31 (dt, J = 2.1, 7.3, 7.3 Hz, 2H, CH_2-5), 2.15 (dt, J = 7.0, 7.3, 7.3 Hz, 2H, CH_2-10), 2.03 (dt, J = 6.4, 6.4, 6.7 Hz, 2H, CH_2-7), 1.59 (tt, J = 7.0, 7.0, 8.0, 8.0 Hz, 2H, CH_2-6), 1.30 (m, 6H, CH_2-11, 12, 13), 0.89 (t, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$) (**19**) δ: 131.1 (=CH-9), 126.4 (=CH-8), 125.5 ($=CH_2$-1), 117.6 (=CH-2), 90.9 (≡C-4), 79.5 (≡C-3), 31.5 (CH_2-12), 29.4 (CH_2-10), 28.7 (CH_2-7), 27.2 (CH_2-11), 26.3 (CH_2-6), 22.6 (CH_2-5), 18.8 (CH_2-13), 14.1 (CH_3-14). MS [EI MSD, m/z (%)] (**19**) 161 (M^+ −29, 5), 133 (35), 119 (25), 105 (50), 91 (100), 79 (35), 67 (30), 41 (50). IR (CCl_4, cm^{-1}) (**19**) 3101 ($=CH_2$), 3009 (*cis*-CH=CH and $=CH_2$), 2227, 2206 (acetylenic), 1833, 972, 913 (vinyl).

(Z)-8-Tetradecen-4-ynol (17). Tetrahydropyranyl ether **16** was deprotected with Dowex 50WX8 (40 mg) to form the alcohol **17** (107 mg, 56% yield based on the starting bromide 15).

^{1}H NMR (200 MHz) δ: 5.35 (dtt, J = 11.0, 7.0, 7.0, 1.0, 1.0 Hz, 2H, CH=CH-8,9), 3.68 (dt, J = 6.5, 5.6, 5.6 Hz, 2H, CH_2-1), 2.44 (tt, J = 6.5, 6.5, 2.5, 2.5 Hz, 2H, CH_2-2), 2.17 (tt, J = 7.0, 7.0, 2.5, 2.5 Hz, 2H, CH_2-5), 2.12 (m, 2H, CH_2-7), 2.04 (m, 2H, CH_2-10), 1.76 (bt, J = 6.5, 6.5 Hz, 1H, OH), 1.55 (tt, J = 4 × 7.2 Hz, 2H, CH_2-6), 1.30 (m, 6H, CH_2-11, 12, 13), 0.89 (t, J = 7.0 Hz, 3H, CH_3). MS [EI MSD, m/z (%)] 179 (M^+-29, 8), 163 (21), 151 (15), 133 (53), 121 (38), 107 (87), 95 (66), 93 (66), 91 (80), 81 (66), 79 (88), 67 (87), 55 (100), 41 (100), 29 (29). IR (CCl_4, cm^{-1}) 3636, 3588 (OH), 3006 (*cis*-CH=CH), 2227 (acetylenic), 1654 (C—O).

(3E,8Z)-3,8-Tetradecadien-1-yl Acetate (3). (8*Z*)-Tetradecen-4-ynol (**17**) (21 mg, 0.1 mmol) was reduced with $LiAlH_4$ (20 mg, 0.5 mmol) in dry diglyme (0.5 ml) at 120°C for 3 hr. The alcohol (**18**) that formed was acetylated with Ac_2O (0.1 ml) and dry pyridine (0.5 ml) and the product was purified on $AgNO_3$ (20%) impregnated silica gel. This procedure afforded 18 mg (72% yield) of pure **3** (97% isomeric purity).

^{1}H NMR (500 MHz) δ: 5.51 (dtt, J = 15.3, 6.8, 6.8, 1.2, 1.2 Hz, 1H, =CH-3), 5.38 (dtt, J = 15.2, 6.6, 6.6, 1.2, 1.2 Hz, 1H, =CH-4), 5.35 (dtt

J = 11, 6.8, 6.8, 1.2, 1.2 Hz, 2H, CH=CH-8, 9), 4.07 (t, J = 6.9, 6.9 Hz, 2H, CH_2-1), 2.31 (dtdt, J = 3 × 6.8, 3 × 1.2 Hz, 2H, CH_2-2), 2.01 (tt, J = 4 × 7.0 Hz, 6H, CH_2-5, 7, 10), 2.04 (s, 3H, $COCH_3$), 1.60–1.20 (8H, m, CH_2-6, 11, 12, 13), 0.88 (t, J = 7.0, 7.0 Hz, 3H, CH_3). ^{13}C NMR (125.7 MHz) δ: 171.1 (C=O), 133.3 (=CH-3), 130.3 and 129.4 (=CH-8, 9), 125.3 (=CH-4), 64.1 (CH_2-1); 32.2 (CH_2-2), 32.0 (CH_2-6), 31.5 (CH_2-12), 29.4 (CH_2-7, 10), 27.2 (CH_2-11), 26.6 (CH_2-5), 22.5 (CH_2-13), 21.0 ($COCH_3$), 14.1, (CH_3-14). MS [EI, m/z (%)], 252 (M^+, 0.5), 192 (M^+ −60, 12), 163 (5), 138 (19), 124 (20), 121 (41), 107 (19), 95 (34), 93 (34), 82 (41), 80 (100), 79 (63), 67 (60), 55 (36), 43 (100), 41 (41). IR (CCl_4, cm^{-1}) 3006 (*cis*=C-H str), 1743 (C=O), 1653 (CH=CH), 1403, 1238, 1035 (C−O), 969 (*trans* CH=CH wag). IR (gas phase, cm^{-1}) 3011 (*cis*=C-H str), 2934 (CH_2), 1761 (C=O), 1231, 1037 (−O−), 967 (*trans* CH=CH wag).

(3E,11Z)-3,11-Tetradecadien-1-yl Acetate (4).

Starting from 1-butyne and 1-bromo-6-(tetrahydropyran-2-yloxy)-hexane (**20**), the (3*E*,11*Z*)-3,11-tetradecadien-1-yl acetate (**4**) was prepared by a synthetic route similar to that used for the preparation of (3*E*,8*Z*)-3,8-tetradecadien-1-yl acetate (**3**). The yield was 15% [17 mg; 96% isomeric purity (GC)] based on the starting material, 1-bromo-6-(tetrahydropyran-2-yloxy)-hexane (**20**).

^1H NMR (500 MHz) δ: 5.51 (dtt, J = 15.1, 6.8, 6.8, 1.2, 1.5 Hz, 1H, =CH-3), 5.35 (m, 3H, CH=CH-4, 11, 12), 4.06 (t, J = 6.8, 6.8 Hz, 2H, CH_2-1), 2.31 (dddt, J = 1.2, 1.2, 6.8, 6.8, 6.8 Hz, 2H, CH_2-2), 2.05 (s, 3H, $COCH_3$), 2.02 (m, 6H, CH_2-5, 10, 13), 1.36–1.24 (m, 6H, CH_2-6, 7, 8, 9), 0.95 (t, J = 7.3, 7.6 Hz, 3H, CH_3). ^{13}C NMR (100.6 MHz) δ: 171.1 (C=O), 133.6 (=CH-3), 131.6 (=CH-12), 129.3 (=CH-11), 125.0 (=CH-4), 64.1 (CH_2-1), 32.6 (CH_2-2), 31.9 (CH_2-6), 29.7 (CH_2-8), 29.3 (CH_2-7), 29.1 (CH_2-9), 29.0 (CH_2-10), 27.1 (CH_2-5), 21.0 ($COCH_3$), 20.5 (CH_2-13), 14.4 (CH_3-14). MS [EI MSD, m/z (%)] 192 (M^+-60, 6), 163 (6), 149 (9), 135 (12), 121 (16), 107 (16), 96 (28), 95 (37), 93 (25), 82 (50), 81 (60), 80 (35), 79 (36), 69 (33), 68 (64), 67 (97), 55 (45), 43 (100), 41 (67). IR (CCl_4, cm^{-1}) 3006 (=CH *cis* str), 1743 (C=O), 1238, 1036 (C−O), 969 (=CH *trans* wag). IR (gas phase, cm^{-1}) 3012 (*cis*=C-H str), 2934 (CH_2), 1761 (C=O), 1231, 1038 (−O−), 968 (=CH *trans* wag).

Wind-Tunnel Experiments

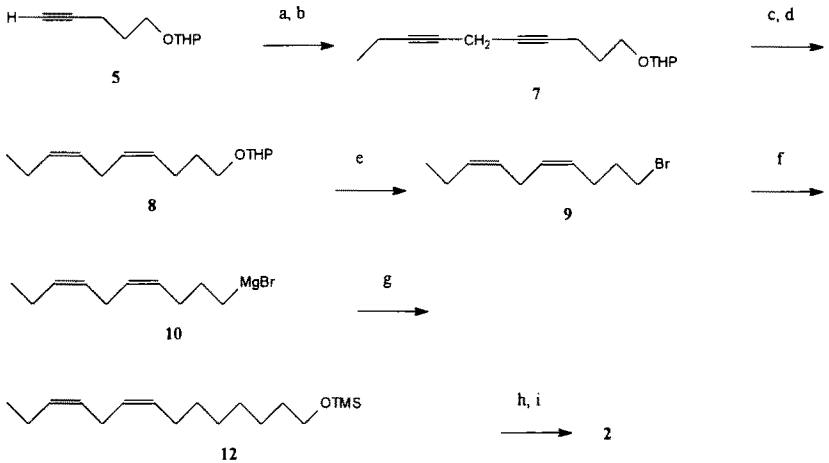
Synthetic samples of test compounds were applied as pentane solutions to rubber septa (cleaned by washing with CH_2Cl_2 for 20 hr in a Soxhlet apparatus), which were used as baits in the wind tunnel. The attractivity of baits loaded with either 100 ng of (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrien-1-yl acetate (**1**), or each individual diene acetate (100 ng, **2–4**), or mixtures of 100 ng of **1** with 10 ng

of each diene acetate **2, 3,** and **4** were compared to that of three calling virgin females (1 to 4 days old) in a cage. The control baits were treated with 100 μl of hexane. The wind tunnel (3.8 × 0.50 m) was operated at a flux speed of 30 cm/sec. The landing platform was 1 m away from the "takeoff" platform. For each experiment, three males (1 to 3 days old) were placed on the takeoff platform and their behavioral responses were observed for 5 min. The test was repeated 10 times for each test sample.

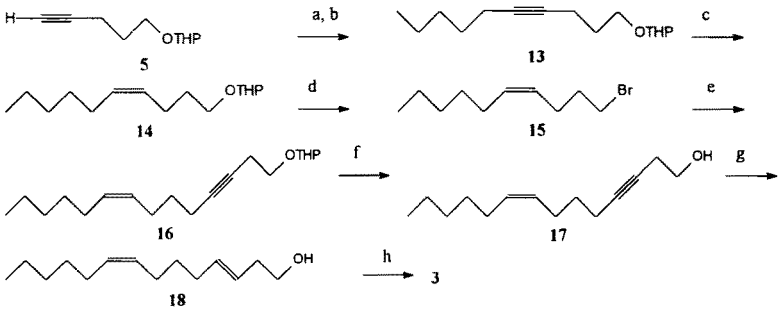
RESULTS AND DISCUSSION

In order to characterize the minor component of the *S. absoluta* pheromone, the dienes **2, 3,** and **4** were synthesized. (8*Z*,11*Z*)-8,11-Tetradecadienyl acetate (**2**) was synthesized according to the reaction shown in Scheme 1.

Intermediates **5–9** were synthesized according to procedures described previously for the synthesis of (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrien-1-yl acetate (**1**) (Attygalle et al., 1996). 1-Iodo-4-trimethylsilyloxybutane (**11**) was prepared in situ from iodotrimethylsilane and an excess of tetrahydrofuran and allowed to react under Cu(I) catalysis with the Grignard reagent **10,** which was prepared freshly from the bromide **9.** The TMS ether (**12**) obtained in this way was deprotected with a THF solution of tetrabutylammonium fluoride and acetylated



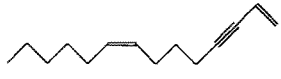
SCHEME 1. (a) EtMgBr/THF; (b) CH_3-CH_2-C≡C-CH_2-OTs (**6**)/Cu(I)Br·Me_2S, −20°C–0°C; (c) Cy_2BH, 4.4 eq.; (d) CH_3CO_2H; (e) PPh_3/Br_2 1.5 eq, CH_2Cl_2; (f) Mg, ether 0–5°C; (g) I-$(CH_2)_4$-OTMS (**11**), CuCN·$(LiCl)_2$, −30°C; (h) tetrabutylammonium fluoride/THF; (i) Ac_2O/pyridine.



SCHEME 2. (a) *n*-BuLi/hexane; (b) CH_3-$(CH_2)_4$-Br, DMPU, 0°C; (c) P-2 Ni/H_2; (d) PPh_3/Br_2 1.5 eq, CH_2Cl_2, 0°C; (e) Li-≡-OTHP, DMPU/THF 0°C; (f) Dowex/MeOH; (g) $LiAlH_4$/diglyme, 120–140°C; (h) Ac_2O/pyridine.

to give the desired dienyl acetate **2** in 34% yield based on the starting bromide **9.**

(3*E*,8*Z*)-3,8-Tetradecadienyl acetate (**3**) was synthesized according to the reaction shown in Scheme 2. This synthesis also employed procedures similar to those used for the synthesis of (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrien-1-yl acetate (**1**), the major pheromone component (Attygalle et al., 1996). An alkylation of the lithiated 1-(tetrahydropyran-2-yloxy)-4-pentyne (**5**) in THF with *n*-pentyl bromide, using 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU) as the base, gave the THP protected alcohol **13.** The reduction of the triple bond in **13** to a *Z* double bond was accomplished by using P2 nickel catalyst (Brown and Ahuja, 1973). The reaction proceeded with high stereoselectivity (98.3% *Z* isomer). Subsequently, using 1.5 eq of $PPh_3 \cdot Br_2$ (Bestmann and Gunawardena, 1992), the tetrahydropyranyl protected hydroxyl group in **14** was replaced by a bromine atom. A coupling of the lithiated 1-(tetrahydropyran-2-yloxy)-3-butyne with the above-prepared bromide **15,** using DMPU as the base, gave the desired THP protected alkeneynol **16.** However, this reaction also produced a nonpolar impurity, which showed a similar R_f value but a different GC retention time compared to those of the starting bromide **15.** This by-product was removed from the bromide **15** by repeated silica gel chromatography. Based on spectral data, this impurity was identified as (*Z*)-1,8-tetradecadien-3-yne (19, Figure 2).



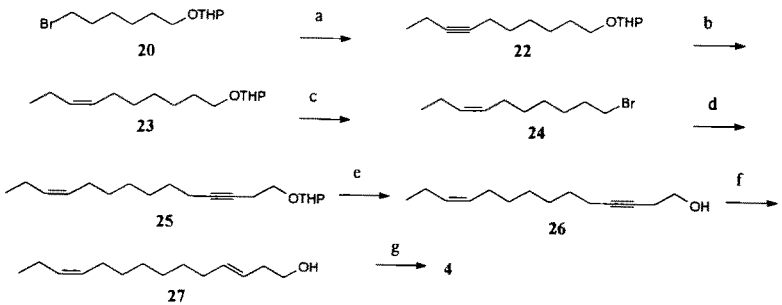
19

FIG. 2. Structure of compound **19.**

This by-product might be expected from a base-catalyzed elimination of the homopropargylic tetrahydropyranyl group. Although this process, to the best of our knowledge, has not been described previously, we have observed other instances in which THP protected 3-alkyn-1-ols give unsaturated impurities even under acidic conditions (TsOH/MeOH also gives rise to the hydrocarbon by-product **19**). In any case, the tetrahydropyranyl protecting group in **16** could be successfully removed, using a Dowex ion-exchange resin in MeOH at room temperature. The product, alcohol **17**, was reduced stereospecifically to dienol **18** with $LiAlH_4$ in diglyme (Rossi and Carpita, 1977). Finally, the reaction mixture containing the dienol **18** was acetylated with Ac_2O/pyridine to give the desired dienyl acetate **3**. Chromatography on a silver-nitrate impregnated silica gel column gave the final product, (3*E*,8*Z*)-3,8-tetradecadien-1-yl acetate **3**, in 97% purity and 17% overall yield (based on lithiated 1-(tetrahydopyran-2-yloxy)-4-pentyne) (Scheme 2).

For the synthesis of (3*E*,11*Z*)-3,11-tetradecadien-1-yl acetate (**4**), we used a procedure similar to that used for the preparation of (3*E*,8*Z*)-3,8-tetradecadien-1-yl acetate (**3**) (Scheme 2). Starting from 1-butynyllithium (**21**) and 1-bromo-6-(tetrahydropyran-2-yloxy)-hexane (**20**), we prepared (3*E*,11*Z*)-3,11-tetradecadien-1-yl acetate in 15% overall yield and in 96% stereoisomeric purity (Scheme 3).

The ion-trap electron-ionization mass spectra of dienes **2**, **3**, and **4** were recorded and compared with those obtained under similar conditions a few months earlier from *S. absoluta* females. Although spectra recorded at different times, even under similar conditions, tend to differ slightly, a comparison of relative intensities within each cluster of *m/z* values provides a more reliable way of asserting whether two spectra represent the same compound or not. An application of this procedure to the spectra presented in Figure 3 disclosed that the



SCHEME 3. (a) Li—≡—/(**21**), DMPU, 0°C; (b) P-2 Ni/H_2; (c) PPh_3/Br_2 1.5 eq, CH_2Cl_2, 0°C; (d) Li—≡—OTHP, DMPU/THF 0°C; (e) Dowex/MeOH; (f) $LiAlH_4$/diglyme, 120–140°C; (g) Ac_2O/pyridine.

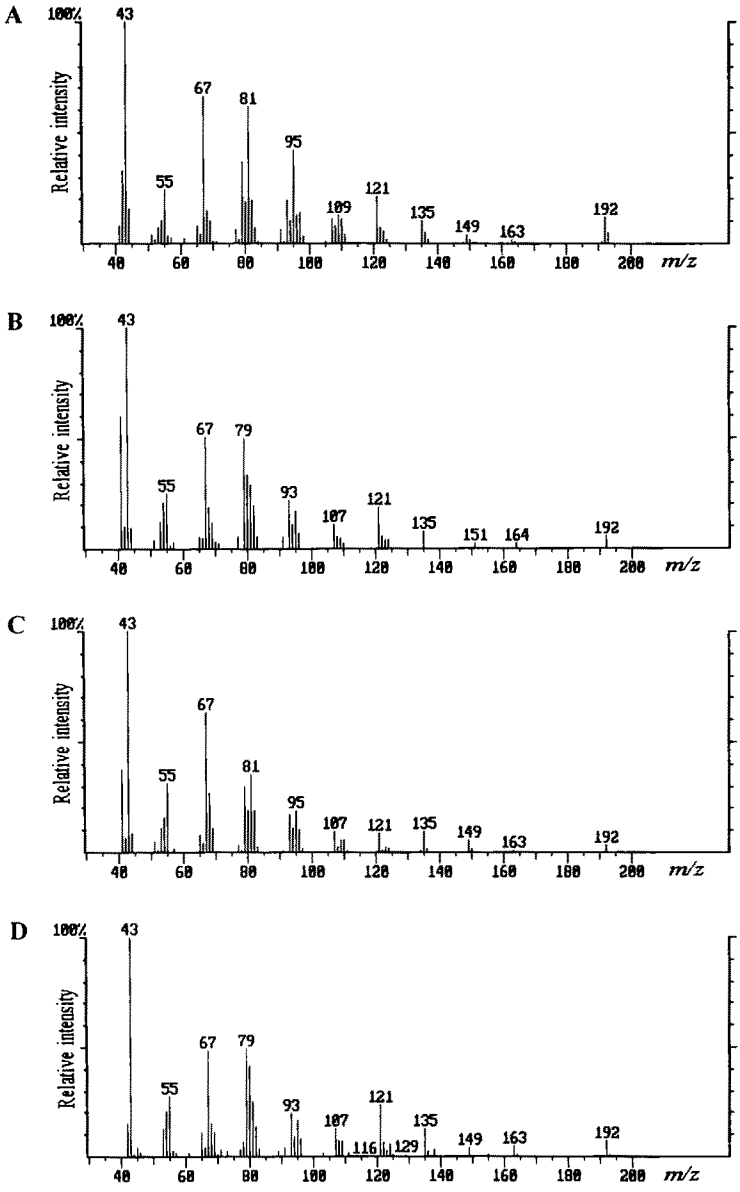


FIG. 3. Ion-trap electron-impact mass spectra of (8*Z*,11*Z*)-8,11-tetradecadien-1-yl acetate (A), (3*E*,8*Z*)-3,8-tetradecadien-1-yl acetate (B), (3*E*,11*Z*)-3,11-tetradecadien-1-yl acetate (C), and the diene acetate from *S. absoluta* (D).

spectrum of synthetic (3*E*,8*Z*)-3,8-tetradecadien-1-yl acetate (**3**) is congruent with that of the natural product. The relative intensities of peaks centered near *m/z* 79 and 91 were particularly useful for characterization of each isomer.

Behavioral activity released by the three synthetic dienes (**2–4**), and by (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrien-1-yl acetate (**1**) was studied in a wind-tunnel bioassay. The diene acetates, although they released some wing-fanning behavior, were essentially inactive, since none of the test insects landed at the source (Figure 4). On the other hand, (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrien-1-yl acetate, the major component of the pheromone, is active by itself, as observed in our previous experiments (Attygalle et al., 1995; 1996). Under the test conditions used in the present study, 53% of test males ($N = 30$) landed at the source baited only with the triene acetate, while three calling females could induce

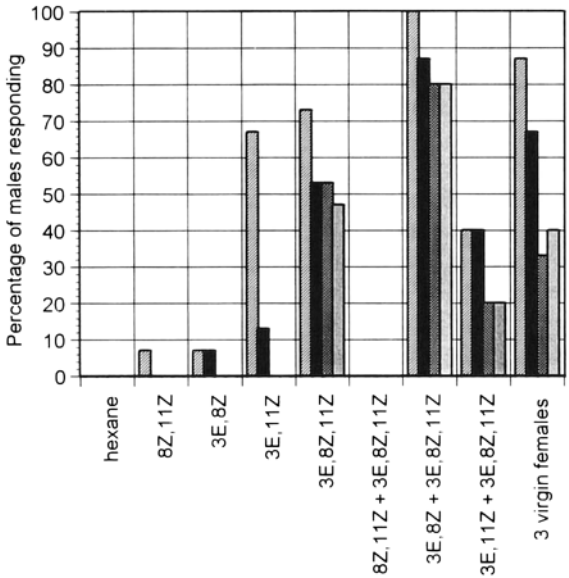


FIG. 4. Percentage of male moths responding in a wind-tunnel behavioral bioassay. Hatched, black, cross-hatched, and stippled bars represent wing fanning, oriented flight, landing at the source, and courtship behavior, respectively. Behavior of 30 males was observed for each test stimulus. Hexane (100 μl) and three calling females were used as control stimuli. Samples of (8*Z*,11*Z*)-, (3*E*,8*Z*)-, and (3*E*,11*Z*)-tetradecadien-1-yl acetate, and (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrien-1-yl acetate were tested individually at the 100-ng level. Binary mixtures contained 10 ng of a diene acetate (**2–4**) and 100 ng of the triene acetate (**1**).

only 33% of the test males to behave similarly. However, the activity released by the binary mixture made of (3*E*,8*Z*)-3,8-tetradecadien-1-yl acetate (**3**) and (3*E*,8*Z*,11*Z*)-3,8,11-tetradecatrien-1-yl acetate (**1**) was significantly better (chi-square analysis, $P < 0.001$) than that of the triene acetate alone (Figure 4). This mixture induced 80% of the males to land at the source and show courtship behavior. Interestingly, the activity of the triene acetate is inhibited dramatically by the presence of (8*Z*,11*Z*)-8,11-tetradecadien-1-yl acetate (**2**). The potency of (8*Z*,11*Z*)-8,11-tetradecadien-1-yl acetate as an inhibitor is unequivocal; a binary mixture (10:1 of **1** and **2**) completely failed even to release wing-fanning behavior.

Over 400 compounds have been characterized as constituents of lepidopteran female sex pheromones (Arn et al., 1992; Mayer and McLaughlin, 1991). However, acetates **1** and **3** of *S. absoluta* have not been identified from any other insect. Although our two-component synthetic mixture was more attractive to *S. absoluta* males than the triene acetate alone, it was clear that *S. absoluta* is far less sensitive to the absence of the minor component than most other Lepidoptera that are characteristically highly sensitive to small qualitative or even quantitative changes in the composition of pheromone blends. As we gather more and more data on the composition of lepidopteran pheromones, it will be interesting to see whether moths using structurally less complicated chemicals as pheromones, such as (*Z*)-11-tetradecenyl acetate (a simple ester shared by many species of Lepidoptera as a component of pheromone blends), are more sensitive to qualitative and quantitative variations in composition than are those using less common, more "information-rich" structures.

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