Synthesis and Field Tests of Sex Pheromone Components of the Leafroller *Argyrotaenia sphaleropa*

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Z. Naturforsch. **59 c**, 708–712 (2004); received April 29/June 1, 2004

Female pheromone glands of the leafroller *Argyrotaenia sphaleropa* were analyzed. Two acetates were identified as (11*Z*,13)-tetradecadien-1-yl acetate and (11*Z*)-tetradecen-1-yl acetate by comparison with synthesized references. The (11*Z*,13)-tetradecadien-1-yl acetate and the aldehyde (11*Z*,13)-tetradecadienal were synthesized via a Wittig reaction. A field-trapping test showed that a lure consisting of a mixture of (11*Z*,13)-tetradecadienal and (11*Z*,13)-tetradecadien-1-yl acetate in a 10:1-ratio produced the highest trap catches.

Key words: (11*Z*,13)-Tetradecadienal, (11*Z*,13)-Tetradecadien-1-yl Acetate, Lepidoptera

Introduction

The leafroller *Argyrotaenia sphaleropa* (Meyrick) (Lepidoptera, Tortricidae) is an important pest of temperate fruit and vine in Brazil and Uruguay. There is a need for an environmentally safe method to control this insect pest. An efficient pheromone lure could be used for population monitoring and for control by mating disruption to reduce the use of insecticides.

Chemical analysis of *A. sphaleropa* pheromone glands has shown the presence of a mixture of (11*Z*)-tetradecenal, (11*Z*,13)-tetradecadienal (**6**), (11*Z*)-tetradecen-1-yl acetate and (11*Z*,-13)-tetradecadien-1-yl acetate (**4**) in the ratio of 1:4:10:40 (Nunez *et al.*, 2002). Previous synthesis of the (11*E*,13)-tetradecadien-1-yl acetate and of the (11*E*,13)-tetradecadien-1-ol has been reported by Bestmann (Bestmann *et al.*, 1981), where the key step in the synthesis was a Wittig reaction between the (*E*)-conjugated aldehyde AcO-$(CH_2)_{10}$-CH=CH-CHO and the ylide $H_2C{=}PPh_3$.

We here report new short and facile syntheses of compounds **4** and **6**. These compounds were synthesized using the inexpensive 11-bromo-1-undecanol (**1**) as starting material. The diene acetate **4** and the dienal **6** have been tested both as single sex pheromone attractants and as two-component lures in apple orchards in South Brazil. The compounds **4** and **6** were also tested in combinations with the monoenes (11*Z*)-tetradecen-1-yl acetate and (11*Z*)-tetradecenal in search for an optimized lure composition.

Results and Discussion

Synthesis and purification of pheromone components

11-Bromo-1-undecanol (**1**) was treated with acetic anhydride in the presence of pyridine to give the corresponding acetate **2**. This acetate was transformed to the phosphonium salt **3** after reaction with PPh_3. A Wittig reaction between **3** and the α,β-unsaturated aldehyde acrolein in the presence of the base $NaN[Si(Me)_3]_2$ gave the (*Z*)-isomer **4**, as the major product. The dienic acetate **4** was solvolyzed to the alcohol **5** by treatment with KOH in MeOH. Finally, oxidation of **5** with pyridinium dichromate (PDC) gave the aldehyde **6**. The synthetic pathway is shown in Fig. 1. The isomeric purity of the compounds **4**, **5** and **6** was increased to < 99.9% by MPLC (medium pressure liquid chromatography) (Baeckström *et al.*, 1987) using silica gel containing silver nitrate (10%).

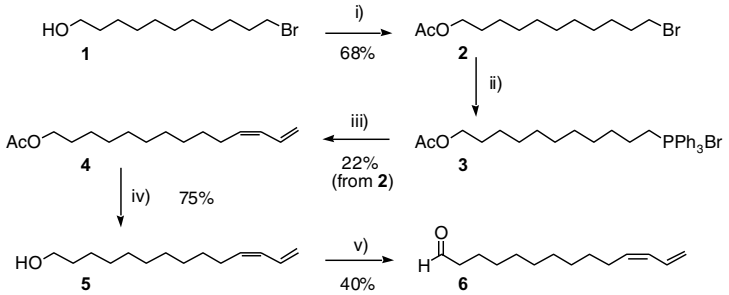

Fig. 1. Synthesis of (11*Z*,13)-tetradecadien-1-yl acetate (**4**) and (11*Z*,13)-tetradecadienal (**6**). Reactions conditions: i) pyridine, acetic anhydride, 0 °C to room temperature (RT); ii) PPh_3, CH_3CN, reflux; iii) $NaN[Si(Me)_3]_2$, THF, reflux then acrolein, – 78 °C to RT; iv) KOH, methanol, 0 °C to RT; v) PDC, CH_2Cl_2, 0 °C to RT.

Gland analysis

Female pheromone gland extracts showed the presence of a diene and a monoene acetate in a 9:1 w/w ratio. The compounds were identified as (11*Z*,13)-tetradecadien-1-yl acetate (*Z*11,13–14Ac) and (11*Z*)-tetradecen-1-yl acetate (*Z*11–14Ac) in comparison with the synthesized references. No aldehyde components were detectable by mass spectroscopy (with a lower threshold for detection of 50 pg/gland).

Field trapping

When tested as single components, neither *Z*11,13–14Ac (**4**) nor (11*Z*,13)-tetradecadienal (*Z*11,13–14Al) (**6**) attracted significant numbers of male moths. Adding the monoenes *Z*11–14Ac to **4**, and (11*Z*)-tetradecenal (*Z*11–14Al) to **6** did not augment male attraction (Table I). However, blends of *Z*11,13–14Ac (**4**) and *Z*11,13–14Al (**6**) captured *A. sphaleropa* males, and the 10:1-blend was significantly more attractive than a blend in the inverse ratio, *i.e.* 1:10.

Table I: Field trapping of *A. sphaleropa* males with synthetic pheromone compounds in Bento Gonçavles, RS, Brazil.

Compound	Trap lure compositions [μg/trap] A	B	C	D	E	F
*Z*11,13–14Al	100		100	10	100	
*Z*11,13–14Ac		100	10	100		100
*Z*11–14Al					10	
*Z*11–14Ac						10
Males/trap lure	2c	1c	43a	17b	0c	1c

The A lure consisted of 100 μg of *Z*11,13–14Al, the B lure consisted of 100 μg of *Z*11,13–14Ac, the C lure consisted of 100 μg of *Z*11,13–14Al and 10 μg of *Z*11,13–14Ac, etc. Trap catches (total number of males caught per trap lure during the experiment) followed by the same letter are not significantly different (Tukey test, $P > 0.05$).

Our results are in contrast with the trap tests carried out by Nunez *et al.* (2002), in which it was found that both *Z*11,13–14Al alone and a blend of *Z*11,13–14Al and *Z*11–14Al attracted *A. sphaleropa* males.

The trap test data shown in Table I indicates that there is a discrepancy between the pheromone gland content and gland emission in *A. sphaleropa* females. An explanation could be that *A. sphaleropa*, similar as *Heliothis virescens* females, produce alcohols in the gland, which are transformed to the corresponding aldehydes by cuticular oxidases on the surface of the gland (Teal *et al.*, 1986). This has not been described in tortricid moths (Witzgall *et al.*, 2004).

Field studies by Bavaresco *et al.* (2004) have shown that several blends of acetate and aldehyde compounds attract *A. sphaleropa* males. Blends of either *Z*11,13–14Ac and *Z*11,13–14Al, or *Z*11,13–14Ac and *Z*11–14Al, or *Z*11,13–14Al and *Z*11–14Ac are equally attractive. It is an intriguing finding that the blend components are interchangeable, provided that the lures contain a dienic 11,13–14 compound, and an aldehyde and acetate, either a monoene or a diene.

Experimental Section

Synthesis: General synthetic methodology

Preparative chromatography was performed on silica gel (Merck 60) or on silica gel impregnated with $AgNO_3$ using cyclohexane/ethyl acetate in a continuous gradient from 0–100% ethyl acetate. The chemical purity of the different products was checked by mass spectroscopy and NMR spectroscopy. ^1H and ^{13}C NMR spectra of $CDCl_3$ solutions were recorded at 250 MHz and 63 MHz, respectively, using a Bruker AC spectrometer.

Chemical shifts were expressed in ppm in relation to $CHCl_3$, followed by numbers of protons, multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; q, quadriplet) and coupling constants (Hz). GC-MS analyses were performed using a Hewlett-Packard instrument, with electron ionization (EI, 70 eV). The isomeric purity was determined by GC-MS.

Acrolein was distilled under inert (N_2) atmosphere before use. The other starting materials employed were purchased from commercial suppliers and used without further purification.

11-Bromo-1-undecyl acetate (**2**): The alcohol **1** (20 g, 80.0 mmol) was dissolved in pyridine (23.2 ml, 286.8 mmol). The reaction mixture was cooled to 0 °C and the acetic anhydride (19.2 ml, 203.5 mmol) was added dropwise. The reaction mixture was stirred at 0 °C for 1 h, then placed in a freezer overnight. After 1.5 h of additional stirring, the mixture was poured into ice-water and extracted with ethyl ether (3 times). The combined organic phases were dried over $MgSO_4$ and the solvent evaporated. The crude acetate was purified by preparative chromatography to give **2** (12.69 g, 68%) as a colorless oil. ^{1}H NMR: δ = 1.15–1.35 (m, 14 H, 7 × CH_2), 1.55 (m, 2 H, CH_2CH_2Br), 1.75 (m, 2 H, CH_2CH_2O), 2.00 (s, 3 H, CH_3), 3.35 (t, 2 H, CH_2Br), 3.9 (t, 2 H, CH_2O). – MS: *m/z* (%) = 232 (1.8) [M^+], 204 (7.4), 190 (2.8), 176 (3.7), 162 (26.1), 148 (46.7), 135 (14.9), 123 (7.4), 111 (20.5), 97 (62.6), 83 (69.1), 69 (76.6), 55 (73.8), 43 (100).

(11-Acetoxy-1-undecyl)-triphenyl phosphonium bromide (**3**): A solution of **2** (8 g, 27.2 mmol) and triphenylphosphine (7.45 g, 28.4 mmol) in dry acetonitrile (16.8 ml) was stirred under reflux for 12 h. After cooling to room temperature, the solvent was evaporated. Some toluene was added and the last traces of acetonitrile were removed by azeotropic distillation. A yellow gel (15.16 g) was obtained and this product was used in the next step without further purification.

(11Z,13)-Tetradecadien-1-yl acetate (**4**): The salt **3** (15.16 g, 27.31 mmol) was dissolved in THF (68 ml) and was the mixture added to a solution of $NaN[Si(Me)_3]_2$ in THF (1 M, 6.01 g, 32.8 mmol, 32.8 ml) under N_2. After reflux for 1 h, the reaction mixture was cooled to – 78 °C and freshly distilled acrolein (3.06 g, 54.64 mmol) dissolved in THF (35 ml) was added slowly. The reaction mixture was stirred for additional 2 h at – 78 °C and 2 h at RT. The reaction mixture was filtrated and the filtrate was poured into a saturated solution of NH_4Cl. The two phases were separated and the aqueous phase was extracted twice with cyclohexane (160 ml). The combined organic phases were dried over $MgSO_4$ and the solvent was evaporated to give a yellow liquid. The crude product was purified by preparative argentum chromatography to give **4** as a colorless liquid (1.53 g, 22% from compound **2**). The isomeric purity was > 99.9%. ^{1}H NMR: δ = 1.27 (m, 14 H, 7 × CH_2), 1.58–1.63 (m, 2 H, CH_2CHCH), 2.03 (s, 3 H, CH_3), 2.13–2.21 (m, 2 H, CH_2CH_2O), 4.04 (t, 2 H, CH_2O), 5.04 5.09 [dd (app. d), 1 H, ^3J = 10.2 Hz, $(C_2H_2)_{trans}CH_{cis}CH_{gem}$H], 5.13 5.19 [dd (app. d), 1 H, ^3J = 16.8 Hz, $(C_2H_2)_{cis}CH_{trans}CH_{gem}$H], 5.39–5.49 [dt (app. q), 1 H, ^3J = 7.9 Hz, ^3J = 10.2 Hz, $CHCHCHCH_2$], 5.94–6.03 [dd (app. t), 1 H, ^3J = 10.9 Hz, ^3J = 10.2 Hz, $CHCHCHCH_2$], 6.55–6.70 [ddd (app. dt), 1 H, ^3J = 10.2 Hz, ^3J = 10.9 Hz, ^3J = 16.8 Hz, $CHCHCHCH_2$]. – ^{13}C{^{1}H} NMR: δ = 20.93, 25.86, 26.87, 27.67, 28.58, 29.19, 29.43, 29.55, 30.14, 32.48 (9 × CH_2 and CH_3CO), 64.59 (CH_2O), 116.58, 129.08, 132.28, 132.96 (all C_{vin}), 171.13 (C=O). – MS: *m/z* (%) = 252 (5.7) [M^+], 209 (1), 192 (12.6), 163 (4.8), 149 (10.2), 135 (20.9), 121 (30.4), 110 (23.3), 95 (47.8), 81 (79), 67 (100), 54 (59), 43 (78.5).

(11Z,13)-Tetradecadien-1-ol (**5**): The acetate **4** (1.53 g, 6.07 mmol) was poured into a solution of KOH in MeOH (10%, w/v, 115 ml) and the reaction mixture was stirred at 0 °C for 1 h and at room temperature overnight. An aqueous saturated solution of NH_4Cl (120 ml) was added to the reaction mixture. After separation of the phases, the aqueous phase was extracted 3 times with cyclohexane. The combined organic phases were dried over $MgSO_4$ and the solvent was evaporated. Purification of the crude product by preparative argentum chromatography gave **5** as a colorless oil (962 mg, 75%). The isomeric purity was > 99.9%. ^{1}H NMR: δ = 1.28 (m, 14 H, 7 × CH_2), 1.53–1.57 (m, 2 H, CH_2CHCH), 2.13–2.21 (m, 2 H, CH_2CH_2O), 3.63 (m, 2 H, CH_2O), 5.05–5.09 [dd (app. d), 1 H, ^3J = 10.2 Hz, $(C_2H_2)_{trans}CH_{cis}CH_{gem}$H], 5.14–5.20 [dd (app. d), 1 H, ^3J = 16.6 Hz, $(C_2H_2)_{cis}CH_{trans}CH_{gem}$H], 5.40–5.50 [dt (app. q), 1 H, ^3J = 7.2 Hz, ^3J = 10.2 Hz, $CHCHCHCH_2$], 5.94–6.03 [dd (app. t), 1 H, ^3J = 11.0 Hz, $CHCHCHCH_2$], 6.56–6.71 (ddd, 1 H, ^3J = 10.2 Hz, ^3J = 11.0 Hz, ^3J = 16.6 Hz, $CHCHCHCH_2$). – ^{13}C{^{1}H} NMR: δ = 25.72, 26.90, 27.72, 29.21, 29.40, 29.50, 29.56, 30.18, 32.80 (9 ×

CH_2), 63.07 (CH_2O), 116.63, 129.10, 132.34, 133.04 (all C_{vin}). – MS: *m/z* (%) = 210 (6.6) [M^+], 192 (1.9), 163 (1.4), 149 (3.3), 135 (9.5), 121 (15.7), 109 (17.1), 95 (47.1), 81 (77.1), 67 (100), 54 (67.1), 41 (50.4).

(11Z,13)-Tetradecadienal (**6**): The alcohol **5** (500 mg, 2.38 mmol) was dissolved in CH_2Cl_2 (15 ml) and the reaction mixture was cooled to 0 °C. PDC (4.48 g, 11.90 mmol) was added in small portions with SiO_2 (2.24 g) and the reaction mixture was stirred overnight (from 0 °C to room temperature). The excess of PDC and SiO_2 was filtered and new SiO_2 (2.24 g) was added. The impregnated silica gel was dried and submitted to preparative argentum chromatography to give **6** as a colorless oil (200 mg, 40%). The isomeric purity was > 99.9%. ^{1}H NMR: δ = 1.27 (m, 14 H, 7 × CH_2), 1.58–1.64 (m, 2 H, CH_2CHCH), 2.37–2.43 (dt, 2 H, ^3J = 1.9 Hz, ^3J = 7.5 Hz, CH_2CHO), 5.04–5.08 [dd (app. d), 1 H, ^3J = 10.0 Hz, $(C_2H_2)_{trans}$-$CH_{cis}CH_{gem}$H], 5.12–5.20 [dd (app. d), 1 H, ^3J = 16.9 Hz, $(C_2H_2)_{cis}CH_{trans}CH_{gem}$H], 5.38–5.49 [dt (app. q), 1 H, ^3J = 7.5 Hz, ^3J = 10.0 Hz, CH$CHCHCH_2$], 5.93–6.02 [dd (app. t), 1 H, ^3J = 11.0 Hz, CHCH$CHCH_2$], 6.54–6.67 (ddd, 1 H, ^3J = 10.0 Hz, ^3J = 11.0 Hz, ^3J = 16.9 Hz, $CHCH$CHCH_2), 9.75 (t, 1 H, ^3J = 1.9 Hz, CHO). – ^{13}C{^{1}H} NMR: δ = 22.03, 27.66, 29.10, 29.27, 29.29, 29.31, 29.51, 30.15, 43.85 (all CH_2), 116.62, 129.09, 132.30, 132.96 (all C_{vin}), 202.88 (C=O). – MS: *m/z* (%) = 208 (9.5) [M^+], 179 (1.9), 165 (3.3), 151 (3.8), 135 (5.7), 121 (7.1), 109 (16.2), 95 (38.5), 81 (77.1), 67 (100), 54 (72.4), 41 (49.5).

Pheromone gland analysis

Insects were field-collected in peach orchards near Bento Gonçalvez (Rio Grande do Sul, Brazil) and were mass-reared in the laboratory on a semisynthetic agar-based diet, (Mani *et al.*, 1978) under a photoperiod of 14h:10h (L:D) at 22 to 25 °C. Pupae were separated by sex according to the number of abdominal segments. Adult insects were kept in 33 cm by 33 cm by 33 cm glass cages. Pheromone glands were dissected from the abdominal tips of 2- to 3-d-old calling virgin females during the first 3 h of the scotophase and were extracted in batches of 30 to 50 (n = 5) in 7 μl of redistilled heptane.

Chemical analysis

Gland extracts were prepared and analyzed according to standard procedures (Cichon *et al.*, 2004). Freshly prepared extracts from 25 female glands (N = 3) were analyzed using a Hewlett-Packard 5970B (Hewlett-Packard, Palo Alto, CA) mass spectrometer (MS) with electron impact ionization (70 eV), interfaced with a Hewlett-Packard 5890 gas chromatograph (GC), equipped with a polar DB-WAX column (30 m x 0.25 mm; J & W Scientific, Folsom, CA, USA). Retention times of the identified gland compounds were compared with synthetic compounds on a Hewlett-Packard 5890 GC, with flame ionization detection, on a DB-WAX column and on a nonpolar SE-54 column (25 m x 0.32 mm; Kupper & Co., Bonaduz, Switzerland). The column temperature was programmed from 80 °C (2 min hold) at 10 °C/min to 220 °C (10 min hold).

Field tests

For field trapping tests, synthetic pheromone compounds in hexane solution were formulated on grey rubber septa (ABS, Dietikon, Switzerland). Tetra traps (PheroNet, Lund, Sweden) baited with blends of synthetic compounds were hung at eye level from green grape branches at Bento Gonçalves, RS, Brazil. Traps within one replicate were 5 m apart, and were inspected once a week for six weeks. Chemical and isomeric purity of commercial compounds (Pherobank, Research Institute for Plant Protection, Wageningen, The Netherlands) were > 99.6%. The number of males trapped was transformed to log(x + 1) and subjected to an analysis of variance (ANOVA), followed by a Tukey test. The significance level was set to 0.05.

Acknowledgement

The authors thank University of Kalmar for financial support. We thank Mr Odimar Zanardi, Embrapa, for help with the field experiments and Professor Ian Nicholls (University of Kalmar) for linguistic advice.

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