CHEMICAL CHARACTERIZATION AND SYNTHESIS OF THE MAJOR COMPONENT OF THE SEX PHEROMONE OF THE SUGARCANE BORER *Diatraea saccharalis*

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(9*Z*,11*E*)-Hexadeca-9,11-dienal (**1**) was identified as a major component of the sex pheromone of the sugarcane borer *Diatraea saccharalis*. The identification is based on mass spectral examination of the extracts prepared from calling females, derivatization experiments with 4-methyl-3,5-dihydro-1,2,4-triazole-3,5-dione (**2**), stereospecific synthesis of dienal **1**, and gas chromatography (GC) with electroantennographic detection (GC-EAD). In GC analysis, the antennaly active component and synthetic dienal **1** show identical retention times on several GC phases. However, the behavioral activity of synthetic **1** was much weaker than that of natural extracts. This suggests that an additional component(s) might be involved in sexual communication of this species.

Keywords: Sex pheromones; *Diatraea saccharalis*; Isolation and identification; Conjugated dienals; Aldehydes; Dienes; Stereoselective synthesis.

Many species of Lepidoptera have developed a remarkably sensitive communication system between the sexes where males can respond to minute amounts of a pheromone produced by virgin females[1]. To date, *ca* 500 Lepidopteran sex pheromones have been characterized chemically and synthesized[2]. In the sugarcane borer *Diatraea saccharalis* Fabricius (Lepidoptera, Pyralidae, Crambinae) the sex pheromone was assigned as (9*Z*,11*E*)-hexadeca-9,11-dienal (**1**) by Hammond in a short conference abstract[3] and a patent application[4] in 1982. However, the described compound has not been

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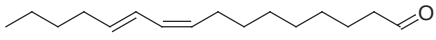
found to be highly attractive for conspecific males in field experiments[5]. These negative results raise doubts about the correct characterization of the sugarcane borer sex pheromone.



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Here we present, for the first time, firm structural evidence, supported by biological experiments, which confirm the original findings[3,4] and we discuss the possible reasons for the low attractiveness of dienal **1** for conspecific males in field experiments.

EXPERIMENTAL

Sample Preparation and Extraction

Laboratory reared *D. saccharalis* calling virgin females (1 to 2 days old) were cooled to –20 °C for 5 min. Female 7th and 8th abdominal segments were excised and washed in hexane for 30 s (*ca* 10 µl per ovipositor, vial with solvent was kept on dry ice) or extracted with dichloromethane at room temperature (10 µl per ovipositor). Thus 10 µl of extracts represent one female equivalent (FE). Extracts were stored at –20 °C.

Preparation of Diels–Alder Adducts[6] (**3**)

A hexane extract of calling females prepared above (100 µl, 10 FE) was treated with a dichloromethane solution of dione **2** (5% w/v, 50 µl) at room temperature for 0.5 h. The resulting violet solution containing **3** was concentrated to a minimum volume in a stream of argon, reconstituted in hexane (5 µl) and 1–2 µl of the solution was injected on GC-MS instrument.

The dichloromethane extract (100 µl, 10 FE) was gradually treated with acetic anhydride (10 µl) and pyridine (10 µl) and after 1 h at room temperature, the obtained solution was concentrated to minimum volume in a stream of argon and treated with a dichloromethane solution of dione **2** (5%, 50 µl) at room temperature for 0.5 h. The obtained solution containing **3** was concentrated to minimum volume in a stream of argon, reconstituted in hexane (5 µl) and 1–2 µl of the solution was injected on GC-MS instrument.

GC-EAD (ref.[7])

Gas chromatographic analysis coupled with electroantennographic detection (GC-EAD) was performed on a Hewlett–Packard HP 5890 gas chromatograph equipped with BPX5 column (30 m × 0.33 mm, i.d.). For EAD detection, the isolated antennal preparation was used. The excised male antenna was placed between two glass Ag/AgCl electrodes filled with insect haemolymph saline. The column was split at the end by a four-arm splitter (Graphpack 3D/2, U.S.A.). The second arm of the splitter was connected to flame ionisation detector (FID), by means of deactivated silica column (0.5 m × 0.33 mm, i.d.). The third arm (0.5 m)

was led through heated transfer line (Effluent Conditioning Assembly, Syntech, Hilversum, The Netherlands) outside the GC and ended in a mixing chamber, from which a continual air stream (1 l min^{-1}) blew onto the antennal preparation *via* PTFE-coated stainless steel flow tube. Through the fourth arm, a make-up gas (N_2, 20 ml min^{-1}) was introduced into the splitter. The split ratio FID/EAD was set to 1 : 1. The capillary column transfer tube was heated up to 180 °C. Approximately 2 μl of concentrated extract (5 to 10 FE) were injected splittless and analysed under a temperature program: The temperature program started at 50 °C held for 2 min, then the temperature of the oven was increased to 140 °C (rate 50 °C min^{-1}), to 240 °C (rate 5 °C min^{-1}) and then to 280 °C (rate 10 °C min^{-1}; 5 min delay at 280 °C). Injector and detector temperature was set at 200 and 290 °C, respectively.

Wind Tunnel Bioassay[8]

The biological activity of extracts and synthetic chemicals was evaluated in wind tunnel. The experiments were performed during 6–7 h of the scotophase. Males of *D. saccharalis* (1–2 days old) were used. Males (pre-conditioned in the laboratory for 2 h) were released individually into an odor plume created by pinning the filter paper disc with test odor onto a holder placed centrally at the upwind end of the wind tunnel (air velocity was 0.2 m s^{-1}). Males were observed during a 5-min period. Male behavior was categorized: 0, no response; 1, activation on starting platform; 2, take-off from the platform; 3, oriented flight towards the odor source; 4, source location; 5, landing on the source and attempted copulation. The percentage of males exhibiting a particular behavior was evaluated. In each experiment, males were allowed to respond to hexane extracts (3 FE) and to a test stimulus. Altogether 25 males were flown in 5 replicates for each treatment.

Chemical Synthesis

Spectral methods. NMR spectra were determined in $CDCl_3$ solutions on a Varian UNITY-500 spectrometer operating at 499.5 MHz for ^1H and at 125.7 MHz for ^{13}C NMR, respectively. Chemical shifts are expressed in ppm (δ-scale) relative to tetramethylsilane for ^1H and relative to $CDCl_3$ signal (77.00 ppm) for ^{13}C NMR, respectively. Coupling constants (*J*) are reported in Hz.

Chromatography. GC analyses were performed on a Hewlett–Packard HP 6890 gas chromatograph equipped with a FID detector (290 °C), split/splitless injection port (200 °C), electronic pressure control (EPC) and HP 6890 automatic injector. A Hewlett–Packard HP-5 capillary column (30 m × 0.32 mm; film thickness 0.25 μm) and helium gas (constant pressure 66 kPa) were used for the separations. The temperature program started at 50 °C held for 2 min, then the temperature of the oven was increased to 140 °C (rate 40 °C min^{-1}) and then to 280 °C (rate 10 °C min^{-1}; 5 min delay at 280 °C). Preparative medium-pressure liquid chromatography (PMPLC) separations were made on Merck 60 silica gel (0.040–0.063 mm) using a Büchi B-680 Prep LC System with ethyl acetate or benzene in hexane. Mobile phase for chromatography of the sensitive conjugated dienic aldehyde **1** was modified with triethylamine (0.3% v/v).

Chemicals. All chemical reactions were run in oven-dried glassware under an argon atmosphere. Tetrahydrofuran (THF), hexane and benzene were distilled from sodium benzophenone ketyl in argon atmosphere. Dichloromethane was distilled from calcium hydride and stored over molecular sieves. All other chemicals were used as purchased. NMR spectra and

elemental analyses of all synthesized compounds were fully consistent with the proposed structures. 10-Tetrahydropyranyloxydec-1-yne (**5**) was previously prepared in our laboratory[9].

(*E*)-1-Iodohex-1-ene[10] (**4**)

A solution of hex-1-yne 3.28 g (40 mmol, 4.58 ml) in dry hexane (20 ml) was treated with diisobutylaluminumhydride solution (1.5 mol l^{-1} in toluene, 30 ml) on ice bath and then the mixture was heated to 55 °C for 5 h, cooled to –55 °C and a solution of iodine (11.16 g, 44 mmol) in dry THF (40 ml) was added during 5 min. After stirring overnight at room temperature the mixture was poured into a separatory funnel containing ice-cold mixture of saturated aqueous solutions of ammonium chloride and sodium thiosulfate (3 : 1, 80 ml). The formed salts were removed on a Celite pad (15 g), which was washed with pentane (3 × 40 ml). The combined extracts were washed with 10% sulfuric acid (30 ml), water (2 × 30 ml) and aqueous sodium chloride solution (30 ml). Solvents were evaporated and the residue was distilled, iodide **4**, b.p.75–78 °C/150 Pa, yield 4.0 g (45%) was obtained in 95% isomeric purity (GC).

(*E*)-16-Tetrahydropyranyloxy-hexadec-9-en-11-yne (**6**)

A suspension of $[Pd(PPh_3)_4]$ (0.17 g, 5 mole %) in dry and deoxygenated benzene (10 ml) in 3-necked round bottom flask purged with argon was treated with (*E*)-1-iodohex-1-ene (**4**; 1.0 g, 4.46 mmol), and after 1 h of stirring at room temperature with CuI (0.1 g, 0.54 mmol) and with a solution of 10-(tetrahydropyranyloxy)dec-1-yne (1 g, 4.42 mmol) in butylamine, freeze-deoxygenated at a reduced pressure (3 ml, 30 mmol). The dark green solution was stirred at room temperature for 16 h (GC control, sample quenched with 2 M HCl–hexane–$MgSO_4$) and the reaction mixture was diluted with ether (10 ml) and quenched with a mixture of ammonia and aqueous ammonium chloride saturated solutions (1 : 2, 10 ml). The organic phase was washed with the ammonia–ammonim chloride mixture (10 ml) and brine (10 ml) and the obtained crude oil was dissolved in hexane (10 ml) and filtered through silica gel/Celite/active charcoal pad. The pad was washed with hexane–ether mixture (5%, 50 ml). The obtained oil (0.70 g) was purified on a silica gel column (100 g) and $AgNO_3$–SiO_2 yielding 0.25 g of the title compound **6** (26%). ^{1}H NMR ($CDCl_3$): 0.98 t, 3 H, J = 7.0 (CH_3CH_2-); 1.24–1.80 m, 18 H (9 × CH_2); 1.50 m, 2 H (CH_3CH_2-); 1.52 m, 2 H (-CH_2CH_2OR); 2.07 dq, 2 H, J = 1.5, 3 × 7.1 (-CH=CHCH_2-); 2.26 dt, 2 H, J = 1.2, 2 × 1.7 (-$CH_2C{\equiv}C$-); 3.40 t, 2 H, J = 6.7 (-CH_2OR); 3.80 m, 2 H (CH_2[6′]); 4.58 bs, 1 H (CH_2[2′]); 5.45 m, 1 H, J = 4 × 1.7, 15.8 (-CH=CH-); 6.03 m, 1 H, J = 4 × 1.7, 15.8 (-CH=CH-). For $C_{21}H_{36}O_2$ (320.5) calculated: 78.70% C, 11.32% H; found: 79.00% C, 11.08% H.

(9*Z*,11*E*)-Hexadeca-9,11-dien-1-ol (**7**)

A solution of cyclohexene (0.6 ml, 6 mmol) in dry THF (3 ml) was treated with a solution of borane–dimethyl sulfide complex in THF (1 mol l^{-1}, 3 ml, 3 mmol) on ice bath for 0.3 h. The mixture was stirred on ice bath for 0.3 h and at room temperature for 2 h. The white slurry was treated with THF solution of **6** (80 mg, 0.25 mmol, 1 ml) on ice bath. The mixture was warmed to room temperature and stirred for 4 h. The formed vinylborane was hydrolyzed with glacial acetic acid (0.2 ml) at 20 °C for 12 h. The reaction mixture was neutralized with NaOH (20%, 0.2 ml) and carefully treated with aqueous H_2O_2 (30%, 0.2 ml). Product was extracted with ether (4 × 5 ml). The crude material was dissolved in methanol

(1 ml) and treated with ion exchange resin (Dowex 50WX, 10 mg) for 5 h. The crude alcohol **7** was purified by chromatography (PMPLC) in hexane yielding dienol **7** (42 mg, 70%) in 95% isomeric purity (GC). ^{1}H NMR ($CDCl_3$): 0.90 t, 3 H, *J* = 7.3 (C**H**$_3$CH$_2$-); 1.24–1.50 m, 12 H (6 × CH_2); 1.41 m, 2 H (CH_3C**H**$_2$-); 1.58 m, 2 H (-C**H**$_2$CH$_2$OH); 2.05 dq, 2 H, *J* = 1.4, 3 × 7.1 (-CH=CHC**H**$_2$-); 2.10 dq, 2 H, *J* = 1.4, 3 × 7.5 (-C**H**$_2$CH=CH-); 3.63 t, 2 H, *J* = 6.5 (-C**H**$_2$OH); 5.30 bdt, 1 H, *J* = 3 × 7.5, 10.8 (-C**H**=CH-CH=); 5.65 dq, 1 H, *J* = 2 × 7.1, 15.1 (=CH-CH=C**H**); 5.94 dtt, 1 H, *J* = 3 × 1.4, 11.0 (-CH=C**H**-CH=); 6.29 ddq, 1 H, *J* = 2 × 1.5, 11.0, 15.1 (=CH-CH=C**H**). HR EI: for $C_{16}H_{30}O$ calculated 238.2297; found 238.2330.

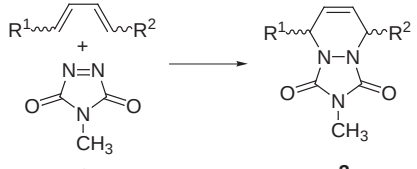
(9*Z*,11*E*)-Hexadeca-9,11-dienal (**1**)

Dienol **7** (20 mg, 0,084 mmol) was injected into a stirred suspension of pyridinium chlorochromate (PCC; 25.8 mg, 0.12 mmol) and anhydrous sodium acetate (2 mg) in dichloromethane (1 ml). The mixture was stirred at room temperature for 90 min, then poured into 50 ml of ether and filtered through combined layer of neutral alumina/charcoal/Celite. Subsequent evaporation of the solvents and PMPLC (0.3% triethylamine in benzene–hexane 1 : 1) afforded 14 mg (70%) of the pure (96%, GC) title compound **1**. ^{1}H NMR ($CDCl_3$): 0.92 t, 3 H, *J* = 7.1 (C**H**$_3$CH$_2$-); 1.24–1.50 m, 12 H (6 × CH_2); 1.41 m, 2 H (CH_3C**H**$_2$-); 2.12 dq, 4 H, *J* = 1.4, 3 × 7.2 (2 × -CH=CHC**H**$_2$-); 2.41 m, 2 H (-C**H**$_2$CHO); 5.35–5.45 m, 2 H (-C**H**=CH-CH=C**H**-); 6.20–6.29 m, 2 H (-CH=C**H**-C**H**=); 9.75 t, 1 H, *J* = 1.8 (-CH=O). EI-MS (*m/z*, rel.%): 236 (12), 135 (8), 109 (15), 96 (35), 81 (76), 79 (39), 67 (100), 55 (29), 54 (28), 41 (43). HR EI: for $C_{16}H_{28}O$ calculated 236.2140, found 236.2117.

RESULTS AND DISCUSSION

The preliminary examinations of hexane extracts of the abdomens of calling sugarcane females using gas chromatography with mass spectroscopic detection (GC-MS) show, beside hydrocarbons and fatty acids methyl esters, one peak possessing a mass spectrum with rather strong molecular ion at *m/z* 236 Da. The observed fragmentation pattern resembled that of a hexadecadienal[11]. In dichloromethane extracts of calling females in addition to aldehyde **1**, the corresponding hexadecadien-1-ol was detected.

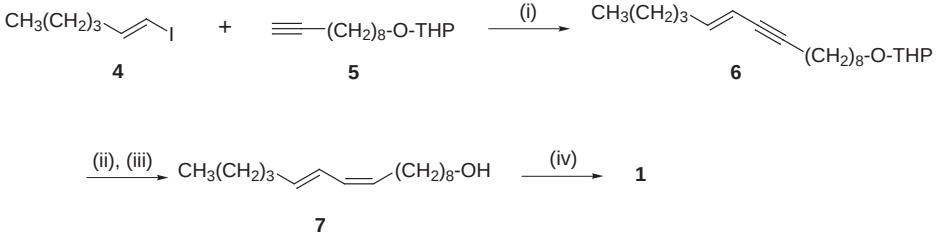
In the mass spectrum of the dienal, a strong molecular ion was observed and it was reasonable to expect[12] that the double bonds are conjugated. For the assignment of the positions of conjugated double bonds, a Diels–Alder reaction of the hexadecadienal with 4-methyl-3,5-dihydro-1,2,4-triazole-3,5-dione (**2**) was performed[6] (Scheme 1).



SCHEME 1

Electron-impact mass spectra of the formed adducts **3** exhibit very characteristic fragmentation patterns, from which the position of carbon–carbon double bonds is directly deducible[6]. When the hexane extract was treated with triazole **2**, the measured mass spectrum shows molecular ion at *m/z* 349 Da and distinct fragment ions at *m/z* 222 and 292. This spectrum was assigned to an adduct of hexadeca-9,11-dienal (Fig. 1A). Additionally, the dichloromethane extract of calling females, presumably containing beside aldehyde **1** its biosynthetic precursor alcohol **7**, was first treated with an acetic anhydride–pyridine mixture to convert the hexadecadien-1-ol to the acetate, and then the reaction mixture was treated with **2**. Again, the 9,11 position of the conjugated carbon–carbon double bonds was clearly assigned (Fig. 1B).

The deduced hexadeca-9,11-dienal can exist as four stereoisomers. Direct spectral characterization of such dienal isomers in a complex matrix is only feasible with a gas-phase Fourier-transform infrared spectroscopy (FTIR) using GC-FTIR instruments at detection limits in the nanogram range[13]. In our case, we dealt with picograms and, therefore, had to rely on less straightforward comparisons of prepared synthetic standards with the natural compound. We have designed a simple synthetic approach[14,15] to prepare aldehyde **1** based on Pd-catalyzed Sonogashira coupling and hydroboration and hydrogenolysis of the formed enyne **6** (Scheme 2).



SCHEME 2

Retention parameters of the synthetic dienal **1** were compared with those of the natural sample on several GC phases (DB1, BPX5, DB WAX) and in all co-injection experiments compound **1** shows identical retention time to the natural hexadeca-9,11-dienal. The MS of synthetic **1** and the hexadeca-9,11-dienal in the hexane extract of calling females were indistinguishable. Amounts of the dienal produced by a calling female were estimated, using a calibration with authentic standard, to 10–50 pg per calling female.

The structural identity of the major component was further supported with electroantennographic measurements performed on a GC–EAD system,

where both hexane extract and synthetic standard **1** showed pronounced antennal activity on the EAD trace, and their retention behavior expressed as Kovats' indices was identical (Fig. 2; KI = 1 842 ± 1 for the hexane extract and 1 843 ± 1 for **1**).

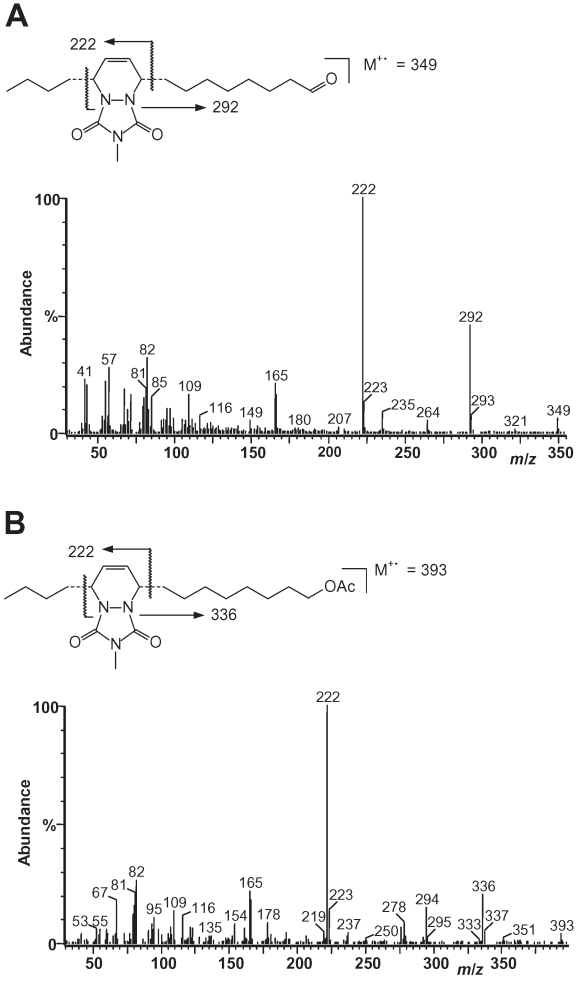


FIG. 1

Spectra of prepared Diels–Alder adducts (**3**) of 4-methyl-3,5-dihydro-1,2,4-triazole-3,5-dione (**2**) with: hexadecadienal in the hexane extract (A) and hexadecadienyl acetate prepared from the dichloromethane extract (B). Both extracts were prepared from calling *D. saccharalis* females

TABLE I

Behavioral reactions of *D. saccharalis* males (n = 25) observed in wind tunnel to hexane extracts of excised pheromone glands expressed as female equivalents (FE) and to different doses of (9*Z*,11*E*)-hexadeca-9,11-dienal (**1**)

No.	Behavioral category	3 FE	1 pg	1 ng	1 μg
0	no response	0	0	0	3
1	activation	25	25	25	22
2	takeoff	25	21	25	20
3	oriented flight	15	11	9	0
4	source location	15	9	1	0
5	landing	15	7	0	0

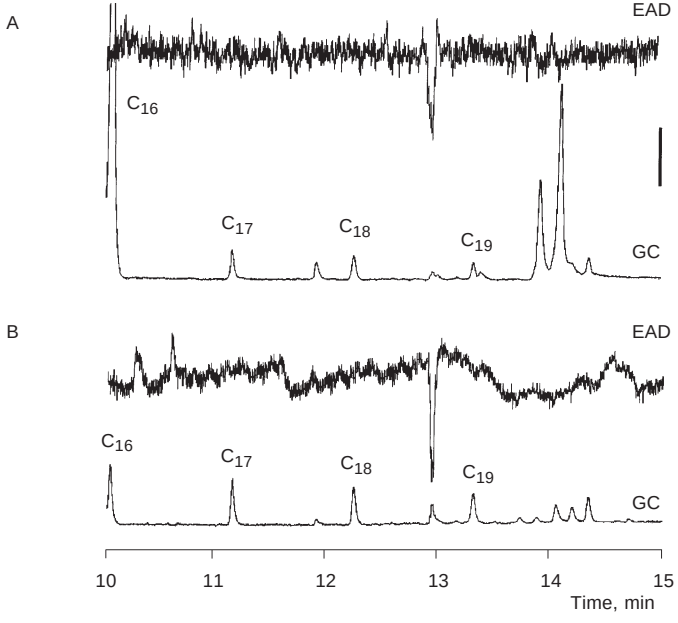


FIG. 2

Sections of GC–EAD–FID traces: hexane extract of *D. saccharalis* female pheromone glands (4 FE) (A) and synthetic (9*Z*,11*E*)-hexadeca-9,11-dienal (**1**) (100 pg) on BPX5 phase (B), both co-injected with hydrocarbon standards. The vertical bar in section a represents 0.2 mV for EAD and 10 mV for FID traces, respectively

In wind tunnel experiments, 1 pg of synthetic **1** displayed high attractiveness for *D. saccharalis* males; however, relatively few males found the source and tried to copulate in comparison with males responding to 3 FE of gland extract (Table I). This observation may indicate that the pheromone produced by females of *D. saccharalis* is very likely a multi-component blend. Our future experiments are aimed towards determination of the missing component(s).

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