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IDENTIFICATION AND BIOASSAY OF SEX PHEROMONE COMPONENTS OF CAROB MOTH, *Ectomyelois ceratoniae* (ZELLER)

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Abstract—Three sex pheromone components of the carob moth were isolated and identified from the extract of female pheromone glands, using a variety of techniques including coupled gas chromatographic–electroantennographic recordings, coupled gas chromatographic–mass spectrometric analysis, microozonolysis, electroantennographic assays of monounsaturated standards, wind-tunnel bioassays, and field trials. The major component was identified as (*Z*,*E*)-9,11,13-tetradecatrienal, a novel lepidopterous pheromone component structure. Two minor components, either one of which improves the upwind flight response of males when blended with the major component, were identified as (*Z*,*E*)-9,11-tetradecadienal, and (*Z*)-9-tetradecenal.

Key Words—Flight tunnel, gas chromatography–electrophysiology, mass

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spectrometry, aldehydes, (*Z*,*E*)-9,11,13-tetradecatrienal, (*Z*,*E*)-9,11-tetradecadienal, (*Z*)-9-tetradecenal, pheromone, Lepidoptera, Pyralidae.

INTRODUCTION

The carob moth, *Ectomyelois ceratoniae*, is a worldwide pest of nuts and fruits, including carobs, almonds, and dates (Gothilf, 1984). It has been expanding its range into new parts of the world. For instance, in the United States this species is a primary pest of dates in the desert valleys of southern California, and it now threatens to move north into the vast almond and pistachio groves of the San Joaquin Valley (Warner, 1988). There is thus an urgent need for a sensitive means of monitoring endemic populations and for detecting new ones, such as is offered by traps with synthetic sex pheromone lures. Opportunities exist also for direct control using mating disruption, because the labor-intensive nature of the crop would allow for application of disruptants at minimal additional labor cost. To provide this needed tool, we undertook to identify the female sex pheromone of this species, and although we provided a brief report on the identification (Baker et al., 1989), the present paper presents more extensive data on the identification and new information about the laboratory and field activity of these compounds.

METHODS AND MATERIALS

Chemical Analysis of Female Pheromone Gland Extract. Moths were obtained from infested dates near Indio in the Coachella Valley of Riverside County, California, in 1985. Larvae were reared on a honey and wheat-bran diet in one-gallon jars in the laboratory (Warner, 1988), the pupae were segregated by sex, and the moths allowed to emerge on a 14:10 light–dark photoperiod regime. The sex pheromone glands of 2- to -5-day-old females were excised into ca. 50 μl of CS_2 in a micro-test-tube during the normal period of female pheromone emission in the fourth through sixth hours of scotophase. The glands from up to 100 females were extracted for 15–45 min, the solvent was recovered with a syringe, and the extract was pooled with other similarly collected samples and stored at −20°C.

Aliquots of the extract, usually 10–20 female equivalents (FE) were injected onto 30-m × 0.25-mm-ID capillary gas chromatographic (GC) columns, coated either with nonpolar DB-1 or polar DB-WAX or DB-225 (J & W Scientific, Folsom, California) for analyses by GC–mass spectrometry (GC-MS) and GC–electroantennography (GC-EAG). Column conditions for the DB-1 and DB-WAX columns were as follows: H_2 carrier gas flow of 1.5 ml/min, injector temperature 250°C, oven temperature program, 2 min at 80°C, then 10°/min to 230°C, and flame ionization detector at 250°C. Conditions for the DB-225 column was: H_2 carrier gas flow of 1.5 ml/min, injector temperature 250°C, oven temperature program 2 min at 80°C, then 15°/min to 180°C, and flame ionization detector at 250°C. GC-MS analyses were performed using a Hewlett-Packard 5890 gas chromatograph with a direct interface to a Hewlett-Packard 5970 mass selective detector (electron impact, 70 eV). All NMR spectra of synthetic samples were obtained with a Bruker WM 400 spectrometer at 400 MHz.

Microozonolysis was performed using an adaptation of the previously published technique (Beroza, 1975). The unknown compound was first collected from the DB-1 column by turning off the flame ionization detector and placing a melting-point tube over the end of the detector jet. A small piece of Dry Ice was touched to the side of the tube so that the effluent would more readily condense onto the glass walls. Ozone was then generated in a 500-ml Ehrlenmeyer flask by introducing oxygen into the flask at ca. 50 ml/min and creating a continuous spark inside the flask with a Tesla coil (high-voltage source) applied to an electrode. The melting point tube then was placed at the outlet of the ozone-flask for no more than 2 sec to expose the adsorbed compound to the ozone, and within ca. 10 sec the tube was rinsed with 5 μl of CS_2 solution. The CS_2 was then removed and injected onto the GC to examine the sample for ozonolysis reaction products, which were identified by comparisons with ozonolysis products from synthetic standards.

Electroantennogram Analysis. To examine antennal sensitivity to geometric and positional isomers, as well as to functional groups, electroantennograms (EAGs) were recorded (Roelofs, 1984) using a series of synthetic standards of acetates and alcohols purchased from Dr. S. Voerman at the Institute for Pesticide Research, Wageningen, The Netherlands. Several 14- and 16-carbon aldehydes were available (Albany International) including tetradecanal, (*Z*)-9-tetradecenal, (*Z*)-9-hexadecenal, (*E*)-9-hexadecenal, (*Z*)-7-hexadecenal, and (*Z*)-11-hexadecenal. Serial dilutions of each compound were made in hexane such that 10 μg were applied to filter paper in 10 μl of solvent, and then the filter paper was placed inside a Pasteur pipet. A male carob moth antenna was placed between two saline-filled glass electrodes, and the signal amplified with a Hansson model 103 amplifier and displayed on a storage oscilloscope. Each sample pipet was inserted into the airstream in a glass tube upwind of the antenna, and 1 ml of air was puffed through the tube from a glass syringe whose plunger was depressed by hand in less than 30 msec; the resulting antennal depolarization was measured on the oscilloscope screen. After every three to four puffs of test compounds, a standard control compound, (*E*)-6-tetradecenyl acetate, was puffed in order to monitor possible deterioration of the preparation.

EAG amplitudes were standardized according to the responses to this internal standard by dividing the amplitude of the EAG generated from the test compound by that from the standard. Within a particular series of isomers having the same chain length and functional group, presentation of the test compounds was randomized.

Simultaneous GC-EAG analysis (Arn et al., 1975) was performed on the DB-1, DB-WAX, and DB-225 30-m × 0.25-mm-ID GC columns (J & W Scientific). Briefly, the effluent was split 1 : 1 so that it entered two 1-m-long deactivated fused silica columns just before they left the GC oven, and make-up gas was added just before the split point to improve splitting and to drive the effluent rapidly through the deactivated columns and to the flame and EAG detectors, respectively. Simultaneous GC and single-cell (GC-SC) analysis (Wadhams, 1984; Van der Pers and Löfstedt, 1983) was performed using the same splitting technique, except that instead of a whole antenna, an electrode was placed over a single sensillum trichodeum (see next section) to record the single neurons' responses to the GC effluent simultaneously with the FID.

Single Cell Antennal Recordings. Recordings from single antennal neurons were made using the cut-sensillum technique of Kaissling (1974; Van der Pers and Den Otter, 1978). An antenna was removed from a male carob moth and its base placed in a saline electrode serving as the ground. A single sensillum trichodeum was then positioned over the edge of a glass knife, and the sensillar tip excised with a second glass knife edge brought down over the first knife edge by means of a micromanipulator. A saline recording electrode was then maneuvered to contact the sensillum and to record from the neurons within after amplifying the signal using a Hansson model 103 amplifier. Recordings of both the AC and DC components of the signal were stored on VCR tape using a Vetter model 420 F four-channel FM recorder and displayed and printed on a Gould model 1604 (DSO) digital oscilloscope/printer.

Wind-Tunnel and Field-Trapping Tests. Behavioral assays were conducted in a 3.5 × 1.0 × 1.0-m wind tunnel previously described in detail by Kuenen and Baker (1982). Two- to five-day-old males were placed in the wind tunnel at least 2 hr before testing in order to acclimate them to the conditions (24–26°C, 0.3 lux, 30–70% relative humidity, 0.5 m/sec wind velocity). Bioassays were conducted 4–6 hr into the scotophase, the optimal sexual activity period for this species. Treatments were loaded onto filter-paper disks (Whatman No. 1) affixed to metal clips 6 cm high complete with metal base. The cork–wire holder for each filter paper was placed on a 15 × 15-cm sheet metal platform 15 cm above the floor of the tunnel, 30 cm from the tunnel's upwind end. Males were released one at a time from screen cages, 4 cm long × 3 cm diameter, 1 m from the odor source and 15 cm above the tunnel floor. The cages were placed on the metal platform in the plume until the male took flight or until 30 sec elapsed. Males were scored for taking flight, locking-on to the plume and progressing upwind, flying to within 50 cm or 10 cm of the source, and contacting the source. Tests were conducted using a randomized, complete-block design so that all treatments were presented on a given day.

In order to see whether or not the reduced attraction with synthetics was due to antagonistic contaminants, we tested the attraction of males to calling females in the wind tunnel compared to calling females plus the 8 : 1 : 1 (333 μg) synthetic blend in polyethylene Beem embedding capsules, size 3 (Ted Pella Inc., Redding, California) (see next section). Six females were placed in a cage in a Pherocon 1C trap just as they were in the field except the trap was placed in the wind tunnel. A second trap was also used, but was loaded with a cage containing six females, to which was also affixed the Beem capsule containing the synthetic blend. Under the same wind-tunnel conditions used in the other tests, 10 males were released downwind and allowed 2 min to approach and enter each trap, presented singly to the males. Males were scored for close approach to the trap (< 10 cm), the duration of hovering in front of the trap, and whether or not they were captured on the sticky surface.

Field-trapping tests were conducted in date gardens in the Coachella Valley near Indio, California, beginning in 1989. Polyethylene Beem capsules, which were left unsealed, were loaded with 333 μg of the 8 : 1 : 1 mixture of synthetic (*Z,E*)-9,11,13-tetradecatrienal, (*Z,E*)-9,11-tetradecadienal, and (*Z*)-9-tetradecenal, and individual capsules were affixed to the bottoms of Pherocon 1C traps. Five virgin female carob moths were placed in screen cages in Pherocon 1C traps as a positive control. They were given continuous access to distilled water on a dental wick. Traps were counted and rebaited twice per week over several weeks. Males were removed and the traps rerandomized whenever counts were taken. Trap bottoms were replaced each week when the Stickem surfaces became coated with dust.

Synthesis. (*Z*)-9-Tetradecenal and (*Z,E*)-9,11-tetradecadienal were prepared from the corresponding alcohols by oxidation with pyridinium dichromate–molecular sieve (Herscovici et al., 1982) and by Swern oxidation (Mancuso et al., 1978). The crude products were purified by HPLC (RP-18; low-pressure gradient; methanol–water) or by flash chromatography on silica gel (5% ether in hexane) followed by Kugelrohr distillation (0.1 mm Hg, oven temperature 100°C). Syntheses of (*Z,E*)-9,11,13-tetradecatrienal have been published elsewhere (Baker et al., 1989; Millar, 1989, 1990).

The trienal was very unstable and usually trimerized or polymerized in the space of a few weeks even at −20°C under Ar, either neat or in dilute solution (hexane, benzene, ether). When freshly prepared, the trienal was >98% stereoisomerically pure by capillary GC (DB-5). However, the trienal was contaminated with 2–5% of a rearrangement product that coeluted with the trienal on

silica gel flash chromatography or HPLC. This rearrangement product has not yet been isolated or fully characterized. The trienal was also thermally unstable and decomposed when subjected to GC injector temperatures above 250°C.

RESULTS

EAGs of Monounsaturated Standards. Standardized EAG responses revealed that male antennae were far more responsive to 14-carbon aldehydes than to other molecules to which they were exposed. Although we had a limited array of aldehydes to test, by far the most active compound was (*Z*)-9-tetradecenal, generating a response 3.8 times (±0.91 SD; $N = 8$) as great as that to the standard compound, (*E*)-6-tetradecenyl acetate. In comparison, the next most active compound, either monounsaturated or saturated, was tetradecanal with a response of 2.3 (±0.45 SD; $N = 8$), followed by (*Z*)-9-tetradecenyl acetate with a response of 2.1 (±0.19 SD; $N = 5$) (Figure 1). Responses to an array of 16-carbon aldehydes, including (*Z*)-11-hexadecenal, hexadecanal, (*E*)-9-hexadecenal, (*Z*)-9-hexadecenal, and (*Z*)-7-hexadecenal all were below a standardized response of 1.3. Thus, the high responses to 14-carbon aldehydes suggested that at least one of the sex pheromone components contained an aldehyde moiety and was 14 carbons in length. Although lower than those of the 14-carbon aldehydes, the EAG responses to the series of 14-carbon acetates (Figure 1) and alcohols, for which complete sets of standards from Δ4 to Δ12 were available, were illuminating, because they highlighted potential involvement of molecules having a (*Z*)-9 configuration and also an (*E*)-11 configuration.

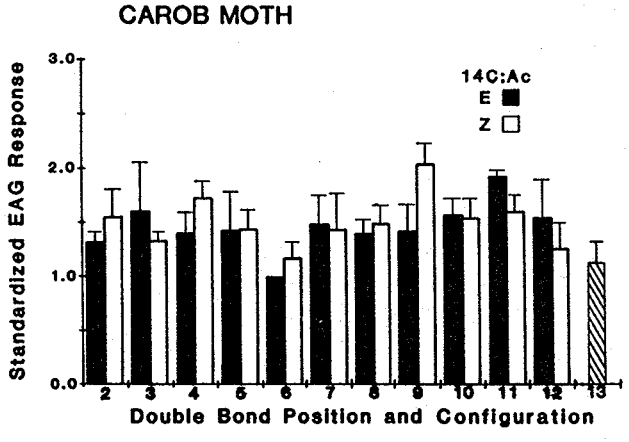

FIG. 1. Standardized EAG amplitudes in response to a series of tetradecenyl acetates ($N = 5$ antennae). Compounds were dispensed at a loading of 10 μg on a filter paper strip and placed in Pasteur pipets. One milliliter of air was puffed through each pipet into an airstream and over the male antenna, the series of compounds being tested in a randomized, complete-block design. The standard response was the millivolt response to a test compound divided by the millivolt response to the most recent puff of (*E*)-6-tetradecenyl acetate, such puffs being interspersed with the test puffs every three to four compounds.

Analysis of Pheromone-Gland Extract. Combined GC-EAG analysis of the extract from ca. 10 FE of pheromone-gland extract consistently revealed three peaks from the FID corresponding to EAG activity on either the DB-1 or DB-WAX columns in GC-EAG trials (Figures 2 and 3, respectively). These three peaks were labeled compounds I, II, and III; the order of elution, based on the amplitude of the EAG's, was the same on the two columns, with a compound corresponding to a small EAG peak eluting first, a compound evoking a larger EAG response eluting second, and the compound with the largest EAG response eluting last (Figures 2 and 3). The compounds were present in a ratio of 8:1:1

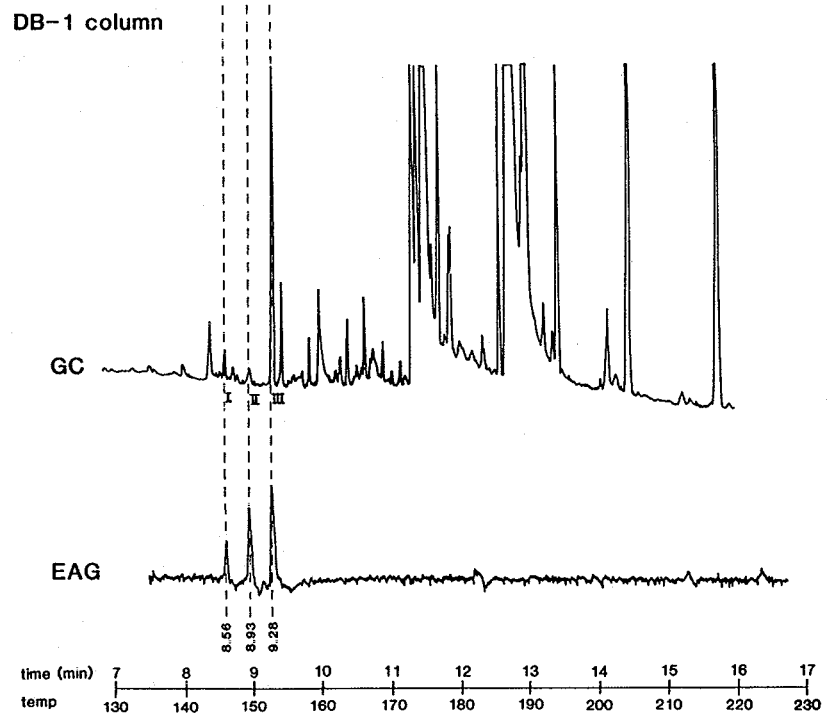

FIG. 2. GC-EAG response to 10 FE of female carob moth sex pheromone gland extract injected onto a 30-m DB-1 capillary GC column. The EAG-active peaks at 8.56, 8.93, and 9.28 min were labeled compounds, I, II, and III, respectively.

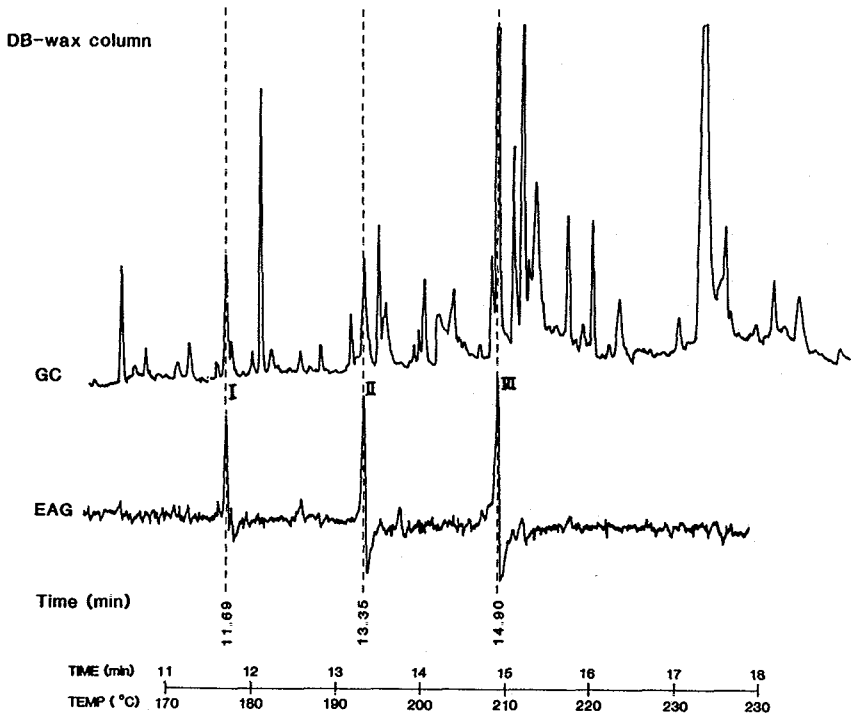

FIG. 3. GC-EAG response to 10 FE of female carob moth sex pheromone gland extract injected onto a 30-m DB-WAX capillary GC column. The EAG-active peaks at 11.69, 13.35, and 14.90 min were labeled compounds I, II, and III, respectively.

(III:II:I). The retention time of compound I corresponded precisely to that of (*Z*)-9-tetradecenal on both columns. The retention indices of compounds II and III relative to I were 1.043 and 1.048 on DB-1 and 1.142 and 1.274 on DB-WAX, respectively. The retention indices of II and III on the two columns relative to (*Z*)-9-tetradecenal and to the other compounds in our library indicated that compounds II and III possibly involved conjugated double bonds since nonconjugated systems elute very close to monounsaturated compounds (c.f., Heath and Tumlinson, 1984). Since the pattern of EAG responses to monounsaturated standards indicated that two configurations evoked high activity [(*Z*)-9 and (*E*)-11] and only one GC-EAG active peak was present in the monounsaturated region on GC in the extract, we reasoned that in at least one of the compounds, (either II or III), a (*Z*,*E*)-9,11 dienal would be involved.

GC-MS analysis of compound I revealed a mass spectrum identical to that of a synthetic standard of (*Z*)-9-tetradecenal. The mass spectrum of compound II showed a base peak at *m/z* 67 plus a significant signal at *m/z* 208, consistent

with the molecular ion of a tetradecadienal (Figure 4A). Conjugated double bonds stabilize the molecular ion in long-chain aliphatic compounds, whereas M+ is virtually missing in monounsaturated analogs or analogs with isolated double bonds (Löfstedt and Odham, 1984; Ando et al., 1988). Compound III had a mass spectrum characterized by a base peak at *m/z* 79 and an intense signal at *m/z* 206 (Figure 4B), suggesting a tetradecatrienal or a tetradecenynal with at least some conjugation.

Ozonolysis of the fraction of female-gland extract containing compound III, collected from the DB-1 column at 9.1–9.4 min, yielded a new peak at 6.70 min, which upon comparison with the bifunctional ozonolysis product of synthetic (*Z*)-9-tetradecenal proved to be 1,9-octanedial. This showed that the first double bond in compound III was at the C9 position and that any other double bonds must occur farther out on the alkyl chain. Excluding allenes and enynes, this meant that the other two double bonds could only be in the 11 and 13 positions. We thus suspected that compound III was (*Z*,*E*)-9,11,13-tetradecatrienal and that compound II was (*Z*,*E*)-9,11-tetradecadienal.

Synthetic II and III. The mass spectrum of synthetic (*Z*,*E*)-9,11-tetradecadienal was identical to that of the natural compound II from the extract (Figure 4A). Moreover, combined GC-EAG recordings on both the 30-m DB-WAX and DB-225 columns demonstrated that (*Z*,*E*)-9,11-tetradecadienal had a retention time identical to compound II from the extract and that was EAG active, while the *E*,*E* isomer eluted later and was relatively inactive. Likewise, the retention time of (*Z*,*E*)-9,11,13-tetradecatrienal was identical to that of nat-

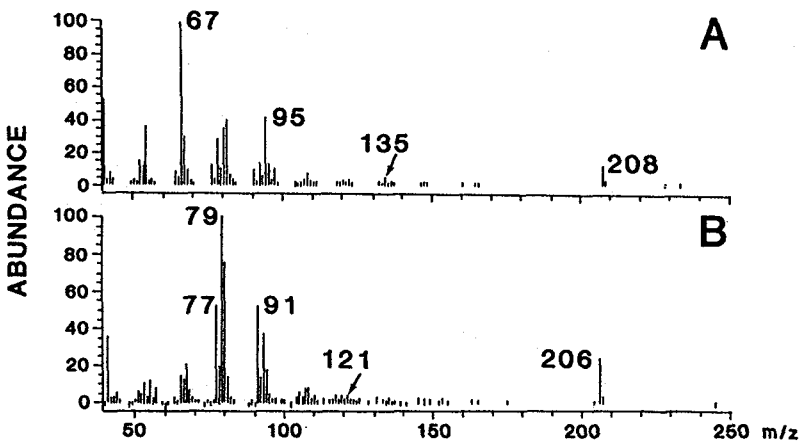

FIG. 4. (A) Mass spectrum (EI 70 eV) of compound II, identical to that of (*Z*,*E*)-9,11-tetradecatrienal. (B) Mass spectrum (EI 70 eV) of compound III, identical to that of (*Z*,*E*)-9,11,13-tetradecatrienal.

ural compound III, and it was EAG-active, whereas the *E,E* isomer eluted later and was relatively inactive on EAG. The mass spectrum of compound III was identical to that of synthetic (*Z,E*)-9,11,13-tetradecatrienal (Figure 4B), confirming the structure of III to be (*Z,E*)-9,11,13-tetradecatrienal.

Although the evidence strongly indicated that compound I was (*Z*)-9-tetradecenal, current knowledge of pheromonal biosynthetic pathways in the Lepidoptera allowed for the alternative possibility that compound I could be unsaturated in the 11 position. This possibility is enhanced when considering that the retention time of (*E*)-11-tetradecenal would likely be very similar to (*Z*)-9-tetradecenal on most columns, and it would exhibit a virtually identical mass spectrum. A small amount of (*E*)-11-tetradecenal was therefore synthesized from (*E*)-11-tetradecenol, and it consistently had a retention time of 9.77 min on the DB-225 column compared to 9.86 min for (*Z*)-9-tetradecenal. The natural extract exhibited no FID peak at 9.77 min and no EAG activity at that elution time in two different GC-EAG analyses on DB-225, whereas there was a small FID peak at 9.87 min plus strong EAG activity at 9.86 min during the same GC-EAG analyses of this extract. These results, coupled with the earlier GC-MS and GC-EAG analyses on the two other columns again strongly support (*Z*)-9-tetradecenal as the structure of compound I.

As was the case for compound I, for which no ozonolysis results could be obtained, possible double bond positions and geometries different from the indicated (*Z,E*)-9,11-tetradecadienal existed also for compound II. However, the same GC-EAG analyses of the natural extract on the DB-225 column revealed a small peak at 11.20 min with correspondingly strong EAG activity at 11.20 min and no other EAG activity except for the usual single peaks corresponding to compounds I and III. Synthetic (*Z,E*)-9,11-tetradecadienal exhibited a retention time of 11.19 min on this same column. These results, coupled with the earlier GC-MS and GC-EAG analyses on the two other columns again strongly support our assignment of (*Z,E*)-9,11-tetradecadienal as the structure of compound II.

GC–single-cell recordings using synthetic I, II, and III confirmed that the retention times and single-cell activity corresponded to (*Z*)-9-tetradecenal, (*Z,E*)-9,11-tetradecadienal, and (*Z,E*)-9,11,13-tetradecatrienal, respectively (Figure 5). Moreover, the monoenal and dienal both caused a large-spike-amplitude cell to fire in some of the sensilla, whereas the trienal caused a smaller-spike-amplitude cell in the same hair to fire. The fact that the trienal excites one kind of receptor cell and the monoenal and dienal excite a second type of cell is consistent with the behavioral data (below) that indicates that the trienal alone is not as active behaviorally as a blend of the trienal plus either I or II.

Wind-Tunnel and Field-Trapping Studies. The activity of synthetic compound III in evoking upwind flight by carob moth males was confirmed in wind-

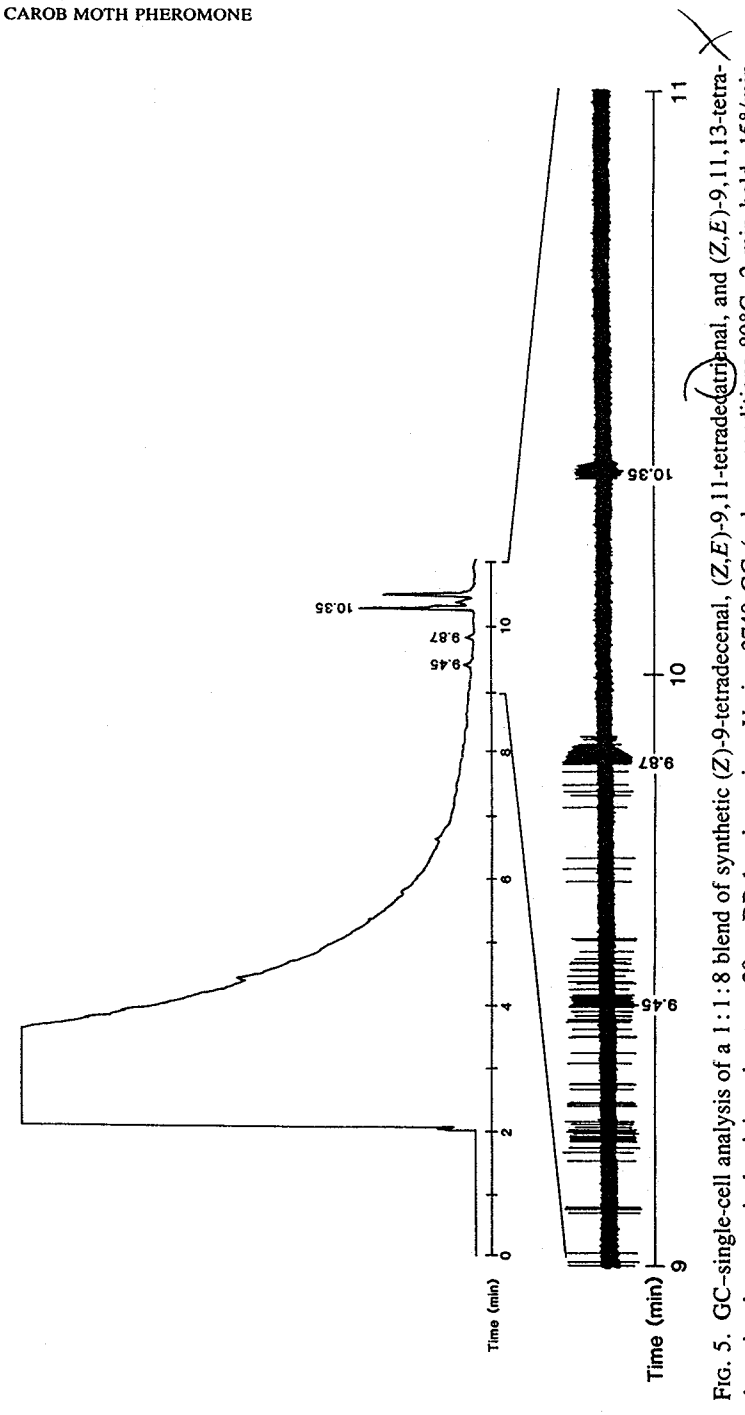

FIG. 5. GC–single-cell analysis of a 1:1:8 blend of synthetic (*Z*)-9-tetradecenal, (*Z,E*)-9,11-tetradecadienal, and (*Z,E*)-9,11,13-tetradecatrienal, respectively, injected onto a 30-m DB-1 column in a Varian 3740 GC (column conditions 80°C, 2 min hold, 15°/min to 180°C; He carrier flow of 1.5 ml/min; injector temperature 200°C; FID detector temperature 250°C). The first two compounds, (monoenal and dienal), evoked action potentials from a large-spiking cell in the sensillum, whereas the trienal caused action potentials to be discharged from a smaller-spiking cell in the same sensillum.

tunnel studies. In an initial test, compounds I and II (0.5 ng each) were inactive by themselves in evoking upwind flight from 3 m downwind (0/18 and 0/19 males flew upwind, respectively), but 0.5 ng of the triene alone did cause upwind flight and source location (9/23 flew upwind, 4/23 touched the source). In further experiments, a blend of the three compounds (40 ng, 5 ng, 5 ng, respectively) approximating the 8:1:1 ratio found in gland extract was nearly as good as 5 FE of gland extract in eliciting upwind flight and source contact (Figure 6). The trienal by itself was poorer in causing source contact than blends that included either the monoenal, the dienal, or both (χ^2 2 × 2 test of independence, $P < 0.05$). Thus it appeared that either the monoenal or dienal or both were behaviorally important components of the sex pheromone blend, in which the trienal plays the primary role in attracting males.

Further wind-tunnel experiments with a wider variety of ratios (source loading, 40 ng of trienal) confirmed that the 8:1:1 ratio approximating that found in the female gland extract was significantly better at causing complete upwind flight and source contact than the 1:1:1 or 1:0:0 ratios (Figure 7; χ^2 2 × 2 test of independence, $P < 0.05$). Although there was a trend for the other ratios to be slightly poorer than 8:1:1, it appears that the trienal needs to exceed the amounts of the other two components by 2:1:1 or greater to be optimal. As in the first experiment, none of the synthetic blends was quite as

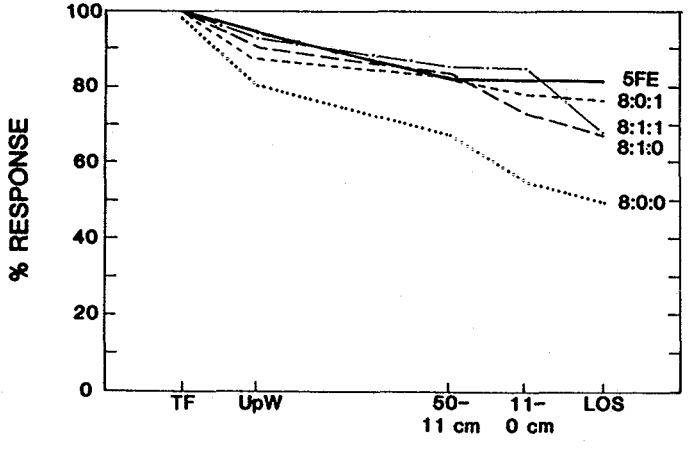

FIG. 6. Percentages of male carob moths flying in the wind tunnel in response to various blends of (*Z*,*E*)-9,11,13-tetradecadienal, (*Z*,*E*)-9,11-tetradecadienal, and (*Z*)-9-tetradecenal, or 5 FE of gland extract. Source loading on filter paper was 40 ng of the trienal applied in 10 μl of hexane. $N = 28$ males tested to each treatment. TF = take flight in plume, UpW = lock on and fly upwind in plume, 50–11 cm = flight to within between 11 and 50 cm of the source, 11–0 cm = flight to within between 0 and 11 cm of the source, and LOS = male lands on the source.

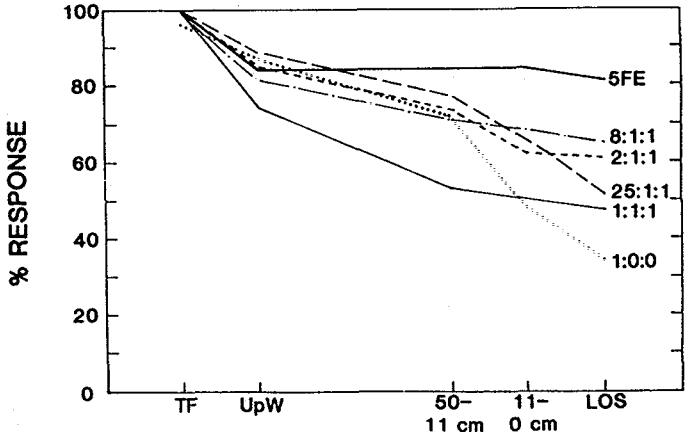

FIG. 7. Percentages of male carob moths flying in the wind tunnel in response to various blends of (*Z*,*E*)-9,11,13-tetradecatrienal, (*Z*,*E*)-9,11-tetradecadienal, and (*Z*)-9-tetradecenal, compared to 5 FE of gland extract. Source loading on filter paper was 40 ng of the trienal applied in 10 μl of hexane. Abbreviations are the same as in Figure 6. $N = 35$ males tested to each treatment.

effective as the natural extract in evoking source contact. Again the trienal alone evoked lower levels of source contact than blends containing the monoenal or dienal (Figure 7). A series of dosages of the 1:1:8 blend of I, II, and III was tested to determine if higher amounts of synthetic compounds could better mimic the natural extract. Although there was no dosage that was excessively high among the ones we tested, none of these source loadings caused levels of source contact as high as the natural extract (Figure 8) (χ^2 2 × 2 test of independence, $P < 0.05$).

In the field, the 8:1:1 mixture of the three components, dispensed at a loading of 333 μg of the major component in polyethylene Beem capsules, captured significant numbers of carob moth males. Traps containing five live females consistently captured greater numbers of males [18.6 ± 17.32 (SD) and 30.2 ± 34.1 (SD) $N = 10$ and 9, respectively] than the synthetics, which captured a mean of 9.9 ± 12.1 SD and 6.5 ± 9.9 SD males ($N = 30$ and 27, respectively). Blank traps captured 0 males in all tests.

The reduced attraction of males to the synthetic blend was not apparently due to contaminants in the synthetic compounds. In the wind tunnel six live calling females attracted and captured an average of 5.13 (±1.89 SD, $N = 8$) out of every 10 males that were released, whereas the addition of the synthetic blend on Beem capsules did not reduce the capture; rather this latter treatment increased the capture slightly, causing 5.85 (±2.17, $N = 8$) out of every 10 males released to become ensnared. In response to the females plus synthetics,

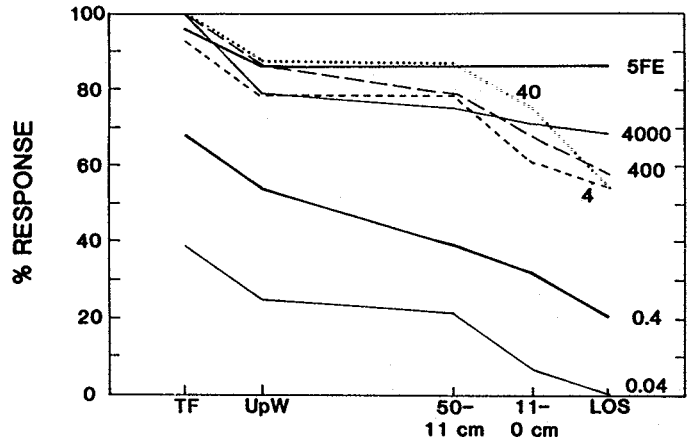

FIG. 8. Percentages of male carob moths flying in the wind tunnel in response to various source loadings of the 8 : 1 : 1 blend of (*Z*,*E*)-9,11,13-tetradecatrienal, (*Z*,*E*)-9,11-tetradecadienal, and (*Z*)-9-tetradecenal, compared to 5 FE of gland extract. Source loadings were 0.04, 0.4, 4, 40, 400, and 4000 ng of the trienal, applied in 10 μl of hexane. *N* = 60 males tested to each treatment. Abbreviations are the same as in Figure 6.

males only spent 7.96 sec (±6.90 SD) hovering in front of the trap, whereas they spent 11.47 sec (±13.71 SD) hovering in front of traps in response to live calling females. A mean of 6.25 (±1.83 SD, *N* = 8) males out of every 10 released was observed hovering in front of the trap containing synthetics plus females, whereas 4.75 (±2.12 SD, *N* = 8) out of 10 were seen hovering in front of traps containing females only.

DISCUSSION

The (*Z*,*E*)-9,11,13-tetradecatrienal identified as the major component of the carob moth sex pheromone in this study represents a novel structure with regard to previously identified lepidopteran sex pheromone components. Indeed, among the known acetate, alcohol, and aldehyde sex pheromone components of moths, only a few compounds possess more than two double bonds, and only recently were conjugated trienes reported. Tellier and Descoins (1990) confirmed our earlier findings (Baker et al., 1989) on *E. ceratoniae* and identified (*Z*,*E*)-9,11,13-tetradecatrienal and the corresponding acetate in the saturniid moth, *Stenoma cecropia*. (*E*,*E*,*E*)-10,12,14-Hexadecatrienyl acetate is a sex pheromone component of the mulberry pyralid, *Glyphodes pyloalis* (Ando et al., 1988; Honda et al., 1990) and (*E*,*E*,*Z*)-10,12,14-hexadecatrienal was identified as a component of the sex pheromone of *Manduca sexta* (Tumlinson, 1989; Tumlinson et al., 1990).

Although the activity of the 8 : 1 : 1 blend under field conditions appears to be low relative to calling females, we believe that this reduced activity can be explained in part by the lability of the trienal, which loses its integrity rapidly even in solution when stored in a freezer. The trienal appears to be the major component of the pheromone, without which attraction does not occur. The activity of freshly prepared lures in wind-tunnel tests is nearly equal to that of female extract, and so it appears that in order for reliable field lures to be prepared for monitoring, the problem of decomposition of the trienal must be overcome. However, even with this limitation and the relatively low trap capture levels relative to females, the synthetic lures emitting the 8 : 1 : 1 blend of trienal, dienal, and monoenal have already produced useful seasonal data for use by date growers attempting to accurately time their single application of malathion dust to protect the date crop during ripening. Because of the labor-intensive nature of date-growing, development of a stable formulation holds promise for disruption of mating, because dispensers could easily be hand-placed and replaced during the season by workers who tend the crop, all with minimal extra labor costs.

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