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AGGREGATION PHEROMONE OF DRIEDFRUIT BEETLE, *Carpophilus hemipterus* Wind-Tunnel Bioassay and Identification of Two Novel Tetraene Hydrocarbons

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Abstract—A male-produced aggregation pheromone was demonstrated in *Carpophilus hemipterus* (L.) (Coleoptera: Nitidulidae) using a wind-tunnel bioassay. Both sexes responded to the pheromone, but the beetles flew in the wind tunnel only after they had been starved for at least several hours. The attractiveness of the pheromone was greatly enhanced by volatiles from a food source, and combinations of pheromone and food volatiles typically attracted 3–10 times more beetles than either source by itself. A variety of food-related sources of volatiles were effective. These included apple juice; a mixture of baker's yeast plus banana; the pinto bean diet used for rearing this beetle; the chemicals propyl acetate, ethanol; and a mixture of acetaldehyde, ethyl acetate, and ethanol. The pheromonal activity resided with a series of 10 male-specific, unsaturated hydrocarbons of 13, 14, and 15 carbon atoms. These were partially separated by HPLC. No single compound was absolutely required for pheromonal activity to be observed, and various subsets of these compounds were active. The most abundant component was (2*E*,4*E*,6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-decatetraene. One minor component was (2*E*,4*E*,6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-undecatetraene. These structures were proven by synthesis. Together, the synthetic compounds were as active in the wind tunnel as the beetle-derived pheromone.

Key Words—Pheromone, aggregation, synergism, hydrocarbon, driedfruit beetle, *Carpophilus hemipterus*, Coleoptera, Nitidulidae, (2*E*,4*E*,6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-decatetraene, (2*E*,4*E*,6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-undecatetraene.

INTRODUCTION

Carpophilus hemipterus (L.) (Coleoptera: Nitidulidae) is a cosmopolitan pest that attacks a large number of agricultural commodities both before and after harvest (Hinton, 1945). It also is able to vector microorganisms responsible for the souring of figs (Hinton, 1945), and mycotoxin-producing fungi that contaminate corn (Wicklow, 1989).

Field traps have been used to monitor or control this and other nitidulid species, and much research has gone into the development of trap baits. Smilanick et al. (1978) determined that a 1:1:1 mixture of acetaldehyde, ethyl acetate, and ethanol is an effective bait for *C. hemipterus*. Alm et al. (1985, 1986) demonstrated that esters such as propyl propionate and butyl acetate are effective baits for *Glischrochilus quadrisignatus* (Say), another economically important nitidulid. Attractive chemicals such as these are produced by the host plant or associated microorganisms. Curiously, no pheromones have been reported for nitidulid beetles, even though attractants of this type would probably be very useful in insect control, and pheromones are known to exist in a large number of other beetle species.

We now report an aggregation pheromone in *C. hemipterus* and describe a wind-tunnel bioassay. Synergistic effects of the pheromone and host plant volatiles are also documented, and the chemical identification and synthesis of two novel pheromone components are presented.

METHODS AND MATERIALS

Beetles. C. hemipterus was reared on a pinto bean diet as described by Dowd (1987). The culture provided beetles for pheromone production and for bioassays. Insects used for pheromone production were separated by sex when 0–1 week old.

Bioassays. All bioassays were conducted in a wind-tunnel olfactometer that measured 0.60 × 0.60 m in cross section by 1.35 m long. The sides and top of the wind tunnel were Plexiglas, and the floor was plywood. The ends were covered with 30-mesh steel screen. Air was forced through the wind tunnel by an electric fan that was connected by a duct to the upwind end. Laminar flow was achieved by passing the air through several layers of cheesecloth mounted at the upwind screen, as described by Baker and Linn (1984). The linear air flow rate was 0.3 m/sec. The temperature was kept at 27°C; the relative humidity was not controlled but was in the range of 30–40%. The wind tunnel was lit by four 40-W fluorescent tubes mounted 10 cm above the top.

About 24 hr before bioassays were to begin, cultures containing a total of 200–400 beetles, 0–1 week old and of mixed sex, were placed in a fume hood

for 8 hr. During this time, the diet medium dried down to about 75% of its original volume. The beetles were then transferred to the wind tunnel and kept without food for an additional 16 hr. Lights and air flow were left off during this time but were turned on before beginning bioassays. Beetles treated in this way appeared healthy and usually were ready to respond to attractive baits within 1 hr after the wind-tunnel lights and fan had been turned on.

Test treatments were suspended from a horizontal wire 0.4 m above the floor of the wind tunnel, perpendicular to the air flow and 0.2 m from the upwind screen. Treatments were always tested in pairs, separated by 0.3 m. The numbers of flying beetles landing at each treatment during the test period were recorded. Initially, observation periods lasted several hours, but as a structured bioassay method evolved, the test periods were decreased to 5 min, then to 3 min. Tests were always replicated, and each bait was tested at both locations, to avoid any bias resulting from position effects. When comparisons among three or more treatments were desired, all possible two-way tests were run using a balanced incomplete block design. Tests were separated in time by 2–5 min.

In initial experiments, whole cultures were used as baits. These were held in 30-ml plastic cups and contained ca. 30 beetles. Each cup was covered with cloth, which allowed volatiles to escape. In one experiment, a second, open cup containing fresh diet was attached immediately below the bait cup, so that any attracted beetles would be able to feed and would remain at the bait, allowing them to be captured and sexed after the test.

Extracts or chromatographic fractions to be used as baits were applied to 7-cm circles of filter paper, which were folded into quarters and secured with a paper clip. Concentrations of test solutions were adjusted so that the application volume was in the range of 10–30 μl. Further details of specific tests are given with results.

Statistical Analysis. Data were transformed to the $\log(X + 1)$ scale before analysis to stabilize variance. Balanced incomplete block experiments involving comparisons among three or more treatments were analyzed by the method of Yates (1940). Paired t tests were used when only two treatments were compared.

Extraction. As a typical example, 300 male beetles, 9–12 days old, and the diet medium from the four rearing cups that held them were extracted by soaking in 100 ml of methylene chloride for 15 min. The extraction was repeated twice more, and the combined extracts filtered and dried over sodium sulfate. The extract was reduced in volume to 10 ml by rotary evaporation. Concentrations of extracts were calculated as beetle equivalents per milliliter, based on counts of beetles and extract volumes.

Volatile Collection. A 50-ml filtering flask was fitted with a cork, into which a Tenax trap was inserted. The Tenax trap was prepared from a 10 cm $\times$ 0.5 cm (ID) piece of soft glass tubing. A piece of brass screen (100 mesh)

was sealed into one end by heating. The tube was filled to a depth of 0.5 cm with Tenax porous polymer (60–80 mesh, Alltech, Deerfield, Illinois), which had been cleaned by extraction with hexane in a Soxhlet apparatus. A plug of glass wool was placed over the Tenax. About 15 ml of pinto bean diet was placed into the flask and the tip of the Tenax trap adjusted to about 1 cm above the diet. A vacuum was applied to the Tenax trap so that volatiles within the flask were drawn through the trap. A second Tenax trap was attached to the side arm of the flask to clean the air drawn into the flask. Approximately 100 male beetles 1–2 weeks old were added to the flask, and the air flow through the flask was adjusted to 50 ml/min. The flask was kept in an incubator at 27°C and 40% relative humidity. At this humidity the diet dried out slowly over a week. With the diet in this condition, the beetles remained active and healthy, but the growth of mold was retarded. The beetles received 14 hr of light each day. Eighteen such flasks were operated in the incubator at one time. Pheromone collections were quantified in terms of beetle-days, defined as the average amount of pheromone collected from one bettle in one day. Volatile collections were also made from female beetles and from diet medium without beetles.

To extract volatiles from the Tenax traps, each trap was back-flushed three times with 200 μl hexane. Before returning the trap to its flask, air was passed through the trap to evaporate residual solvent. Traps were extracted every two or three days.

Chromatography. Column chromatography on silica gel was used for all initial purifications. Columns were usually 5 cm × 0.5 cm, and these were adequate for extracts with 100 beetle equivalents, including diet medium. Before chromatography, the solvent was carefully removed from these samples under nitrogen and the samples taken up in hexane. Columns were eluted with two column volumes (2 ml) of these solvents: hexane; 5%, 10%, and 50% ether in hexane; and 10% methanol in methylene chloride. Each solvent was collected as a separate fraction. Larger columns were used for extracts with greater numbers of equivalents.

The rinses from the Tenax traps were also applied to silica gel columns; collections containing 3000 beetle-days did not overload a 5-cm × 0.5-cm column.

Silica gel containing 25% $AgNO_3$ was also used as a packing in open columns (5 cm × 0.5 cm). The samples were applied in hexane and the columns eluted sequentially with hexane; 5%, 10%, and 25% ether in hexane; and finally, with ether.

All chromatographic separations and syntheses were monitored by gas chromatography (GC), using a Varian 3700 gas chromatograph that was equipped with flame ionization detector, splitless injector for capillary columns, effluent splitter for preparative GC on a packed column, and effluent collector

(Brownlee and Silverstein, 1968). The gas chromatograph was interfaced to a Hewlett-Packard 3396A integrator. Two columns were used: The first was a 15-m × 0.25-mm (ID) DB-1 capillary with a 1.0-μm film thickness (J & W Scientific, Folsom, California). For many samples, this column was programmed from 100° to 200°C at 10°C/min, although lower starting temperatures or higher final temperatures were sometimes required. Beetle-derived samples were usually concentrated to 1–5% of the original volume by careful evaporation under N_2, so that the 1- to 2-μl injections would contain enough material to be easily detected (>1 ng/component). Concentrations of compounds in extracts and fractions were estimated by using integrator units; the integrator was calibrated using standard solutions of heptadecane in hexane. The other column, used for preparative GC, was a 2-m × 2-mm (ID) glass column, packed with 3% OV-101 on Chromosorb WHP 100/120 (Alltech).

Retention indices (I) relative to n-alkane standards were determined for the male-specific hydrocarbons. The DB-1 column was programmed from 100° to 200°C at 10°C/min, and the retention indices calculated by linear interpolation (Poole and Schuette, 1984).

High-performance liquid chromatography (HPLC) was conducted isocratically using a Waters Associates model 6000 pump and R401 refractometer detector. Two columns were used. The first was a 30-cm × 0.75-cm (ID) PLGEL 50A 10-μm size-exclusion column (Polymer Laboratories, Shropshire, U.K.) eluted with hexane. The other column was a 25-cm × 0.46-cm (ID) Lichrosorb Si60 silica column (5 μm particle size) (Alltech), coated with $AgNO_3$ as described by Heath and Sonnet (1980). This column was eluted with 25% toluene in hexane. The void volumes for the two columns were estimated to be 8 and 3.5 ml, respectively. The beetle-derived samples were not concentrated enough to be detected by the refractometer. Effluent was collected as 0.5- or 1-ml fractions, which were later analyzed by GC and bioassayed.

Spectroscopy. Mass spectra were obtained on a Finnigan 4535 quadrupole mass spectrometer. Sample introduction was always by GC [15-m × 0.25-mm (ID) DB-1 capillary with a 0.25-μm film thickness]. An ionizing potential of 70 eV was used for electron impact (EI) spectra. Isobutane was the reagent gas for chemical ionization (CI) spectra. NMR proton spectra were obtained on a Bruker 300-MHz instrument. Samples were dissolved in deuterobenzene, and shifts were calculated relative to tetramethylsilane. Nuclear Overhauser enhancements (NOEs) were measured for some samples, and the difference spectra were obtained as described by Sanders and Mersh (1982). Further experimental details are given with results. Ultraviolet spectra were taken with a Perkin Elmer Lambda 4B high-performance UV spectrophotometer. The solvent was hexane.

Hydrogenation. Saturated derivatives of male-derived hydrocarbons were

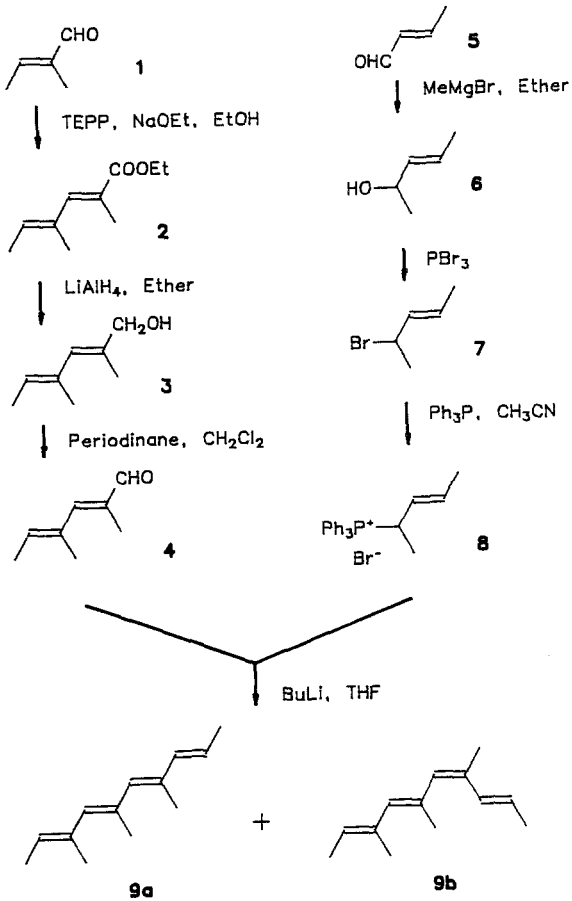

FIG. 1. Synthetic scheme for (2*E*,4*E*,6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-decatetraene (*EEEE*-13, **9a**) and (2*E*,4*E*,6*Z*,8*E*)-3,5,7-trimethyl-2,4,6,8-decatetraene (*EEZE*-13, **9b**). TEPP is an abbreviation for triethyl 2-phosphonopropionate.

prepared by the method of Parliment (1973), except that methylene chloride was used as the solvent. Palladium (10%) on carbon was used as the catalyst in the initial reactions, but PtO_2 was later found to be preferable.

Synthesis of 13-Carbon Tetraenes. (2*E*,4*E*,6*E*,8*E*)-3,5,7-Trimethyl-2,4,6,8-decatetraene (*EEEE*-13) and (2*E*,4*E*,6*Z*,8*E*)-3,5,7-trimethyl-2,4,6,8-decatetraene (*EEZE*-13) were prepared as standards for comparison with the major male-specific compound from beetles. The synthesis is outlined in Figure 1. The reactions were monitored by GC and mass spectrometry. Except for the phosphonium salt, each intermediate product was used in the next reaction with-

out purification, other than drying over sodium sulfate and removal of solvent. Generally, each reaction produced a single major compound which, by GC, accounted for over 90% of the volatile reaction products. The exception was the final reaction, which produced the two stereoisomers, *EEEE*-13 and *EEZE*-13, in approximately equal proportions.

Tiglic aldehyde [**1,** 2-methyl-2(*E*)-butenal] was converted to the ethyl ester of acid **2** in a Wittig-Horner condensation with triethyl 2-phosphonopropionate, by the procedure of Gallagher and Webb (1974). This reaction is primarily *E*-directed (Boutagy and Thomas, 1974), and by GC, only one isomer was observed. Ester **2** was reduced with $LiAlH_4$ to alcohol **3** as described by Mori (1976) for a different ethyl ester. Alcohol **3** was then oxidized to aldehyde **4** with periodinane reagent (Dess and Martin, 1983). The other half of the target hydrocarbon was constructed by alkylating crotonaldehyde [**5,** 2(*E*)-butenal] with methylmagnesium bromide to form alcohol **6,** as described by Brooks and Snyder (1955), except that a commercially prepared Grignard reagent was used. The alcohol **6** was converted to bromide **7** with PBr_3 by the procedure of Noller and Dinsmore (1943), except that bromide **7** was recovered by extraction with hexane rather than distillation. Allylic bromide **7** was treated with triphenylphosphine in refluxing acetonitrile to produce phosphonium salt **8.** This salt was crystallized by washing the product repeatedly with dry ether. Finally, aldehyde **4** and phosphonium salt **8** were linked in a Wittig reaction (Sonnet, 1974) to form isomers **9a** (*EEEE*-13) and **9b** (*EEZE*-13) of the conjugated tetraene. Initial purification was on silica gel (elution with hexane). There appeared to be some decomposition on this column (formation of yellow color, which remained on the column), but both isomers were recovered.

One tetraene was thermally labile. On DB-1 (100–200°C at 10°C/min), it produced sharp peaks at 3.99 min (I = 12.06) and 5.47 min (I = 13.26), with a broad hump between these peaks. The initial peak, due to rearrangement in the injector, could be eliminated by cooling the injector temperature to 100°C, and the hump (which indicated on-column thermal rearrangement) could be eliminated by using a thinner film column (0.25 μm versus 1.0 μm), which allowed the compound to elute at a cooler temperature (ca. 115°C versus 155°C). Thus, I = 13.26 appeared to represent the intact compound. This compound was assigned the structure, *EEZE*-13, because other conjugated systems with internal *Z* double bonds have been known to undergo rearrangement at GC temperatures or lower (for example, Näf et al., 1975; Huisgen et al., 1967). The other synthetic isomer was assigned the structure, *EEEE*-13, and it was stable under capillary GC conditions (I = 13.83).

In order to confirm assignment of structures to the synthetic compounds, *EEEE*-13 was prepared by a second method, which formed the 6(*E*) double bond stereoselectively (Boutagy and Thomas, 1974). Aldehyde **4** (Figure 1) was subjected to a second Wittig-Horner reaction with triethyl 2-phosphonopro-

pionate, again followed by reduction to the alcohol with $LiAlH_4$ and oxidation to the aldehyde with periodinane. The resulting aldehyde, (2*E*,4*E*,6*E*)-2,4,6-trimethyl-2,4,6-octatrienal, was then coupled in a Wittig reaction with ethyltriphenylphosphonium bromide. The major product had GC retention index, I = 13.83, and the other isomer in the first synthesis (I = 13.26) was not detected at all. Thus, I = 13.83 did correspond to *EEEE*-13.

EEZE-13 and *EEEE*-13 could be completely resolved by HPLC on the $AgNO_3$ column. *EEZE*-13 eluted at 5.5 ml after injection, and *EEEE*-13 at 6.5 ml. Collected peaks were uncontaminated by the other isomer, and purities of the separated isomers exceeded 98%, by capillary GC. The concentration of *EEEE*-13 was determined by GC, using heptadecane as a quantitative internal standard, and an aliquot was diluted to 1 ng/10 μl for bioassay.

The following NMR data were obtained for *EEZE*-13: δ7.00 (1H, dqd, J = 15.7, ~1, ~1), 6.12 (1H, br s), 5.90 (1H, br s), 5.71 (1H, dqd, J = 15.7, 6.7, ~1), 5.55 (1H, qqd, J = 6.8, ~1, ~1), 1.98 (3H, br s), 1.93 (3H, d, J = 1.4), 1.74 (3H, br s), 1.72 [3H, dd (half concealed), J = 6.6, 1.6], 1.63 (3H, d, J = 6.8). The UV spectrum contained maxima at 224 nm (ϵ = 2.1×10^4) and 285 nm (ϵ = 2.1×10^4). The mass spectrum was indistinguishable from that of *EEEE*-13. The NMR, UV, and mass spectral data for *EEEE*-13 were identical to the insect-derived compound and are presented in the results section.

Synthesis of 14-Carbon Tetraenes. (2*E*,4*E*,6*E*,8*E*)-3,5,7-Trimethyl-2,4,6,8-undecatetraene (*EEEE*-14) and (2*E*,4*E*,6*Z*,8*E*)-3,5,7-trimethyl-2,4,6,8-undecatetraene (*EEZE*-14), were synthesized as in Figure 1, except that 2(*E*)-pentenal served as structure **5** in place of crotonaldehyde. Again, one isomer was labile under GC conditions (the intact compound eluting at I = 14.06), and by analogy to the 13-carbon tetraenes, it was assigned the structure *EEZE*-14. *EEEE*-14 was stable at GC temperatures (I = 14.76). These isomers were also separable by HPLC on the $AgNO_3$ column. Elution volumes for *EEZE*-14 and *EEEE*-14 were 5.4 and 6.3 ml, respectively. Purities were 85% and 98%, respectively. A bioassay solution of *EEEE*-14 was prepared as described above.

The following NMR data were obtained for *EEEE*-14:δ6.27 (1H, dq, J = 15.5, ~1), 6.06 (1H, br s), 6.04 (1H, br s), 5.71 (1H, dt, J = 15.5, 6.6), 5.53 (1H, qqd, J = 6.7, ~1, ~1), 2.12 (2H, qdd, J = 7.3, 6.6, ~1), 2.02 (3H, d, J = 1.3), 1.99 (3H, d, J = 1.1), 1.75 (3H, dq, J = ~1, ~1), 1.64 (3H, d, J = 6.8), 1.03 (3H, t, J = 7.4). The UV spectrum had maxima at 224 nm (ϵ = 1.1×10^4) and 287 nm (ϵ = 2.5×10^4). The mass spectrum was as in Figure 3 below (lower).

NMR data were also obtained for *EEZE*-14: δ7.03 (1H, dq, J = 15.7, ~1), 6.13 (1H, br s), 5.93 (1H, br s), 5.79 (1H, dt, J = 15.7, 6.6), 5.55 (1H, qqd, J = 6.9, ~1, ~1), 2.10 (2H, qdd, J = 7.4, 6.6, ~1), 1.98 (3H, d, J = ~1), 1.95 (3H, d, J = ~1), 1.74 (3H, br s), 1.63 (3H, d, J = 6.9), 0.99

(3H, t, $J = 7.4$). The UV spectrum had maxima at 225 nm ($\epsilon = 2.1 \times 10^4$) and 286 nm ($\epsilon = 2.1 \times 10^4$). The mass spectrum was indistinguishable from that of *EEEE*-14.

Chemicals. The compounds and reagents for chemical syntheses were obtained from Aldrich Chemical Co. (Milwaukee, Wisconsin) and were used as received for reactions. Solvents for syntheses were dried over 4 Å molecular sieves, except ether, which was dried over sodium metal.

RESULTS AND DISCUSSION

Evidence for Aggregation Pheromone. Development of a flight-oriented bioassay for attractants was the initial goal of this research. Because these insects are excellent fliers and, presumably, colonize new food sources primarily in this way, we believed a wind-tunnel assay would be more relevant to the ecology of the beetles than the more classical "pitfall" bioassay used for many stored-product beetles (Phillips and Burkholder, 1981; Oehlschlager et al., 1988). We observed very little flight when *C. hemipterus* beetles were transferred from cultures directly into the wind tunnel. Instead, they walked to the edges and corners and formed aggregations. After several hours, however, the aggregations dispersed and the beetles began to fly spontaneously. When a beetle culture (in a small cup) was hung in the upwind end of the wind tunnel, the flying beetles were moderately attracted to it. Responding beetles approached the culture from downwind, with a casting, hovering flight and eventually landed on the cup. These flights typically lasted 1–3 min and covered the entire length of the wind tunnel. This behavior was typical of long-range orientation to an odor source (Cardé, 1984). A cup with beetles and diet was visited more frequently than one with only diet medium; during 7 hr of observation, landings on the two types of baits were 97 and 2, respectively. This was the first indication that a pheromone was operating.

In a subsequent study, culture cups containing males and diet were far more attractive than ones containing females and diet. During 5 hr, 77 beetles landed on cups with males while only eight landed at cups with females. Both sexes responded to the baits, however. During two 16-hr periods, 142 beetles were collected after flying to cups containing males, and of the responding beetles, 44% were males. (During this time, the corresponding cups containing females attracted 24 beetles, of which 58% were males). Thus, there was evidence of a male-produced pheromone to which both sexes responded about equally.

A methylene-chloride extract of the culture cups with only males was more attractive in the bioassay than one derived from females. In 10 tests spanning a total of 80 min, 71 beetles landed on the male-derived bait, while only one

landed on the comparable female-derived bait (1 beetle equivalent per test). Thus, the pheromone could be extracted into solvent.

A more rapid bioassay response was needed so that the wind tunnel could be used to monitor pheromone isolation. We discovered that by starving the bioassay beetles for a longer period of time (e.g., 16 hr) responses to attractive baits in the wind tunnel would occur with greater frequency (e.g., >10 landings in a 5- or 3-min period). With these initial results, we began a more systematic investigation of the aggregation pheromone.

Pheromone Isolation. After column chromatography of the extract of male cultures on silica gel, none of the five fractions were active in the bioassay, compared with the original extract. However, the recombined fractions were highly attractive, indicating that the active compounds had eluted from the column but that more than one chemical was required for attraction.

We hypothesized that both male-derived and diet-derived volatiles were responsible for the activity of the culture cups. To identify which fraction of the male-derived extract contained the pheromone, we tested combinations of the five chromatographic fractions (hexane; 5%, 10%, and 50% ether–hexane; and 10% MeOH–CH_2Cl_2) in this way: In each combination, one of the fractions was derived from males and the remaining four, from females; all were used at 1 beetle equivalent per test. Each combination was tested against the whole extract of the female culture (the control in this experiment), also used at 1 beetle equivalent per test. Thus, all the bioassay treatments would contain the full complement of diet compounds as well as any "general" metabolites produced by beetles of both sexes. The combination of fractions would be expected to differ from the control only if the single male-derived fraction contained the pheromone. From Table 1, it is clear that the hexane fraction was the primary source of male-specific attractant(s). Furthermore, because one male-derived fraction was sufficient for the combination to be a potent attractant, the pheromone appeared not to include components of widely different polarity.

The diet-derived coattractant used to bioassay the male-derived silica gel fractions, although successful, was more elaborate than necessary. Table 2 demonstrates that the activity of the male-derived hydrocarbon fraction was greatly enhanced by a wide variety of food-related volatiles, not just by volatiles from the beetles' own cultures. Because reproduction occurs at feeding sites in these beetles, the enhanced attraction to combined host- and beetle-derived volatiles is undoubtedly of great ecological importance.

For consistency, we continued to use the female-derived diet extract as the coattractant in subsequent bioassays of chromatographic fractions; however, propyl acetate (10% in mineral oil) was found later to be equally effective. Because it was better defined and easier to prepare, it became the coattractant of choice in the later bioassays.

The active compound(s) from the male beetles appeared to have at least

TABLE 1. ACTIVITY OF SILICA GEL FRACTIONS OF MALE-DERIVED EXTRACT IN WIND TUNNEL

Male-derived fraction	Mean bioassay count ($N = 6$)	
	Fraction combination[a]	Control[b]
Hexane	23.3*	0.5
5% Ether–hexane	1.5	0.5
10% Ether–hexane	1.5	1.8
50% Ether–hexane	1.2	0.8
10% MeOH–CH_2Cl_2	2.2	1.3

[a] Each male-derived fraction was combined with the four complementary fractions derived from females (all at 1 beetle equivalent per test). *The hexane fraction was the only one to show significant activity compared with the control ($P < 0.01$, t test).
[b] The control for this experiment was the whole extract of a culture of females (1 beetle-equivalent per test).

one double bond because the 10% ether–hexane fraction from the $AgNO_3$ column contained most of the activity (Table 3). A hydrocarbon without double bonds would have eluted with hexane. Further purification by HPLC with the size-exclusion column yielded two consecutive 1-ml fractions that were quite

TABLE 2. ENHANCEMENT OF ACTIVITY OF MALE-DERIVED HYDROCARBONS BY VARIOUS COATTRACTANTS

Coattractant	Mean bioassay count[a]		
	Male-derived hydrocarbons[b]	Coattractant	Male H-Cs + coattractant
Extract of culture of female beetles	2.0 b	4.0 b	17.0 a
Apple juice	1.3 b	1.2 b	15.6 a
Banana + baker's yeast	3.5 b	2.2 b	30.4 a
Propyl acetate[c]	2.2 c	4.7 b	18.8 a
Ethanol[c]	4.5 b	0.4 c	32.1 a
Ethanol + acetaldehyde + ethyl acetate (1 : 1 : 1)[c]	4.5 c	9.2 b	29.6 a

[a] Each line of table is a balanced incomplete block experiment, in which the treatments were tested in pairs, in all possible combinations; $N > 8$ in each line except the first, for which $N = 4$. In each line, means followed by the same letter were not significantly different (LSD, 0.05).
[b] Hydrocarbons were extracted from a culture of male beetles and used at 1 beetle equivalent per test.
[c] Tested as 10% solutions or suspensions in mineral oil.

TABLE 3. ACTIVITY OF CHROMATOGRAPHIC FRACTIONS DERIVED FROM MALE *C. hemipterus* HYDROCARBONS.[a]

Fraction description	Mean bioassay count ($N = 4$)	
	Fraction + coattractant[b]	Coattractant[b]
$AgNO_3$ fractions (open column, from culture extract)		
Hexane	1.0	1.3
5% Ether–hexane	15.0*[c]	2.0
10% Ether–hexane	33.3*	1.3
25% Ether–hexane	6.7	2.5
Ether	1.3	2.0
Size-exclusion fractions (HPLC, from $AgNO_3$ 10% ether-hexane fraction, above)		
8–10 ml after injection	0.8	1.0
10–11 ml	12.0*	1.5
11–12 ml	9.3*	1.0
12–13 ml	3.0	1.0
13–14 ml	1.0	1.8
14–15 ml	1.0	1.3
15–16 ml	1.5	1.0
$AgNO_3$ fractions (HPLC, from Tenax collections)		
3.0–4.5 ml after injection	0.0	0.3
4.5–5.0 ml	0.0	0.0
5.0–5.5 ml	0.5	0.0
5.5–6.0 ml	12.8*	0.3
6.0–6.5 ml	12.0*	0.0
6.5–7.0 ml	25.8*	0.8
7.0–7.5 ml	4.8*	0.3
7.5–8.0 ml	0.8	0.3

[a] Hydrocarbons were isolated by column chromatography on silica gel prior to separations listed in the table. Fractions were used at 1 beetle equivalent per test (culture extract) or 3 beetle-days per test (Tenax collections).

[b] In first two experiments, coattractant was the extract from female beetles + diet; in the last experiment, coattractant was propyl acetate (10% in mineral oil, 10 μl per test).

[c] Active fractions indicated by (*); t tests, $P < 0.05$.

active (Table 3). Male-derived Tenax collections also provided active hydrocarbons, and these were fractionated by HPLC on the $AgNO_3$ column. Four consecutive 0.5-ml fractions had activity (Table 3). As with the separation on the open column, the retention of active fractions indicated unsaturation in the pheromone.

Parallel chromatographic fractions derived from female beetles were prepared, and the fractions from both sexes were analyzed by GC. In the active, male-derived HPLC fractions were at least 10 compounds that were absent from the females (Figure 2, Table 4). While the activity spanned four consecutive

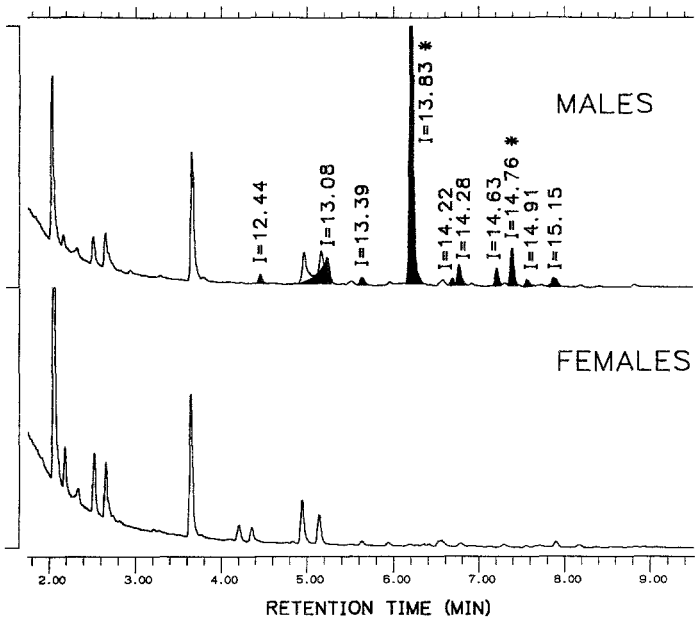

FIG. 2. Gas chromatograms of hydrocarbon fractions from male (upper) and female (lower) *C. hemipterus*. The samples were derived from Tenax collections. They were purified first by column chromatography on silica gel (elution with hexane) and then by HPLC on the $AgNO_3$ column (the GC traces represent the eluant between 5.5 and 7.5 ml). Structures of components indicated by (*) have been identified and proven by synthesis. Retention indices are listed above male-specific peaks.

$AgNO_3$–HPLC fractions (Table 3), no single compound was detected in more than two consecutive $AgNO_3$ fractions (Table 4). Thus, no single compound was absolutely required for activity and more than one subset of male-specific hydrocarbons was sufficient to elicit attraction in the wind tunnel. However, complete separation of these compounds was not obtained by any HPLC method. Preparative GC did not provide pure compounds either because many were too similar in GC retention or were too labile to survive this technique. Thus, it was not known whether all of the compounds in Table 4 were pheromonally active, and determination of the activity of individual compounds had to wait until synthetic compounds were available.

One difficulty in working with these compounds was that they were obtained only in minute amounts. In the extract of male cultures, 1 beetle equivalent contained approximately 1 ng of the major component (I = 13.83). In a typical Tenax collection, 1 beetle-day represented ca. 0.5 ng of this component. Because the beetles could live for several months in the aeration flasks, the

TABLE 4. MALE-SPECIFIC HYDROCARBONS IN *C. hemipterus*.[a]

Retention index (*I*) (DB-1 capillary GC column)	Approx. amount (pg) per male-equivalent	HPLC retention (ml)		Molecular weight	Formula
		Size exclusion	$AgNO_3$		
12.44	30	10.0-11.0*	6.0-6.5*	176	$C_{13}H_{20}$
13.08	200	10.5-11.5*	6.0-6.5*	176	$C_{13}H_{20}$
13.39	50	11.0-12.0*	5.0-5.5	176	$C_{13}H_{20}$
13.83	1000	11.0-12.0*	6.5-7.5*	176	$C_{13}H_{20}$
14.22	30	10.0-11.0*	5.5-6.5*	190	$C_{14}H_{22}$
14.28	50	10.5-11.5*	5.5-6.5*	190	$C_{14}H_{22}$
14.63	50	10.5-11.5*	6.5-7.0*	190	$C_{14}H_{22}$
14.76	100	11.0-12.0*	6.0-7.0*	190	$C_{14}H_{22}$
14.91	20	10.0-11.0*	6.0-6.5*	204	$C_{15}H_{24}$
15.15	40	10.0-11.0*	5.5-6.0*	204	$C_{15}H_{24}$

[a] Retention indices relative to *n*-alkanes. Presence of compounds in HPLC fractions determined by GC; many retention volumes represent two consecutive fractions which both contained the compound. Active HPLC fractions denoted by (*). Compounds with $I = 13.08$ and $I = 13.39$ also appear wherever the major component ($I = 13.83$) occurs; thus, they may be decomposition products. In Tenax collections, 1 beetle-day represents about 500 pg of the major component ($I = 13.83$).

Tenax collections were the richer source of active hydrocarbons, and furthermore, these were relatively easy to purify.

Mass Spectra. The EI mass spectrum of the most abundant compound ($I = 13.83$) is shown at the top in Figure 3. The apparent molecular weight, 176, was confirmed by the CI mass spectrum, in which the major peaks were 177 (M+H) and 233 (M+57, due to the isobutane reagent gas). The molecular weight is consistent with the formula, $C_{13}H_{20}$, indicating four double-bond or ring equivalents. The other male-specific peaks had similar fragmentation patterns, indicating hydrocarbons of 13, 14, or 15 carbons, all with four double-bond equivalents (Table 4). The EI spectrum for one minor component ($I = 14.76$) is also shown in Figure 3.

Hydrogenation of the Major Component. The saturated derivatives of the major component provided important structural information, but interpretation was complex. As shown in the gas chromatogram at the top of Figure 4, at least 12 distinct products were formed from the single parent compound. A typical mass spectrum of hydrogenation products is presented in Figure 3. The products were of two types that overlapped broadly: those with molecular weights of 182

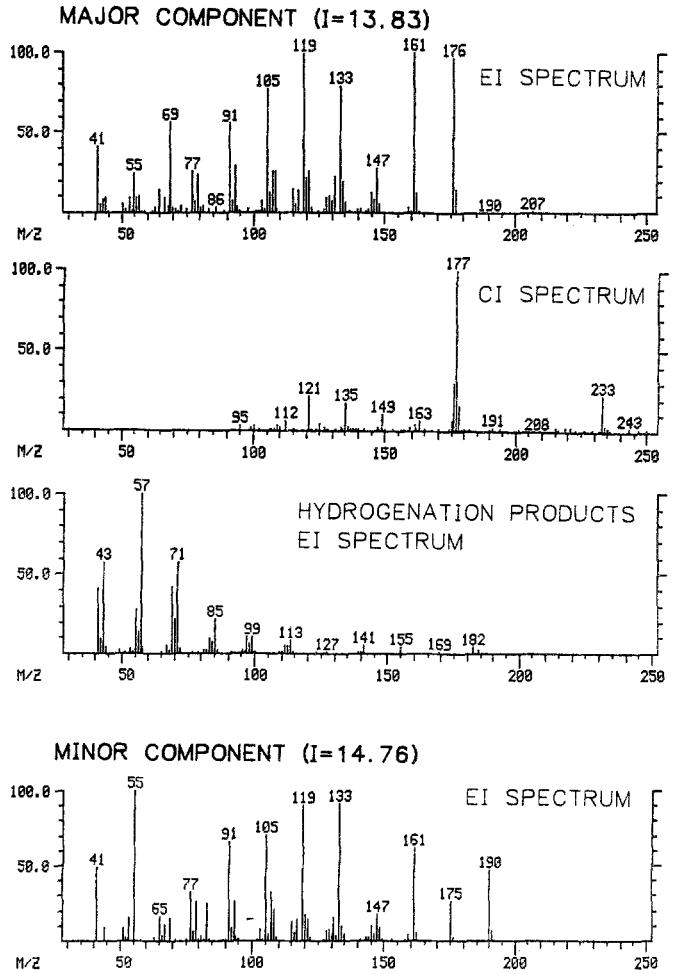

FIG. 3. Mass spectra of beetle-derived tetraenes and hydrogenated derivatives. The spectrum for the hydrogenated derivative contains peaks derived from both the cyclic (MW = 182) and acyclic products (MW = 184); see text.

and those with 184. The latter group was more informative. A molecular weight of 184 resulted from the uptake of eight hydrogens, indicating that the parent compound had four double bonds and no rings, if no triple bonds were present. (The compounds with molecular weights of 182 were also produced each time the reaction was run, and these never did hydrogenate further. Apparently, cyclic rearrangement competed with simple hydrogenation. PtO_2 as catalyst gave a

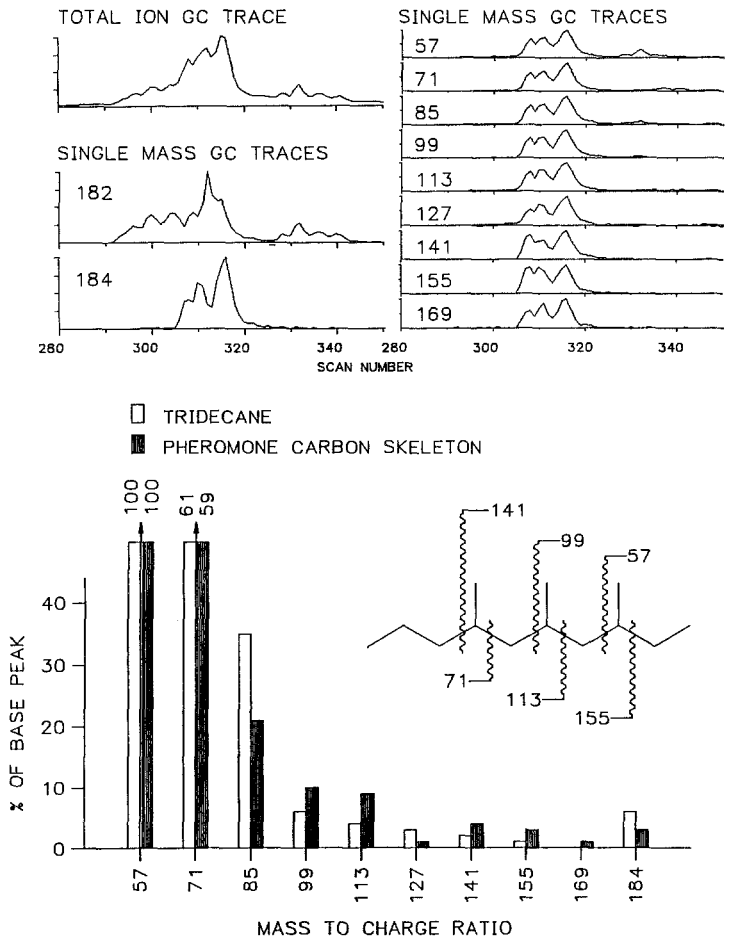

FIG. 4. Mass spectral analysis of hydrogenation products for major component (I = 13.83). Gas chromatogram of hydrogenation products is shown at top. These products had molecular weights of either 182 or 184 (second and third traces). Single mass GC traces for key fragment ions shown at upper right. GC temperature was increased at 10°/min; data acquired at the rate of 1 scan/sec. Fragmentation pattern compared with tridecane shown below.

greater proportion of the desired acyclic products than palladium did; thus PtO_2 was the catalyst of choice).

Both the number of distinct acyclic products (with molecular weight of 184) and their MS fragmentation patterns provided information about the parent compound. As shown in Figure 4, the GC trace for m/z 184 has three peaks.

The right-hand peak is broad and represents two compounds, which just began to separate; thus, there were apparently four acyclic saturated hydrocarbons derived from the parent compound. The retention indices for these three peaks were 11.50, 11.56, and 11.66. Being about 1.4 carbon units less than the *n*-alkane, these indices suggested a high degree of branching.

To make use of the MS fragmentation patterns, it had to be established whether the important fragments were derived specifically from the acyclic products. Key fragment ions in the mass spectra of acyclic saturated hydrocarbons are the series, m/z = 57, 71, 85, . . . , 169 (see Nelson, 1978). These correspond to $C_nH_{2n+1}^+$. GC traces for these masses, as well as for the molecular ions of the acyclic (184) and cyclic (182) hydrogenation products are given in Figure 4. Because the GC patterns for the key fragments followed that for 184 very closely, but not that for 182, these fragments were derived almost entirely from the acyclic products. Although the cyclic and acyclic products overlapped broadly in GC retention, the cyclic products were "transparent" and did not interfere with analysis of the $C_nH_{2n+1}^+$ fragments from the acyclic products.

Based on the $C_nH_{2n+1}^+$ fragments, all the acyclic products had nearly identical mass spectra. Compared with tridecane, the mass spectral peaks at m/z = 99, 113, 141, and 155 were relatively enhanced; while those at m/z = 85 and 127 were relatively suppressed (Figure 4). Interpretation of these data according to Nelson (1978) suggested 3,5,7-trimethyldecane as a likely structure. 3,5,7-Trimethyldecane has three asymmetric centers. If the original compound had double bonds involving the 3, 5, and 7 positions, then catalytic hydrogenation would create these asymmetric centers without stereoselectivity. The resulting eight optical isomers would produce, at most, four peaks on an achiral GC column, explaining the GC pattern we observed.

UV Spectrum of the Major Component. The UV spectrum of the natural material possessed a maximum at 287 nm ($\epsilon = 2.2 \times 10^4$) and another at 223 nm ($\epsilon = 1.0 \times 10^4$). The maximum at the longer wavelength suggested that three or four double bonds were in conjugation, but because steric and other factors can affect UV absorbance (Silverstein and Bassler, 1967), the exact number of conjugated double bonds was ambiguous.

NMR Spectrum of the Major Component. The NMR spectrum provided important structural information, but the lability of the compound made acquiring the data difficult. The initial NMR sample of about 20 μg was purified by preparative GC and transferred to a capillary NMR tube. The final purity of this sample was only 72%, however, by capillary GC, primarily because the compound had rearranged or decomposed to a significant extent on the preparative GC column. Nevertheless, the largest impurity was only 7% of the sample, so useful NMR data could still be obtained. A total of 30,000 scans were acquired. A subsequent NMR sample, containing about 30 μg, was prepared by HPLC on the size-exclusion column. After evaporating the hexane and add-

ing deuterobenzene, the sample was 90% pure, by capillary GC. A standard (5 mm) tube was used for this sample, and 3200 scans provided a reasonably good spectrum (Figure 5).

Spectral interpretation was complicated by sample degradation. The compound rearranged, polymerized, or both during acquisition of the spectra (in the latter sample, totally, within 10 hr). Peaks belonging to the original compound were distinguished from those due to decomposition by observing changes in the spectra over time. At first, no peaks were present in the region of 0.8–1.4 ppm, but over time, peaks in this area grew to become the dominant spectral features. Nevertheless, both NMR samples produced identical spectra when the peaks due to sample degradation were ignored. The observed resonances were: δ6.25 (1H, dq, J = 15.4, ~1), 6.03 (2H, br s), 5.63 (1H, dq, J = 15.4, 6.7), 5.53 (1H, qqd, J = 6.7, ~1, ~1), 2.00 (3H, br s), 1.98 (3H, br s), 1.74 (3H,

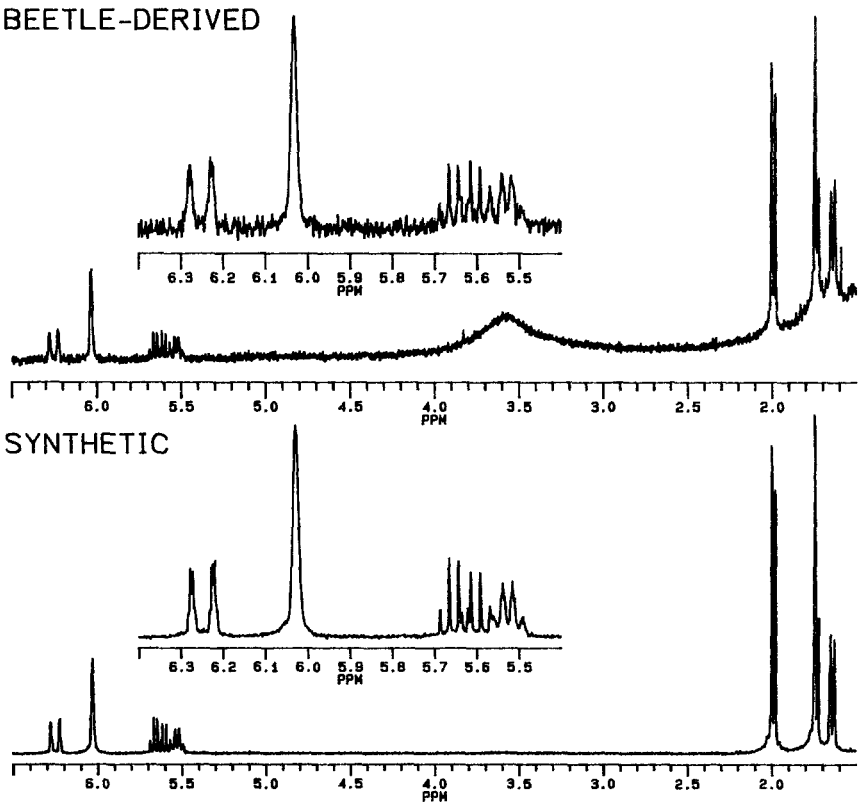

FIG. 5. Proton NMR spectrum of major beetle-derived tetraene (upper) and synthetic *EEEE*-13 (lower). Insets show expansion of olefinic region.

br s), 1.73 [3H, d (half concealed), $J = 6.7$], and 1.64 (3H, d, $J = 6.6$). All the resonances appeared to represent either olefinic protons or olefinic methyl groups. For five olefinic methyl groups to exist on the carbon skeleton in Figure 4, all four double bonds must be in conjugation (not just three, as suggested by the UV spectrum). Thus, the data suggested that the compound was a 3,5,7-trimethyl-2,4,6,8-decatetraene. The double bond at the 8 position had the *E* configuration because of the large coupling constant ($J = 15.4$ Hz) between the olefinic protons, but the configurations at the three trisubstituted double bonds could not be determined without having model compounds for comparison.

In retrospect, the observed sample degradation probably occurred because the NMR tubes had been prepared for use by heating in an oven at 110°C to drive off residual water. Synthetic tetraenes placed in similarly dried tubes also decomposed as noted above, but when the tubes were simply rinsed with hexane and dried under a stream of nitrogen, no decomposition of tetraene occurred.

Determination of Remaining Double-Bond Configurations of the Major Component by Synthesis. Once the model compounds were synthesized, it was clear that the unknown hydrocarbon was (2*E*,4*E*,6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-decatetraene (*EEEE*-13), because this hydrocarbon matched the beetle-derived compound in every way: GC retentions and mass spectra (of the hydrogenated derivatives as well as the parent compound), NMR spectrum, UV spectrum, and retention on the $AgNO_3$ and size-exclusion HPLC columns.

Identification of One 14-Carbon Minor Component. The mass spectrum of the minor component with $I = 14.76$ (Table 2) indicated a hydrocarbon with 14 carbon atoms and four degrees of unsaturation (Figure 3). Hydrogenation resulted in the uptake of eight hydrogen atoms, thus four double bonds were again indicated. Mass spectra of the hydrogenated products were interpreted as for the major component. Single-mass GC traces indicated that there were four products with molecular weight 198. These had retention indices of 12.52, 12.55, 12.62, and 12.63. The data suggested that the carbon skeleton was 3,5,7-trimethylundecane (Figure 6). Sufficient pure material was never available for an NMR spectrum of the original compound, but the similarity of retention on the $AgNO_3$ HPLC column suggested that this minor component and the major component had similar double bond systems. By analogy to the major component, (2*E*,4*E*,6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-undecatetraene (*EEEE*-14) was chosen as the first synthetic target. Fortuitously, the synthetic compound matched the insect-derived component exactly, by GC, HPLC on the $AgNO_3$ column, MS, and by GC and MS of hydrogenated derivatives.

NOE Measurements. Nuclear Overhauser enhancement (NOE) was used to confirm in another way the unusual *EEEE* configurations of the pheromone components. The synthetic tetraenes, which were identical in all respects to the pheromone components and which were available in much larger quantities, made the NOE experiments possible. This NMR technique measures spatial

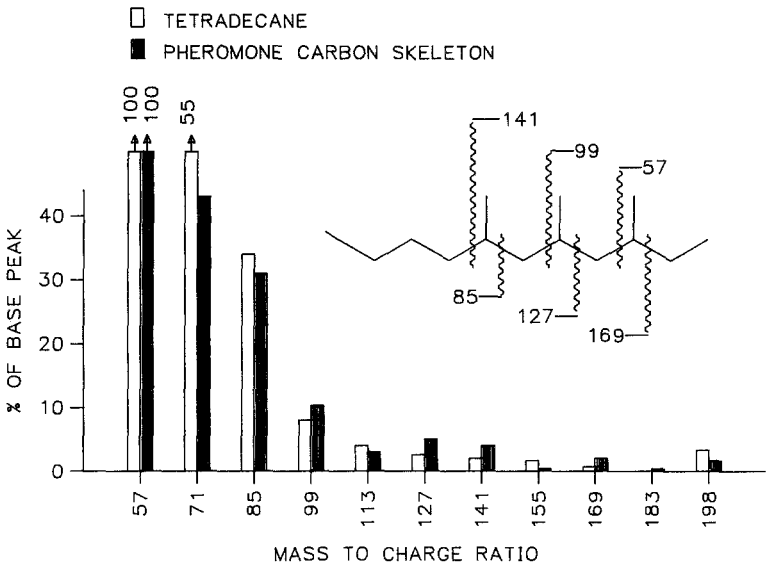

FIG. 6. Mass spectral analysis of the carbon skeleton of the 14-carbon minor component at $I = 14.76$.

relationships among protons and can determine the configurations of trisubstituted double bonds. Anet and Bourn (1965) reported a 17–18% enhancement of an olefinic proton signal when it was *cis* to an irradiated olefinic methyl group, but a *trans* proton experienced a decrease in signal intensity of 2–4%. Thus, by NOE, a clear-cut difference between *E* and *Z* double bonds in our synthetic tetraenes was expected. In order to conduct and interpret the NOE experiments, the proton shifts were first assigned (Figure 7), based on double irradiation experiments. (These assignments were later supported by NOE data).

With *EEEE*-14, irradiation at the δ1.75, 1.99, and 2.02 methyl signals produced NOEs of the protons at δ5.53, 6.04, and 6.06 of 0%, 0–2%, and 0–2%, respectively. The latter two NOE measurements were somewhat uncertain because the proton shifts were so close together, but all three values clearly supported the E configuration. Irradiation at δ2.02 did enhance the proton signal at δ5.71 by 9%. This result supported the 8(*E*) configuration because the 7 methyl group and the proton at the 9 position can be close in space only if the double bond at the 8 position is *E*.

Analysis of *EEEE*-13 was more complicated, because two proton resonances overlapped (δ6.03). When either the 5 or 7 methyl group was irradiated, a 2% enhancement of the δ6.03 peak was observed (4% if due solely to changes in one proton). These values were too small to indicate the presence of a *Z* double bond, but it was impossible to tell whether the enhanced proton was on the same double bond as the irradiated methyl group or on the adjacent one.

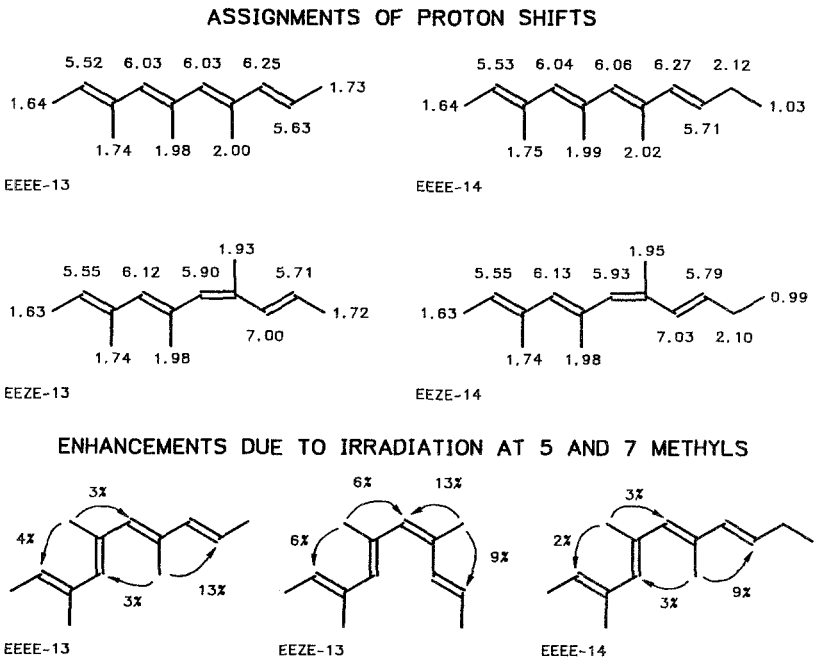

FIG. 7. Assignments of proton shifts for four synthetic tetraenes and NOE enhancements observed when the 5 and 7 methyl groups of three tetraenes were irradiated.

EEZE-13 was analyzed for comparison. Irradiation at the δ1.93 methyl signal caused a 13% enhancement of the proton signal at δ5.90, confirming the 6(*Z*) double bond, and irradiation at δ1.98 led to a 2% decrease in the signal at δ6.12, which supported the 4(*E*) configuration.

The major enhancements for these tetraenes following irradiation at the 5 and 7 methyl groups are shown in Figure 7 (lower). The NOE assignments for the protons at δ6.03 in *EEEE*-13 were made by analogy to *EEEE*-14. The NOE data suggested molecular conformations in benzene solution similar to those figured, but the double bonds were probably not coplanar. Molecular models indicated that a fully planar conformation would be strained and that in this conformation the enhancement of the proton at the 2 position should equal or exceed that at the 9 position. The enhancement of the proton in the 4 position would be similarly large in the *EEEE* tetraenes. Rotation about single bonds so that the 2, 4, and 6 double bonds were no longer coplanar would relieve the steric strain, would explain the fairly low observed NOE values, and may contribute to the unexpected maxima at 223–225 nm in the UV spectra.

Bioassays with Synthetic Hydrocarbon. Both *EEEE*-13 and *EEEE*-14 were active in the bioassay (Table 5). The major compound in the beetles, *EEEE*-13, showed significant synergistic activity with propyl acetate, although it was

TABLE 5. ACTIVITY OF SYNTHETIC HYDROCARBONS

Treatment[a]	Mean bioassay count[b]
Activity of *EEEE*-13 (N = 12)	
Control	0.1 c
EEEE-13 (1 ng)	0.5 bc
Propyl acetate (coattractant)	0.8 b
EEEE-13 (1 ng) + propyl acetate	5.7 a
Activity of *EEEE*-14 (N = 12)	
Control	0.1 c
EEEE-14 (1 ng)	1.8 b
Propyl acetate	2.0 b
EEEE-14 (1 ng) + propyl acetate	23.2 a
Comparative activities of *EEEE*-13 and *EEEE*-14 (N = 12)	
Propyl acetate	1.3 d
EEEE-13 (1 ng) + propyl acetate	4.7 c
EEEE-14 (200 pg) + propyl acetate	14.1 b
EEEE-13 + *EEEE*-14 (1 ng + 200 pg) + propyl acetate	25.0 a
Comparison of male-derived hydrocarbons with *EEEE*-13 (N = 8)	
Propyl acetate	0.4 c
Propyl acetate + *EEEE*-13 (1 ng)	5.5 b
Propyl acetate + male-derived H-Cs (1 ng of *EEEE*-13)	18.0 a
Comparions of male-derived H-Cs with combined *EEEE*-13 and EEEE-14 (N = 8)	
Propyl acetate	3.3 b
Propyl acetate + *EEEE*-13 + *EEEE*-14 (1 ng + 200 pg)	27.5 a
Propyl acetate + male-derived H-C's (1 ng of *EEEE*-13)	24.4 a
Synergistic activity of the *EEEE*-14 and a combination of ethanol, ethyl acetate, and acetaldehyde (N =12)	
Ethanol + ethyl acetate + acetaldehyde[c]	8.0 b
EEEE-14 (1 ng)	0.8 c
EEEE-14 (1 ng) + ethanol + ethyl acetate + acetaldehyde	31.2 a

[a] *EEEE*-13 = (2*E*,4*E*,6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-decatetraene; *EEEE*-14 = (2*E*,4*E*,6*E*,8*E*)-3,5,7-trimethyl-2,4,6,8-undecatetraene; except where otherwise indicated, propyl acetate was the coattractant (used as 10% solution in mineral oil, 10 μl per test). Male-derived hydrocarbons were from Tenax collection, hexane fraction from silica gel. Amount tested contained 1 ng of *EEEE*-13.

[b] Each experiment was a balanced incomplete block with the treatments tested in pairs. Tests lasted 3 min. Analysis was in the log (X + 1) scale, conducted by the method of Yates (1940). Means were converted back to the numerical scale for presentation. In each of the six experiments, means followed by the same letter were not significantly different (LSD, 0.05).

[c] The three components were used in a 1:1:1 mixture as a 10% solution with mineral oil (see Smilanick et al., 1978).

not active by itself at the level corresponding to one male equivalent. The 14-carbon minor component, *EEEE*-14, was also synergistic with propyl acetate and, in addition, was significantly active by itself. Surprisingly, *EEEE*-14 was more active than *EEEE*-13, even when tested at one fifth the dose. The 14-carbon compound is a minor constituent in the male-derived hydrocarbons (about 10–20% as abundant as the major component), but it accounted for a large proportion of the pheromonal activity. Furthermore, these hydrocarbons synergized each other in much the same way as host-derived volatiles synergized the whole pheromone.

The fourth and fifth experiments in Table 5 demonstrate in another way that just the major 13-carbon component, *EEEE*-13, is insufficient for maximum activity. This compound attracted only about one third as many beetles as the whole, male-derived hydrocarbon mixture. However, when *EEEE*-13 and *EEEE*-14 were combined, the combination compared favorably with the male-derived attractant.

The final experiment in Table 5 shows the tremendous increase in activity that is possible by adding *EEEE*-14 to a previously reported "best" attractant (Smilanick et al., 1978).

Qualitatively, the two model compounds that do not occur in the beetles, *EEZE*-13 and *EEZE*-14, were not active in the wind tunnel. Bioassay details for these and other synthetic tetraenes will be published later along with the structures of the remaining male-specific hydrocarbons.

SUMMARY

This is the first report of an aggregation pheromone in a nitidulid species. A wind-tunnel bioassay was described that permits efficient monitoring of chromatographic fractions and evaluation of pure compounds. Two novel, conjugated tetraene hydrocarbon pheromone components were identified and synthesized. Insect pheromones with four conjugated double bonds have not been reported previously, although related compounds have been found in plants. For example, Boland et al. (1987) reported a series of 2,4,6,8-undecatetraenes in the marine alga *Giffordia mitchellae*, but these compounds do not have the methyl branches found in the *C. hemipterus* pheromone components. Interestingly, the cyclic mycotoxin, citreomontanin, from the fungus, *Penicillium pedemontanum*, contains the *EEEE*-13 structure as part of a side chain (Patel and Pattenden, 1985).

The pheromone was most active when used in combination with host-type volatiles. This enhancement is similar to that observed in other beetle groups (e.g., Walgenbach et al., 1987; Oehlschlager et al., 1988; Birch, 1984) and also in fly species that use aggregation pheromones (Bartelt et al., 1986). Fur-

ther research will explore the chemical nature of host-derived coattractants for *C. hemipterus*. The pheromone, used with the optimum coattractants, may become a useful pest management tool.

Acknowledgments—Chris Weber of this laboratory maintained the beetle culture for this research. The identity of the species in culture was kindly confirmed as *C. hemipterus* by J.M. Kingsolver of the USDA Systematic Entomology Laboratory.

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