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Unusual mechanism of hydrocarbon formation in the housefly: Cytochrome P450 converts aldehyde to the sex pheromone component (*Z*)-9-tricosene and CO_2

(hydrocarbon biosynthesis/fatty acyl-CoA)

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ABSTRACT **An unusual mechanism for hydrocarbon biosynthesis is proposed from work examining the formation of (*Z*)-9-tricosene (Z9-23:Hy), the major sex pheromone component of the female housefly, *Musca domestica*. Incubation of (*Z*)-15-[1-^{14}C]- and (*Z*)-15-[15,16-$^{3}H_2$]tetracosenoic acid (24:1 fatty acid) with microsomes from houseflies gave equal amounts of [^{3}H]Z9-23:Hy and $^{14}CO_2$. The formation of CO_2 and not CO, as reported for hydrocarbon formation in plants, animals, and microorganisms [Dennis, M. & Kolattukudy, P. E. (1992) *Proc. Natl. Acad. Sci. USA* 89, 5306–5310], was verified by trapping agents and by radio-GLC analysis. Incubation of (*Z*)-15-[15,16-$^{3}H_2$]tetracosenoyl-CoA with microsomal preparations in the presence of NADPH and O_2 gave almost equal amounts of (*Z*)-15-$^{3}H_2$]tetrasosenal (24:1 aldehyde) and Z9-23:Hy. Addition of increasing amounts of hydroxylamine (aldehyde trapping agent) caused a decrease in hydrocarbon formation with a concomitant increase in oxime (aldehyde derivative) formation. The 24:1 aldehyde was efficiently converted to (*Z*)-9-tricosene only in the presence of both NADPH and O_2. Bubbling carbon monoxide (20:80 CO/O_2) or including an antibody against housefly cytochrome P450 reductase inhibited the formation Z9-23:Hy from 24:1 aldehyde. These data demonstrate an unusual mechanism for hydrocarbon formation in insects in which the acyl-CoA is reduced to the corresponding aldehyde and then carbon-1 is removed as CO_2. The requirement for NADPH and O_2 and the inhibition by CO and the antibody to cytochrome P450 reductase strongly implicate the participation of a cytochrome P450 in this reaction.**

Long-chain hydrocarbons are abundant components in the cuticular lipids of plants and insects (1, 2), where they function to prevent water loss from the surface of the organism. In some insect species, including the housefly, *Musca domestica*, hydrocarbon components function as sex pheromones. The main component of the sex pheromone produced by the female housefly is (*Z*)-9-tricosene (Z9-23:Hy) (3), which functions as a short-range attractant and stimulant (4). In vertebrates, hydrocarbons function in the myelin sheath of peripheral nerves (5) and as components of uropygial gland secretions (6).

The mechanism for hydrocarbon biosynthesis has proven to be elusive. Studies in the 1920s (7) suggested that two fatty acids condense head-to-head to form a ketone that is then reduced to the alkane. In an elegant series of experiments in the 1960s (reviewed in ref. 8), Kolattukudy and coworkers demonstrated that hydrocarbons are formed by the elongation of fatty acids, which are then converted to hydrocarbon by the loss of the carboxyl group, which was presumed to be a decarboxylation reaction (9, 10). More recently, Kolattukudy and coworkers have obtained evidence from studies in a microorganism (11, 12), a plant (13), a vertebrate (6), and an insect (14) that long-chain fatty acyl groups are reduced to aldehydes and then converted to hydrocarbons by a reductive decarbonylation mechanism. This mechanism does not require reduced pyridine nucleotides, and the carbonyl carbon is released as CO. In contrast, Gorgen and coworkers (15, 16) presented evidence that 1-alkenes are formed by a decarboxylation mechanism in both plants and insects. We present evidence in this paper that hydrocarbon formation in the housefly occurs by the reduction of a long-chain acyl-CoA to an aldehyde that is then converted to the hydrocarbon and CO_2 by a reaction that requires NADPH and O_2 and involves cytochrome P450.

MATERIALS AND METHODS

Houseflies. Insects were obtained and handled as described (17).

Chemicals and Materials. Bio-Sil A was obtained from Bio-Rad. Pyridinium chlorochromate was purchased from Aldrich. Ecolume (biodegradable liquid scintillation solution) was obtained from ICN. Triton X-100 was purchased from Calbiochem. All solvents except diethyl ether (Fisher) were redistilled in glass prior to use. All other chemicals were obtained from Sigma. Antibody to cytochrome P450 reductase (18) was a gift from René Feyereisen, Tucson, AZ.

Labeled Substrates. 15-Tetracosynoate and 19-octacosynoate methyl esters (ME) (19) were reductively tritiated to (*Z*)-15-[15,16-$^{3}H_2$]tetracosenoate ME (24:1-ME) and (*Z*)-19-[19,20-$^{3}H_2$]octacosenoate ME (28:1-ME) (60 Ci/mmol; 1 Ci = 37 GBq) by Amersham. The labeled fatty acid ME derivatives were converted to free fatty acids by hydrolysis in 0.5 M KOH in ethanol for 1 hr at 80°C, acidified, extracted with $CHCl_3$, and purified by TLC on silica gel developed in hexane/diethyl ether/acetic acid, 90:10:1 (vol/vol), and by HPLC on an Econosphere C_8 reverse-phase column with a solvent system of acetonitrile/water, 80:20 (vol/vol). The method of Bergstrom and Reitz (20) was used to convert fatty acids to their CoA derivatives with the modification that a $NaHCO_3$ buffer was added to the reaction as described in Biship and Hajra (21). The final acyl-CoA product was >98% pure and contained <0.5% free fatty acid.

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Abbreviations: Z9-23:Hy, (*Z*)-9-tricosene; 24:1 fatty acid, (*Z*)-15-tetracosenoic acid; 28:1 fatty acid, (*Z*)-19-octacosenoic acid; 24:1 aldehyde, (*Z*)-15-tetracosenal; 24:1-CoA, (*Z*)-15-tetracosenoyl-CoA; ME, methyl ester.

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(*Z*)-15-[15,16-$^3H_2$]Tetracosenal (24:1 aldehyde) was obtained by the reduction of [15,16-$^3H_2$]24:1-ME with $LiAlH_4$ in anhydrous diethyl ether. The alcohol product was oxidized to the aldehyde with pyridinium chlorochromate (13). The aldehyde was dissolved in 0.3 mM Triton X-100 detergent before addition to the assay mixtures.

(*Z*)-15-[1-^{14}C]Tetracosenoic acid (24:1 fatty acid) was synthesized from (*Z*)-14-tricosenoate ME (23:1-ME) and $K^{14}CN$ (57.5 mCi/mmol, purchased from American Radiolabeled Chemicals, St. Louis) by the method described by Bjostad *et al.* (22).

Preparation of Microsomes. Four-day-old flies of a single sex were immobilized at −20°C but not frozen. Microsomes from whole flies, abdomens, or epidermal tissue were prepared as described (17) after being homogenized in a chilled mortar and pestle, with the microsomal fraction isolated by centrifugation at 165,000 × *g* for 60 min. Protein was determined as described by Bradford (23).

Assay for Hydrocarbon Biosynthesis with Acyl-CoA as a Substrate. Final concentrations in the reaction mixture, except where otherwise indicated, were 30 μM acyl-CoA, 1 mg of microsomal protein, 0.1 M potassium phosphate, 0.25 mM sucrose, 2 mM $MgCl_2$, 2 mM sodium ascorbate, 1 mM dithiothreitol, and 1 mM NADPH at pH 7.2. The total volume was 1 ml. Incubations were run either anaerobically or aerobically as indicated and stopped after 10 min by the addition of 200 μl of 2 M HCl, unless otherwise specified. Lipid was extracted four times with 1 ml of chloroform. The combined chloroform extracts were dried over anhydrous Na_2SO_4, and the chloroform was evaporated under a gentle stream of N_2. Hydrocarbon was isolated either by column chromatography on 6 cm × 0.5 cm i.d. columns of activated Bio-Sil A eluted with 3 ml of hexane or by TLC in hexane/ether, 85:15 (vol/vol). Radioactivity was assayed as described (17).

Identification of the Hydrocarbon Product. Hydrocarbon products were fractionated by TLC developed in hexane/diethyl ether/7.4 M NH_4OH, 70:30:1 (vol/vol). Radioactivity was detected by a radio-TLC plate scanner (Bioscan Imaging Scanner Systems, 200-IBM; Bioscan, Washington, DC). The hydrocarbon fraction, which was near the solvent front (R_f = 0.87), was recovered by scraping the silica gel into a test tube and extracted from the silica gel with diethyl ether. Hydrocarbon was subjected to radio-HPLC on an Econosphere C_8 reverse-phase column having a particle size of 5 μm. A flow-through liquid scintillation detector (Flo-one by Radiomatic Instruments and Chemicals, Tampa, FL) was used to detect radioactive material. The mobile phase was composed of hexane and acetonitrile, 5:95 (vol/vol). Samples of hydrocarbon were also analyzed by radio-GLC as described (24).

Assay for Hydrocarbon Biosynthesis from Aldehyde. [15,16-$^3H_2$]24:1 aldehyde (2.5 μM, 437,500 dpm per assay) was used as the substrate in the presence of 0.5 mM NADPH, 0.5 mg of microsomes (from abdominal integument), and 0.3 mM Triton X-100 in 1 ml. The aldehyde was suspended in a 0.3 mM Triton X-100 solution by sonication for 30 sec. All other incubation conditions were the same as that described above, except that the reaction was performed in the absence of sodium ascorbate and dithiothreitol.

Anaerobic conditions were maintained by first flushing stoppered tubes with N_2 for 15 min while simultaneously drawing out gas from the tubes with an aspirator. The tubes were connected in sequence by rubber hoses which were fitted with 21-gauge needles. After 15 min, the aspirator was disconnected and replaced with an outlet needle. The tubes were then transferred to a water bath, and the reaction was initiated as described above.

Identification of an Aldehyde Intermediate in the Metabolism of (*Z*)-15-[15,16-$^3H_2$]-Tetracosenoyl-CoA (24:1-CoA). After terminating the incubation described above, lipid was extracted with $CHCl_3$ and separated by TLC developed in 85:15 hexane/diethyl ether. Aldehyde was localized at R_f = 0.55 by comparison with a standard, recovered by scraping the silica gel from this region into a test tube, and eluted from the silica gel with diethyl ether.

A portion of the aldehyde sample was placed in 1 ml of 95% ethanol containing 1 M $NaBH_4$. After stirring for 1 hr at room temperature, the reaction was stopped by adding 200 μl of 2 M HCl. Lipid was extracted with hexane. The $NaBH_4$ reduction product was separated on TLC developed in 70:30:1 hexane/diethyl ether/7.4 M NH_4OH and analyzed by a radio-TLC plate scanner. A portion of the sample was subjected to radio-HPLC analysis with a solvent system of acetonitrile/water, 90:10 (vol/vol). (*Z*)-15-[15,16-$^3H_2$]-Tetracosen-1-ol (24:1 alcohol) was used as a standard.

Another portion of the aldehyde sample was dissolved in 1 ml of acetone. Chromic acid (3.3 M) was added dropwise until an orange color persisted. The reaction mixture was stirred for 15 min at room temperature. 2-Propanol was added until a green color appeared. One milliliter of water was added, and the lipid was extracted with hexane. The components were separated by TLC developed in 70:30:1 hexane/diethyl ether/7.4 M NH_4OH and analyzed by a radio-TLC plate scanner.

The aldehyde was also treated with 1 mM hydroxylamine (NH_2OH) hydrochloride in 1 ml of phosphate buffer at pH 7.2, mixed in a Vortex, and then kept at room temperature for 15 min. Oximes were extracted with hexane, separated by TLC as described above, and then analyzed by a radio-TLC plate scanner.

Aldehyde Trapping Experiment. Various concentrations (0, 0.02, and 0.08 mM) of the aldehyde-trapping agent hydroxylamine were included in the assay mixture. Incubations were terminated by adding $CHCl_3$ and mixing. After centrifugation, lipids in the lower $CHCl_3$ layer were separated by TLC developed in 70:30:1 hexane/diethyl ether/7.4 M NH_4OH. Oximes (syn and anti isomers) (R_f = 0.22 and 0.26) and hydrocarbon (R_f = 0.87) were detected by a radio-TLC plate scanner.

CO_2 and CO Trapping Experiment. The assay was performed as described above, except that [15,16-^{3}H]24:1 and [1-^{14}C]24:1 fatty acids (60 nmol; 723,000 dpm of ^{3}H and 616,500 dpm of ^{14}C per assay) dissolved in 500 mM Zwittergent 3-08 were used in place of the 24:1-CoA. Final concentrations in the assay were 2 mg of microsomal protein, 50 mM Zwittergent 3-08, 30 μM [15,16-^{3}H]24:1/[1-^{14}C]24:1 fatty acids combined, 0.1 M potassium phosphate, 0.25 mM sucrose, 2 mM $MgCl_2$, 2 mM ATP, 0.5 mM unacylated CoA (CoASH), 2 mM NADPH, 2 mM sodium ascorbate, and 1 mM dithiothreitol at pH 7.2. The total volume was 2 ml. Incubations were done in air in a stoppered 10-ml conical flask equipped with two polypropylene center wells. Each well contained a 0.5 × 1.5 mm glass fiber filter paper strip. One strip was soaked in 200 μl of 1.0 M methylbenzethonium hydroxide in methanol (to trap $^{14}CO_2$); the other strip was soaked in buffer and was coated with ≈10 mg of $RhCl[(C_6H_6)]_3P]_3$ (to trap ^{14}CO) (14). The reaction was allowed to proceed at room temperature for 60 min and was terminated by the addition of 400 μl of 2 M HCl. Lipid was extracted with chloroform, and the hydrocarbon was isolated and quantified as described above. The $^{14}CO_2$ and ^{14}CO were quantified by putting the respective traps directly into scintillation vials and analyzing by liquid scintillation counting.

In a separate experiment, the [1-^{14}C]24:1 fatty acid was incubated under the same conditions in 4-ml stoppered tubes without the CO and CO_2 traps. After the reaction was terminated, 3 ml of gas was drawn from the tubes with an air-tight syringe and was analyzed by radio-GLC on a 2m × 2mm i.d. column containing Carboxen 1000 (Supelco) programmed from 140°C to 210°C at 8°C per min.

Incubations of microsomes were also done with and without NADPH in stoppered 4-ml tubes in the presence of ^{14}CO for 40 min. The ^{14}CO was synthesized from [^{14}C]formate (25). Head-space gas (3 ml) from these incubations was drawn off and injected onto the radio-GLC column described above.

Experiment Testing CO Inhibition of the Conversion of [15,16-$^3H_2$]24:1 Aldehyde to [$^3H_2$]Z9-23:HY Under Light and Dark Conditions. The hydrocarbon assay using [15,16-$^3H_2$]24:1 aldehyde as a substrate was performed as described above except the reaction was bubbled continuously with an 80:20 CO/O_2 mixture. Control tubes were bubbled continuously in the light with an 80:20 N_2/O_2 mixture. A 75-W incandescent bulb was placed 10 cm from the tubes. Dark conditions were achieved by wrapping the tubes with aluminum foil.

RESULTS

Product Characterization. After incubation of male and female microsomes with labeled fatty acyl-CoAs and NADPH, the hydrocarbon products were isolated by column chromatography and were analyzed by radio-HPLC and radio-GLC. The hydrocarbon product from both male and female microsomes incubated with [15,16-$^3H_2$]24:1-CoA had the same retention time as a Z9-23:Hy standard on radio-GLC (Fig. 1*A*) and radio-HPLC (Fig. 1*B*). Similarly, the hydrocarbon product from 28:1-CoA had the same retention time as a (*Z*)-9-heptacosene (Z9-27:Hy) standard as shown by radio-GLC and radio-HPLC (data not shown). Thus, microsomal preparations from both males and females produced C_{23} and C_{27} alkenes when incubated with fatty acyl-CoAs one carbon longer.

Evidence for an Aldehyde Intermediate in the Conversion of 24:1-CoA to Z9-23:Hy. Radio-TLC analyses of the products of either 24:1-CoA or 28:1-CoA incubated with microsomal preparations from male and female houseflies in the presence of NADPH showed that three main products were present from 24:1-CoA (Fig. 1*C*). Radio-GLC and radio-HPLC indicated that two of the products were the corresponding hydrocarbon (one carbon shorter in chain length) and free fatty acid (presumably 24:1). The other product was shown to be the 24:1 aldehyde and the 28:1 aldehyde by radio-HPLC, radio-GLC, and by conversion of the TLC-purified aldehydes to the corresponding alcohols, acids, and oximes, which were characterized by radio-TLC (data for the 24:1 aldehyde are shown in Fig. 1 *D–F*) and radio-HPLC (data not shown).

Incubation of the 24:1-CoA with microsomes in the presence of increasing amounts of NH_2OH showed that as the concentration of NH_2OH increased, the amount of oxime formed increased with a concomitant decrease in the amount of hydrocarbon formed (Fig. 2). At 0.08 mM NH_2OH, no labeled hydrocarbon was detected. These data were interpreted to mean that increasing NH_2OH concentrations resulted in higher proportions of the aldehyde converted to the oxime, and thus less was available for hydrocarbon formation.

Incubation of chemically synthesized [15,16-$^3H_2$]24:1 aldehyde with microsomes under anaerobic conditions in the presence of NADPH resulted in minimal hydrocarbon formation. Optimal activity occurred under aerobic conditions (Fig. 3). Radio-HPLC analyses of the hydrocarbon products from the 24:1 aldehyde showed that the only product formed was Z9-23:Hy (data not shown).

Cofactor Requirement for the Conversion of Acyl-CoA to Hydrocarbon. When NADPH was omitted from the incubation mixture, very little hydrocarbon was formed from either 24:1-CoA or 28:1-CoA, nor was 24:1 aldehyde formed. Increasing amounts of alkenes were formed from both 24:1-CoA and 28:1-CoA as the concentration of NADPH was increased up to 3.0 mM. Very little hydrocarbon was formed from either 24:1-CoA or 28:1-CoA when NADPH was replaced with NADH in the incubation media, except for trace amounts from 24:1-CoA at 3.0 mM NADH. Males formed 7–10 times more hydrocarbon product from 24:1-CoA than from 28:1-CoA, while females formed about 14 times more hydrocarbon product from 24:1-CoA than from 28:1-CoA (data not shown). Thus, there is a requirement of a reduced nucleotide for alkene production.

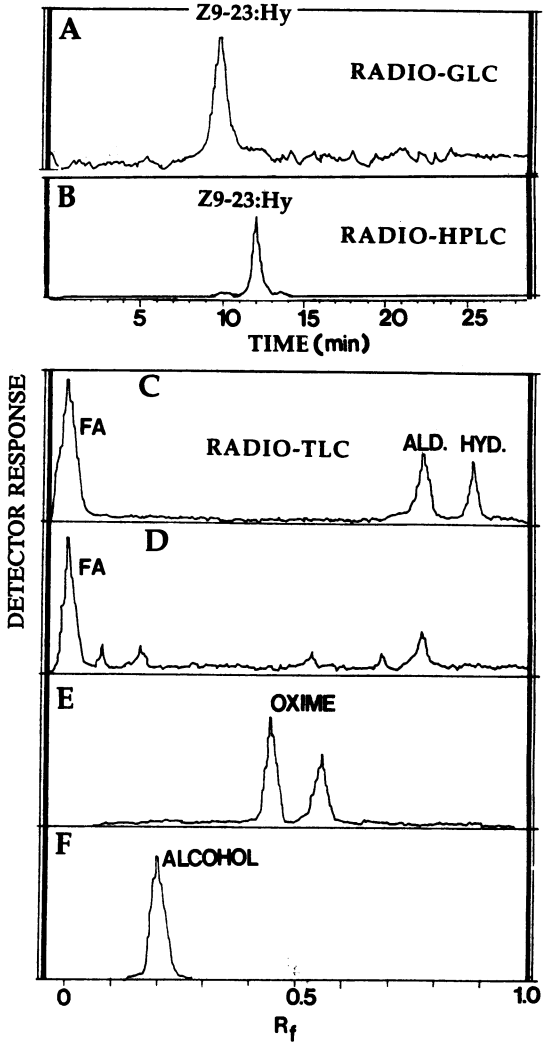

FIG. 1. Radio-chromatograms of the microsomal incubation products of [15,16-$^3H_2$]24:1-CoA. (*A*) Radio-GLC of Z9-23:Hy product. (*B*) Radio-HPLC of Z9-23:Hy product. (*C*) Radio-TLC of products identified as fatty acid (FA), 24:1 aldehyde (ALD.), and Z9-23:Hy. (*D*) Radio-TLC of fatty acid formed by oxidation of the aldehyde product isolated from *C* by TLC. (*E*) Radio-TLC of oximes formed from reaction of aldehyde product isolated from *C* with NH_2OH. (*F*) Radio-TLC of primary alcohol formed by reduction of aldehyde product from *C*. All products were compared against authentic standards. Experiments were performed as described in text.

Cofactor Requirements for the Conversion of Aldehyde to Hydrocarbon. Both NADPH and NADH support hydrocarbon formation from tritiated 24:1 aldehyde. Specifically, 80 pmol (±4) and 100 pmol (±7) of Z9-23:Hy were formed in the presence of 2 mM NADH and 1 mM NADPH, respectively, when [15,16-$^3H_2$]24:1 aldehyde was used as a substrate. Very little hydrocarbon was formed in the absence of reduced pyridine nucleotides.

The Carbonyl Carbon Is Removed as CO_2. When equal amounts of [15,16-$^3H_2$]24:1 and [1-^{14}C]24:1 fatty acids were incubated with microsomes in stoppered tubes containing CO

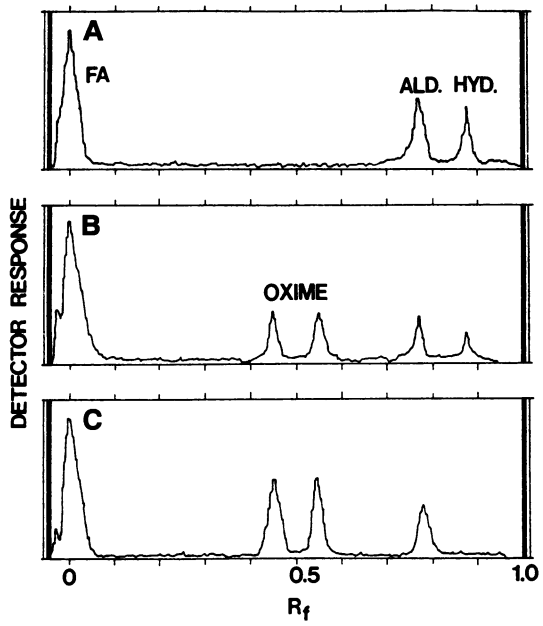

FIG. 2. Effect of increasing amounts of NH_2OH on the conversion of [15,16-$^3H_2$]24:1-CoA to hydrocarbon (HYD), aldehyde (ALD.), oximes, and fatty acid (FA). Chromatograms show the products extracted from incubations with 0.00 mM (*A*), 0.02 mM (*B*), and 0.08 mM (*C*) NH_2OH. Experiments were performed as described in text.

and CO_2 traps, it was found (Fig. 4) that only $^{14}CO_2$ was trapped in the formation of Z9-23:Hy. The amount of Z9-23:Hy extracted from the reaction was stoichiometrically equivalent to that of the trapped $^{14}CO_2$. To confirm that only CO_2 was released, the reaction was repeated with the [1-^{14}C]24:1 fatty acid in stoppered tubes without the traps, and 3 ml of the head-space gas was drawn off and analyzed by radio-GLC (Fig. 4 *Inset*). The results of both experiments showed that only $^{14}CO_2$ was released in the reaction. Thus,

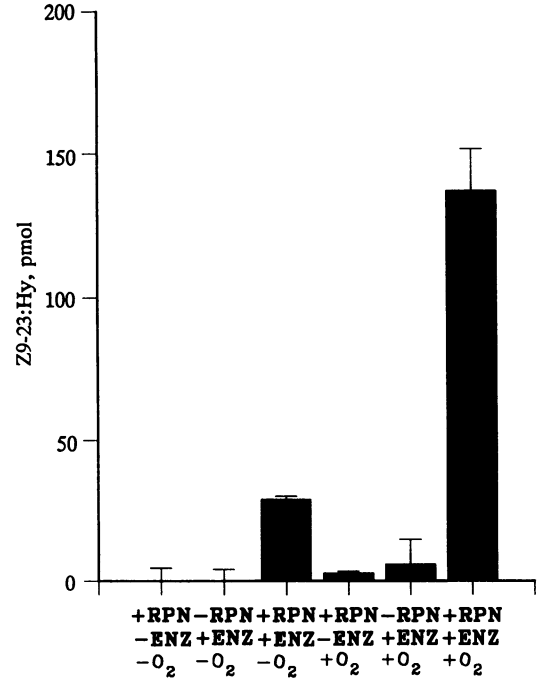

FIG. 3. Effect of O_2 and NADPH (reduced pyridine nucleotide, RPN) on the conversion of [15,16-$^3H_2$]24:1 aldehyde to Z9-23:Hy. Experiments were performed as described in text. ENZ, enzyme.

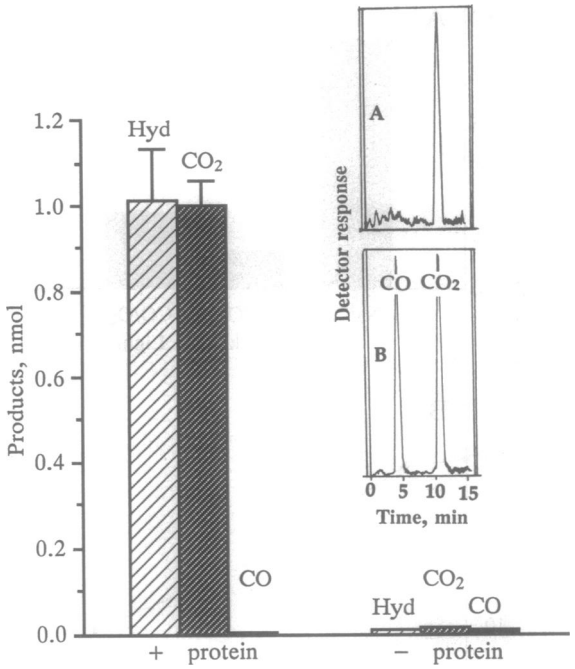

FIG. 4. Formation of [^{3}H]Z9-23:Hy (Hyd), $^{14}CO_2$, and ^{14}CO from equal amounts of [15,16-$^3H_2$]24:1 fatty acid and [1-^{14}C]24:1 fatty acid in microsomal preparations from the housefly. (*Inset*) Radio-GLC of the head-space product from the incubation of [1-^{14}C]24:1-CoA with housefly microsomes. (*Inset A*) Product from microsomes incubated with [1-^{14}C]24:1-CoA. (*Inset B*) ^{14}CO and $^{14}CO_2$ standards. Experiments were performed as described in text.

the use of both CO/CO_2 traps and radio-GLC showed that the carbonyl carbon was given off as CO_2. In addition, radio-GLC analysis of the head-space gas derived from incubations of ^{14}CO with microsomes in the presence or absence of NADPH showed that $^{14}CO_2$ was not formed (data not shown).

Inhibition of Hydrocarbon Formation by CO and Antibody to Cytochrome P450 Reductase. Bubbling 80:20 CO/O_2 through the reaction mixture in the dark markedly inhibited the conversion of the 24:1 aldehyde to Z9-23:Hy (Fig. 5*A*). The inhibition was partially reversed by white light (which contains wavelengths of 450 nm). Inclusion of antibody prepared against cytochrome P450 reductase (18) in the reaction mixture also inhibited the conversion of 24:1 aldehyde to Z9-23:Hy in a dose-dependent manner (Fig. 5*B*). Bovine serum albumin, which was used as a control at twice the concentration of the antibody, did not affect the reaction (data not shown).

DISCUSSION

The mechanism for hydrocarbon biosynthesis in the housefly is similar to that reported by Kolattukudy and his group (6, 11–14) for hydrocarbon formation in algae, plants, and animals in that an acyl-CoA is reduced to the corresponding fatty aldehyde prior to conversion to the hydrocarbon. Evidence for an aldehyde intermediate in the housefly is as follows: (*i*) aldehydes are produced as intermediates *in vitro* in the conversion of acyl-CoA to hydrocarbon; (*ii*) incubation with the aldehyde trapping agent, hydroxylamine, inhibited hydrocarbon formation; and (*iii*) 24:1 aldehyde was directly converted to Z9-23:Hy in the presence of NADPH and O_2. Hydrocarbon biosynthesis in the housefly is different from the mechanism proposed by Kolattukudy and coworkers (6, 11–14) in that in their system, the aldehyde was converted to hydrocarbon without a cofactor requirement under anaerobic conditions with the carbonyl carbon released as CO. The

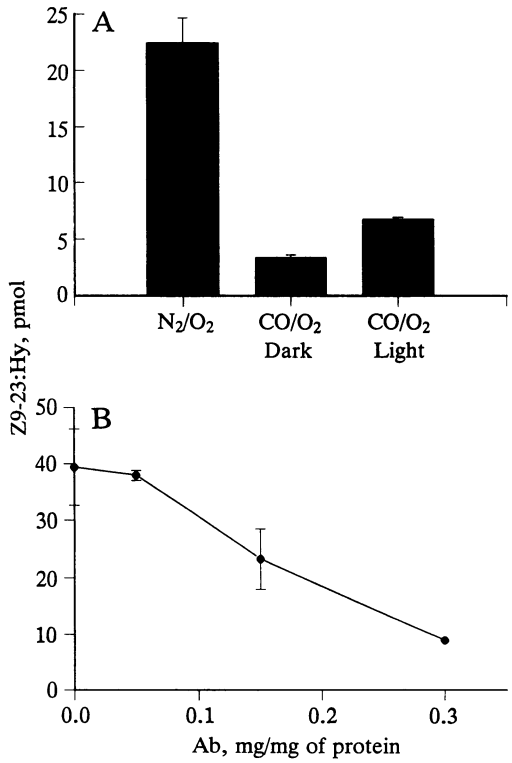

FIG. 5. Effect of CO (*A*) and antibody to housefly cytochrome P450 reductase (*B*) on Z9-23:Hy formation from [15,16-$^3H_2$]24:1 aldehyde. Experiments were performed as described in text.

work reported herein shows that O_2 and NADPH are required and that the carbonyl group is released as CO_2.

There does not appear to be a change in the chain length specificity in the reductive conversion of very long-chain acyl-CoAs to hydrocarbons in the housefly (17). The change in the chain length of hydrocarbons produced as the female housefly becomes vitellogenic and produces C_{23} alkenes and alkanes rather than C_{27} alkenes is accomplished by a change in the chain-length specificity of the fatty acyl-CoA elongation system rather than a change in the chain-length specificity of the reductive conversion of fatty acyl-CoAs to alkenes (17).

The requirements for NADPH and O_2 for the conversion of aldehyde to hydrocarbon and the release of the carbonyl carbon as CO_2 in the housefly suggested the possibility that a cytochrome P450-dependent mixed function oxidase participated in this reaction. The efficient inhibition of the reaction by CO and its partial alleviation by light supports this concept. Further evidence supporting the role of a cytochrome P450 enzyme was provided by the experiments showing that antibody to the housefly cytochrome P450 reductase inhibited the conversion of aldehyde to hydrocarbon. Yoder *et al.* (14) presented evidence that hydrocarbon biosynthesis in another dipteran, the fleshfly *Sarcophaga crassipalpis*, occurs by a decarbonylation mechanism. It is difficult to reconcile their conclusion with our results, since both experiments were performed in dipterans. Yoder *et al.* (14) did use a nonphysiological shorter chain C_{18} aldehyde, rather than aldehydes of the same chain length as the major hydrocarbons, which might account for the differences. Further work is needed to verify the role of a cytochrome P450 in hydrocarbon synthesis in the housefly and to determine how general this mechanism is in insects and other organisms. In addition to serving as the major sex pheromone component, Z9-23:Hy is also the precursor to a C_{23} epoxide and ketone, both of which are formed by cytochrome P450-dependent enzymes (26). Thus, it appears that cytochrome P450 enzymes are involved in several steps of sex pheromone biosynthesis in the housefly.

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