

Major Hydrocarbons of the Post-Pharyngeal Glands of Mated Queens of the Red Imported Fire Ant *Solenopsis invicta*

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ABSTRACT

Thin layer and column chromatographic analyses showed that hydrocarbons were the major lipoidal components of post-pharyngeal glands of mated queens of the red imported fire ant, *Solenopsis invicta*. Gas liquid chromatographic analyses on an OV-17 column showed four major hydrocarbons which have been identified and confirmed by synthesis and comparative mass spectral analyses as 13-methylheptacosane, 13,15-dimethylheptacosane, 3-methylheptacosane, and 3,9-dimethylheptacosane. When microgram quantities of the natural alkanes on filter paper were placed in colonies of ants, the ants clustered on the paper about the sample and proceeded to masticate the paper in the area containing the alkanes.

INTRODUCTION

The post-pharyngeal glands (PPG) that occupy a large portion of the head overlaying the brain with openings in the pharynx (1-3) are an important pair of structures in ants. In the red imported fire ant, *Solenopsis invicta*, the PPG of the queen are more highly developed than the PPG of other castes (B.M. Glancey, unpublished observations) and they become disproportionately large in queens and are filled with fluid prior to the mating flight (B.M. Glancey, unpublished observations). Although no definite roles or functions have been established for the PPG, it has been recently suggested that they function in much the same way as a gastric caecum (4). We have examined the lipids of PPG of queens 1 to 8 weeks after the mating flight and report in this paper on the identification and organic synthesis of the major lipoidal components of the PPG.

EXPERIMENTAL PROCEDURES

Instrumentation

Melting points were observed on a Kofler block, and IR spectra were obtained with a Perkin-Elmer Model 221 prism-grating spectrophotometer. Gas liquid chromatographic (GLC) analyses were made on a Barber-Colman Model 10 chromatograph; the GLC systems were 0.75% SE-30 and 1.0% OV-17 coated on Gas-Chrom P. The mass spectra of the various intermediates and hydrocarbons were obtained by using an LKB Model 9000 (Electron Impact) gas chromatograph-mass spectrometer (GC-MS),

(LKB Produkter AB, Stockholm, Sweden) equipped with a Varian Spectro System 100 MS data system; the samples were introduced directly into the ionization chamber of the mass spectrometer through the GLC system and the ionization energy was 70 eV. The hydrocarbons were also analyzed by an analytical GC-MS LKB Model 2091, Electron Impact/Chemical Ionization GC-MS-EI, using an SE-30 glass capillary column 0.2 mm id x 25 m. The spectrometer was equipped with an LKB-2130 GC-MS Data system. In addition, chemical ionization mass spectra of the hydrocarbons were obtained with a Finnigan 4000 mass spectrometer fitted with 0.25 mm id x 30 m, SP-2100 glass capillary column. Isobutane chemical ionization was used for production of ions. Data were collected and analyzed via an Incos Data system.

Extractions and Isolation of Hydrocarbons from Post-Pharyngeal Glands

The PPG were removed from queens 1 to 8 weeks after the mating flight (ages mixed within this range) and stored and homogenized in benzene (initially, PPG were extracted with chloroform/methanol (2:1); because the major lipid was hydrocarbons, benzene was used for subsequent extractions). The solution was filtered through a fritted disc funnel and the benzene was evaporated under a stream of nitrogen. The residue was chromatographed over 2.0 g of Unisil and the column was eluted with 15 mL of hexane followed by 15-mL fraction of hexane containing 2, 5, 10, 25, and 50% diethyl ether, then 100% diethyl ether.

The fractions were analyzed on two separate thin layer chromatography (TLC) plates; one plate was developed in the solvent system of benzene/hexane (1:1) and the other in benzene/ethyl acetate (9:1). The hexane fraction which contained the hydrocarbons was analyzed by argentation TLC.

Bioassay of PPG Hydrocarbons

Either a 10- or 20- μ g sample in hexane (1 μ g/ μ L) of chromatographically pure hydrocarbons from the PPG was spotted on the center of a 5 \times 5 cm piece of filter paper. A blank was similarly treated with the appropriate volume of hexane. After the hexane had evaporated, the papers with and without hydrocarbons were placed in laboratory colonies of about 10,000 ants. The ants were observed for 2 hr.

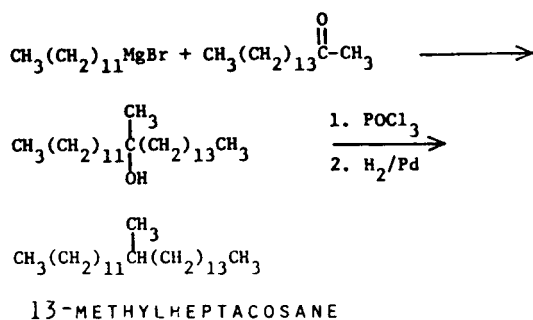
Materials and Chemical Synthesis of Intermediates Used in Schemes I-IV

Silicic acid (Unisil 100 to 200 mesh) was obtained from Clarkson Chemical Co. (Williamsport, PA). Neutral alumina (Woelm) was obtained from ICN Nutritional Biochemicals (Cleveland, OH), and the required amount of water was added to make activity Grade II alumina. Docosanol was purchased from Lachat Chemicals, Inc. (Mequon, WI), Dodecyl bromide was purchased from Aldrich Chemical Co. (Milwaukee, WI) and 3-methyl-1-pentanol from Albany International Chemicals Division (Columbus, OH). The reaction of 3-methyl-1-pentanol with triphenylphosphine dibromide (5) gave 1-bromo-3-methylpentane, bp 47-49 C, 20 mm (used in Scheme III). The reaction of dodecyl magnesium bromide with acetaldehyde gave in nearly quantitative yield 2-tetradecanol, (mp 33-34 C) which, when allowed to react with triphenylphosphine dibromide (5), gave 2-bromotetradecane, bp 152-155 C, 9 mm. This material was used as starting material in Scheme II. The other starting material in Scheme II, 2-methyltetradecanal, was prepared via the reaction of dodecyl bromide and the sodium salt of diethyl methylmalonate (6); this was followed by decarboxylation and treatment of the resulting monocarboxylic acid with diazomethane to give the methyl 2-methyltetradecanoate. Reduction of the ester with lithium aluminum hydride in ether gave the alcohol which, upon oxidation with anhydrous chromium trioxide-pyridine in methylene chloride (7), gave 2-methyltetradecanal. A similar oxidation of docosanol yielded docosanal (starting materials, Scheme III). The required starting material (4-methyl docosanol) in Scheme IV was also prepared by the condensa-

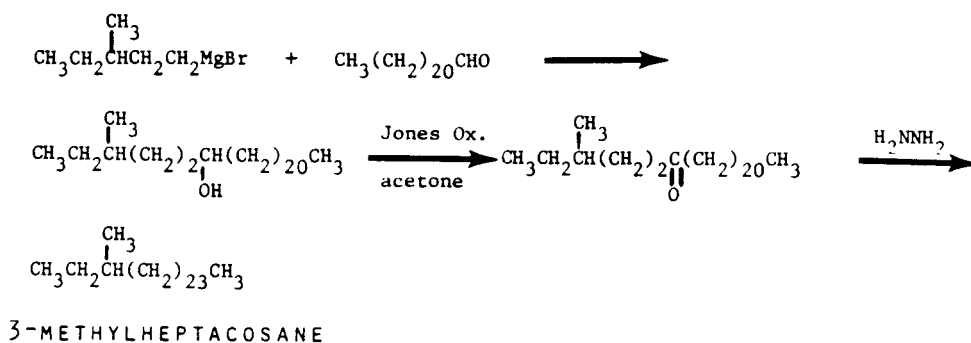
tion of octadecyl bromide and the sodium salt of diethylmalonate (6), followed by decarboxylation to the monocarboxylic 2-methyleicosanoic acid, mp 62-66 C. Treatment of the acid with diazomethane followed by reduction with lithium aluminum hydride gave 2-methyleicosanol, mp 48-50.5 C, which was converted by oxidation with anhydrous chromium trioxide-pyridine in methylene chloride (7) to 2-methyleicosanal. The reaction of this aldehyde with malonic acid in pyridine containing 0.4% piperidine gave the 4-methyl-2-docosenoic acid, which upon treatment with diazomethane and subsequent catalytic reduction with 10% palladium on charcoal in cyclohexane followed by a reduction with lithium aluminum hydride, gave 4-methyl docosanol, mp 48-49 C.

Preparation of 13-Methylheptacosane (Scheme I)

To a solution of dodecyl magnesium bromide, prepared by refluxing 0.4 g of magnesium and 4.15 g of dodecyl bromide in 75 mL of anhydrous diethyl ether until all magnesium had been consumed (4 hr), was added 4.0 g of 2-hexadecanone in 75 mL of ether and the mixture was refluxed for 8 hr. The solution was cooled and 10 mL of water was added by drops, followed by 15 mL of a solution of 6 N sulfuric acid. The ethereal phase was separated, washed with water, dried over anhydrous sodium sulfate and reduced to dryness under vacuum to give 6.28 g of crude 13-methyl-13-heptacosanol. The material was chromatographed over 120 g of hexane-washed neutral alumina and the following 100-mL fractions were collected: 1, hexane, 2, hexane/benzene (9:1), 3, hexane/benzene (1:1), 4, benzene, and fraction 5 and 6 of benzene/ether (3:1). On the basis of TLC analyses, fractions 4-6 were combined to give 4.3 g of material. Recrystallization of a sample from acetone-methanol yielded needles, mp 50-51 C. To 4.2 g of the 13-methyl-13-heptacosanol in 75 mL of dry pyridine and 25 mL of benzene was added 3.0 mL of phosphorus oxychloride and the solution was refluxed for 1 hr and then 40 mL of



SCHEME I. Synthesis of 13-methylheptacosane.

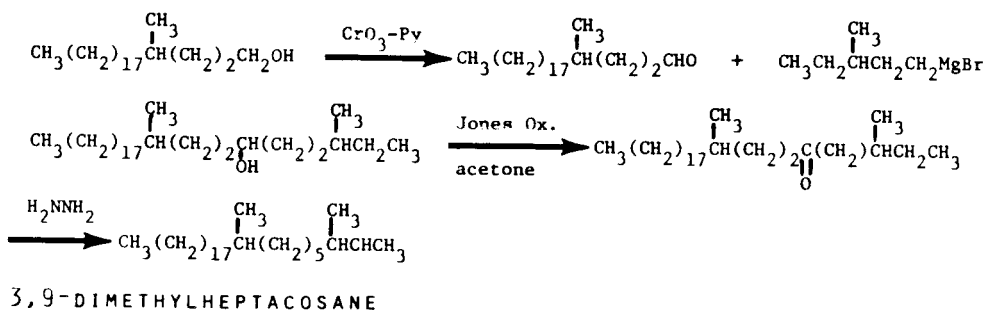


SCHEME III. Synthesis of 3-methylheptacosane.

added 6.0 g of freshly prepared docosanal over a 30-min period. The mixture was refluxed and, when worked up as in the preparation of 13-methyl-13-heptacosanol, yielded 7.65 g of crude alcohol. Purification of the material by column chromatography over 153 g of activity Grade II neutral alumina gave 5.63 g of 3-methyl-6-heptacosanol, mp 62-63 C. The oxidation of 3-methyl-6-heptacosanol (5.0 g) in 200 mL of acetone with an 8 N solution of chromic acid in dilute sulfuric acid (Jones reagent [8]) gave 4.6 g of 3-methyl-6-heptacosanone, mp 54-55 C. To a 3-neck flask equipped with a reflux condenser and a thermometer was added 150 mL of triethylene glycol and 0.5 g of sodium and the mixture was heated below 90 C until the sodium had been consumed (9). The mixture was cooled, 4.5 g of 3-methyl-6-heptacosanone in 50 mL of anhydrous diethyl ether and 4.0 mL of hydrazine was added; the mixture was gently heated and the ether was removed by distillation. The temperature of the solution was gradually raised to 140 C, maintained between 140-145 C overnight, then gradually raised to 212 C and kept at that temperature for 3 hr. After cooling, the solution was poured into a cold solution of dilute hydrochloric acid; the precipitate was collected and dried to give 4.3 g of 3-methylheptacosane, mp 45-46 C. A sample recrystallized from acetonitrile/ether melted at 46-47 C.

Preparation of 3,9-Dimethylheptacosane (Scheme IV)

To a magnetically stirred solution of 8.3 g of pyridine in 200 mL of methylene chloride was added 5.25 g of chromium trioxide and the deep burgundy solution was stirred for 15 min at room temperature (7). At the end of this period, a solution of 3.0 g of 4-methyl-docosanol in 75 mL of methylene chloride was added and the mixture was stirred for an additional 15 min. The solution was decanted from the tarry black deposit which was washed with 200 mL of ether. The combined organic phases were washed with solutions of 5% sodium hydroxide, 5% hydrochloric acid, 5% sodium bicarbonate and then with water, and dried over sodium sulfate. After solvent removal under vacuum, 2.6 g of the 4-methyl-docosanal was obtained. The aldehyde (2.6 g) in 100 mL ether was added to a refluxing solution of 1.45 g of 3-methylpentyl magnesium bromide in 50 mL of ether and the mixture was refluxed for 8 hr. The work-up of the Grignard in the usual manner gave the crude 3,9-dimethyl-6-heptacosanol, which, when chromatographed over 75 g of activity Grade II neutral alumina and eluted with the solvent systems used in the chromatography of 13-methyl-13-heptacosanol (Scheme I), yielded 2.2 g of chromatographically pure 3,9-dimethyl-6-heptacosanol, mp 37-39 C. The oxidation of 3,9-dimethyl-6-heptacosanol (2.1 g) in 100 mL



SCHEME IV. Synthesis of 3,9-dimethylheptacosane.

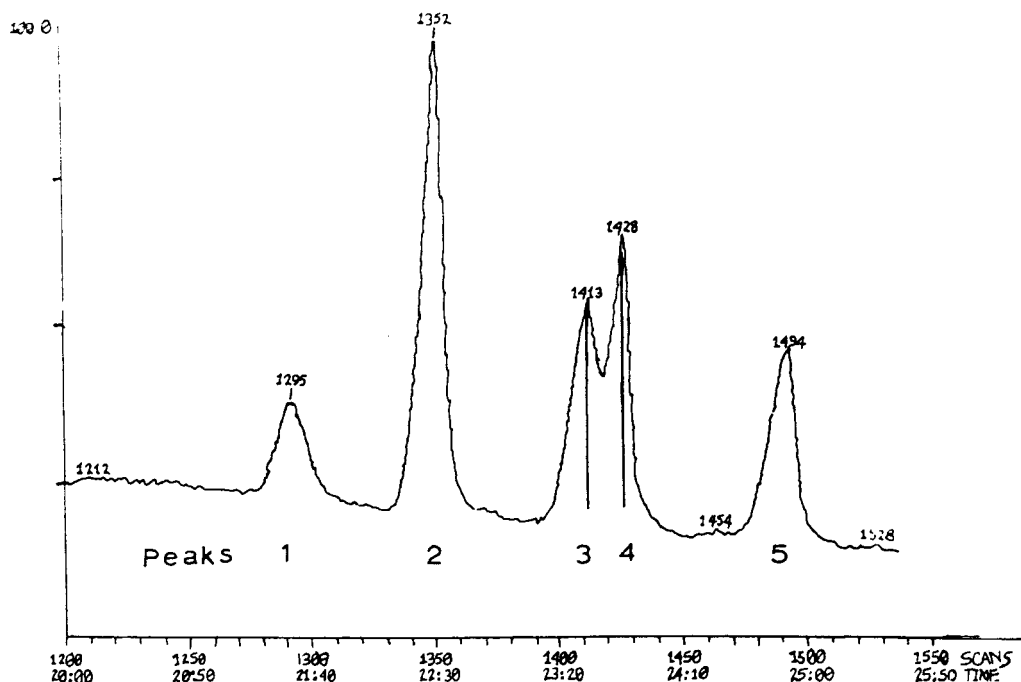


FIG. 1. GC-MS total ion monitor trace of the hydrocarbons from PPG of *S. invicta* obtained with a Finnigan 4000 mass spectrometer.

of acetone with Jones reagent (8) gave 2.0 g of 3,9-dimethyl-6-heptacosanone, mp 30-31 C. A modified Wolf-Kishner reduction of 3,9-dimethyl-6-heptacosanone (9) as in preparation of 3-methylheptacosane (Scheme III) gave 1.69 g of crude product, which, when filtered in hexane through 30 g of Unisil, gave in the second 50 mL fraction 1.57 g of 3,9-dimethylheptacosane, refractive index at 15 C 1.4519.

RESULTS

Surprisingly, TLC and column chromatographic analyses showed that the major lipoidal components of PPG from queens 1 to 8 weeks after the mating flight were hydrocarbons. In a typical experiment, 15 PPG yielded 1,000 μ g of lipids, which, when chromatographed over 2 g of Unisil and the residue from each fraction weighed, showed that the fraction eluted with hexane yielded 601 μ g of saturated hydrocarbons as indicated by argentation TLC analyses. The remaining 40% of lipids consisted of triglycerides, waxes, sterol esters, alcohols and sterols. GLC analyses of the hydrocarbons on SE-30 showed a minor peak with an equivalent chain length (ECL) of 27 and three major peaks with ECL of 27.5, 27.7 and 28, respectively. The GLC analyses of the hydrocarbons on an OV-17 column, however, yielded a minor peak with an ECL of 27 and four major peaks (peaks 2, 3, 4, and 5) with ECL of 27.3, 27.5, 27.7 and 28, respectively. Thus, the second

major peak on the SE-30 column separated into two peaks, the third and fourth peaks on the OV-17 system. Peaks 1 through 5 on the OV-17 column represented ca. 6, 35, 17, 22 and 15%, respectively, of the total hydrocarbons. Minor peaks eluting both before and after accounted for the remaining 5%.

The hydrocarbons, when analyzed by GC-MS on a Finnigan spectrometer equipped with an SP-2100 glass capillary column, also separated into four major peaks similar to those on the OV-17 column. A typical total ion monitor trace is shown in Figure 1. The chemical ionization (CI) mass spectra of these hydrocarbons are shown in Figures 2 and 3. The mass spectra of hydrocarbon peaks 2-5 (Fig. 1) indicated that all were methyl branched alkanes. The CI-mass spectrum of peak 1, with a base peak at $(M-1)^+$ at m/z 379 and the ECL of 27, identified peak 1 as heptacosane. The EI spectrum (Fig. 4) also supports this structure. The $(M-1)^+$ peak at m/z 393 of the compound with an ECL of 27.3 (peak 2, Fig. 1) and fragment ion pairs at m/z 224/225 and 196/197 which arise from cleavage on each side of a methyl branch, established the structure for this component as 13-methylheptacosane (Fig. 2, spectrum 2).

The second major branched alkane (peak 3, Fig. 1) with an ECL value of 27.5 on OV-17 was identified as 13,15-dimethylheptacosane (Fig. 2, spectrum 3). The $(M-1)^+$ peak at m/z 407 established the molecular weight as 408.

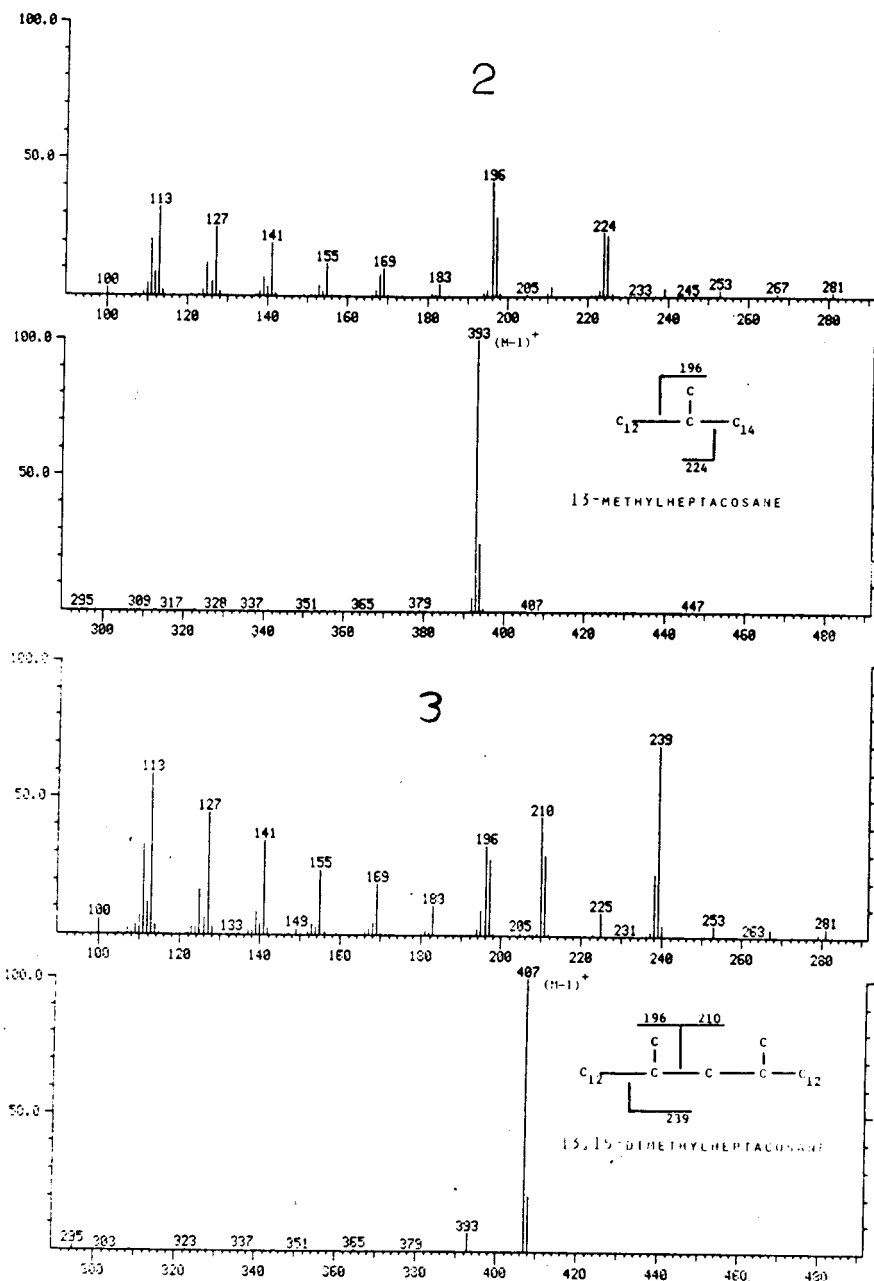


FIG. 2. CI mass spectra of GLC hydrocarbon peaks 2 and 3, respectively, from PPG of *S. invicta*, (2) 13-methylheptacosane and (3) 13,15-dimethylheptacosane.

Cleavage internal to either methyl branch yielded a secondary carbonium ion that gave the ion pairs at m/z 196/197 whereas cleavage external to the methyl branch gave the 17-carbon secondary carbonium ion at m/z 239. The ion pairs at m/z 210/211 resulted from cleavage internal to the methyl branch which formed a 15-carbon primary carbonium ion. The small peak in the spectrum at m/z 393 ($M-1$)⁺ most likely results from a small amount of contamination of the alkane of peak 4.

The GC-CI-MS analyses of peak 4 (Fig. 1) with an ECL of 27.7 showed ($M-1$)⁺ ion peak at

m/z 393 and a strong fragment ion at m/z 365 which indicated cleavage at the methyl branch (Fig. 3, spectrum 4). Thus, this compound was identified as 3-methylheptacosane.

The CI-mass spectrum of peak 5 (Fig. 1) showed a ($M-1$)⁺ at m/z 407 which indicated a carbon number of 29. Thus, the ECL of 28 and the mass spectral data of this hydrocarbon suggested a methyl branch at 2, 3 or 4 positions and a second methyl group farther down the chain. The ion pairs at m/z 155/154 and 281/280 suggested that the major hydrocarbon of peak 5 was 3,9-dimethylheptacosane (Fig. 3,

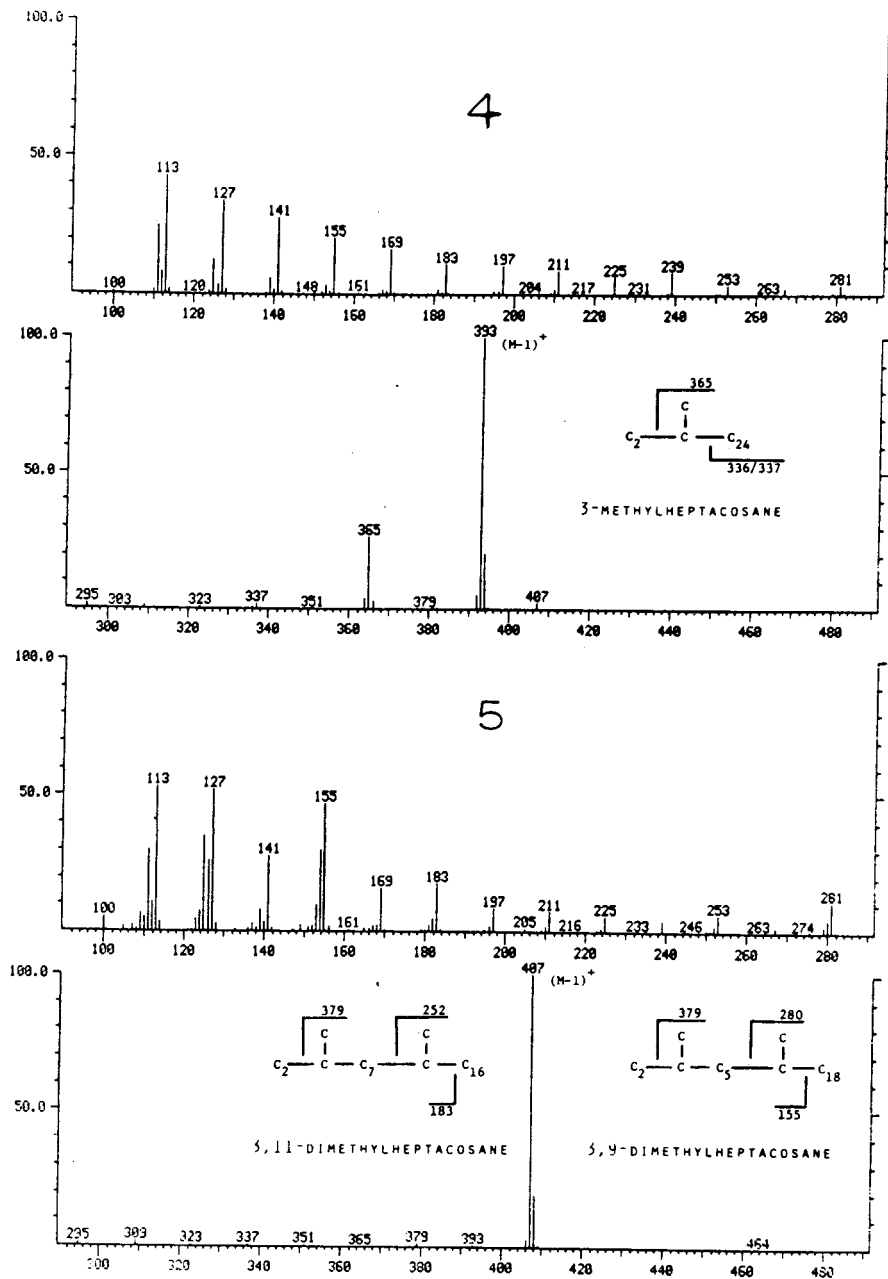


FIG. 3. CI mass spectra of GLC hydrocarbon peaks 4 and 5, respectively, from PPG of *S. invicta*, (4) 3-methylheptacosane and (5) 3,9-dimethylheptacosane and 3,11-dimethylheptacosane.

spectrum 5). Unlike the CI-mass spectra of the other hydrocarbons, the mass spectrum of this peak was not readily interpretable; that is, fragments indicating branch points were not very obvious. The EI-mass spectrum (Fig. 8), however, confirms that the major hydrocarbon of this peak is indeed 3,9-dimethylheptacosane. The fragment ion pairs at m/z 183/182 and 253/252 indicate another component in peak 5 with the structure of 3,11-dimethylheptacosane.

The EI-mass spectral analyses of the hydro-

carbons of peak 1-5 and of respective synthetic compounds (Fig. 4-8) confirm the structures and identities of the major hydrocarbons of the PPG of the red imported fire ant. The ECL of the synthetic and naturally occurring respective hydrocarbons were identical.

Schemes I-IV show the syntheses of the red imported fire ant hydrocarbons and all were obtained in reasonable yields except for the 13,15-dimethylheptacosane. The structures of intermediates and final products were confirmed by IR and mass spectroscopy of the

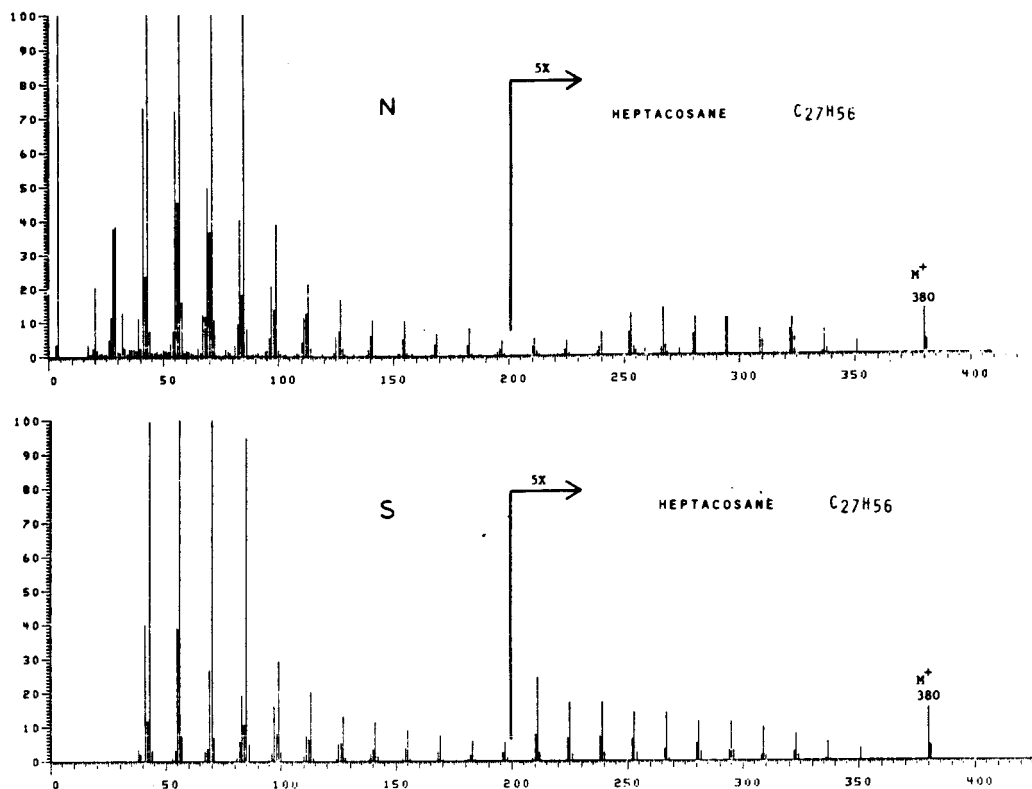


FIG. 4. EI mass spectra of GLC peak 1. (N) Heptacosane from PPG of *S. invicta* and (S) synthetic heptacosane.

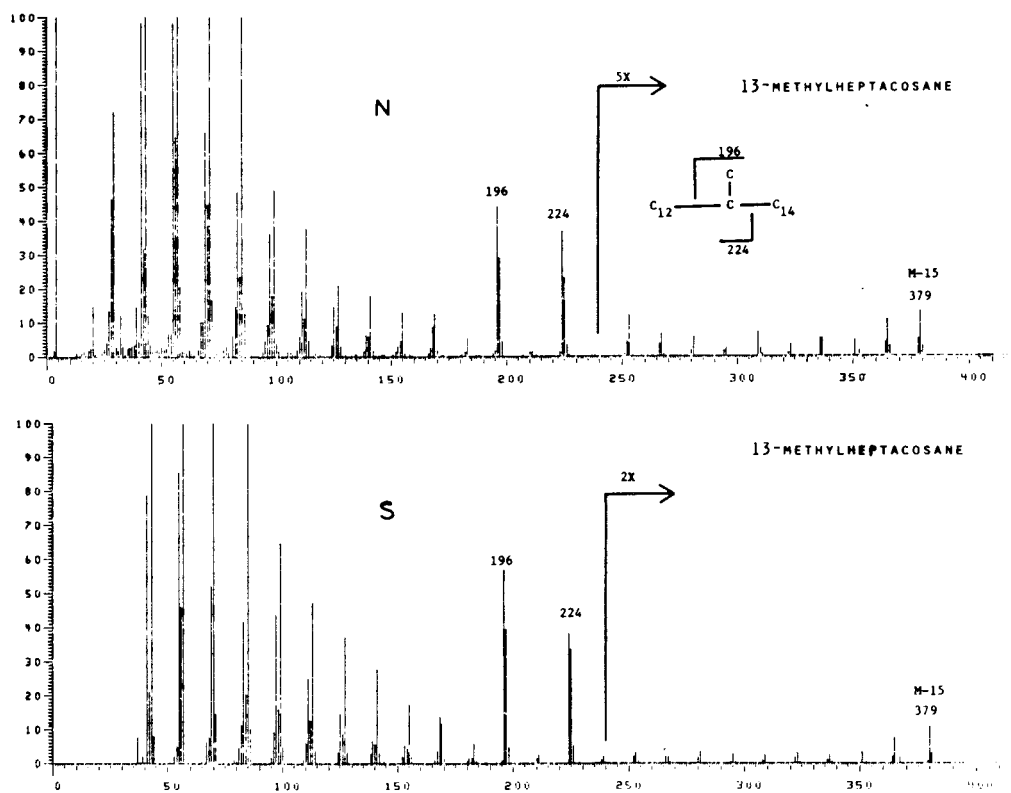


FIG. 5. EI mass spectra of GLC peak 2. (N) 13-Methylheptacosane from PPG of *S. invicta* and (S) synthetic 13-methylheptacosane.

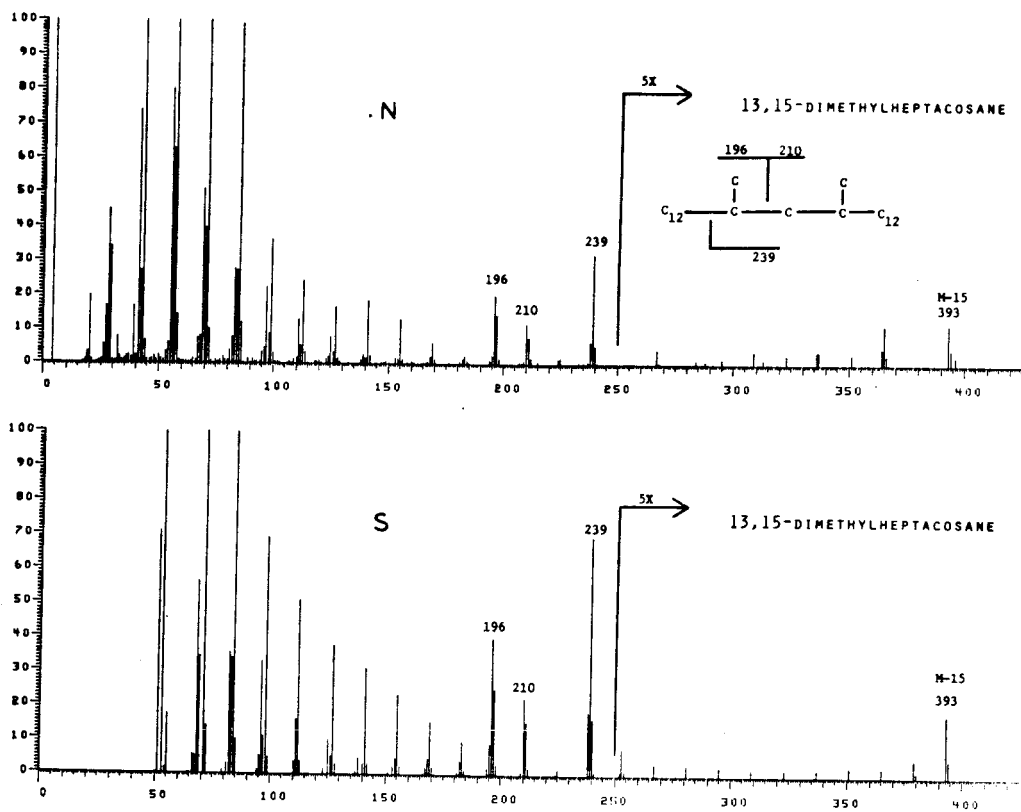


FIG. 6. EI mass spectra of GLC peak 3. (N) 13,15-Dimethylheptacosane from PPG of *S. invicta* and (S) synthetic 13,15-dimethylheptacosane.

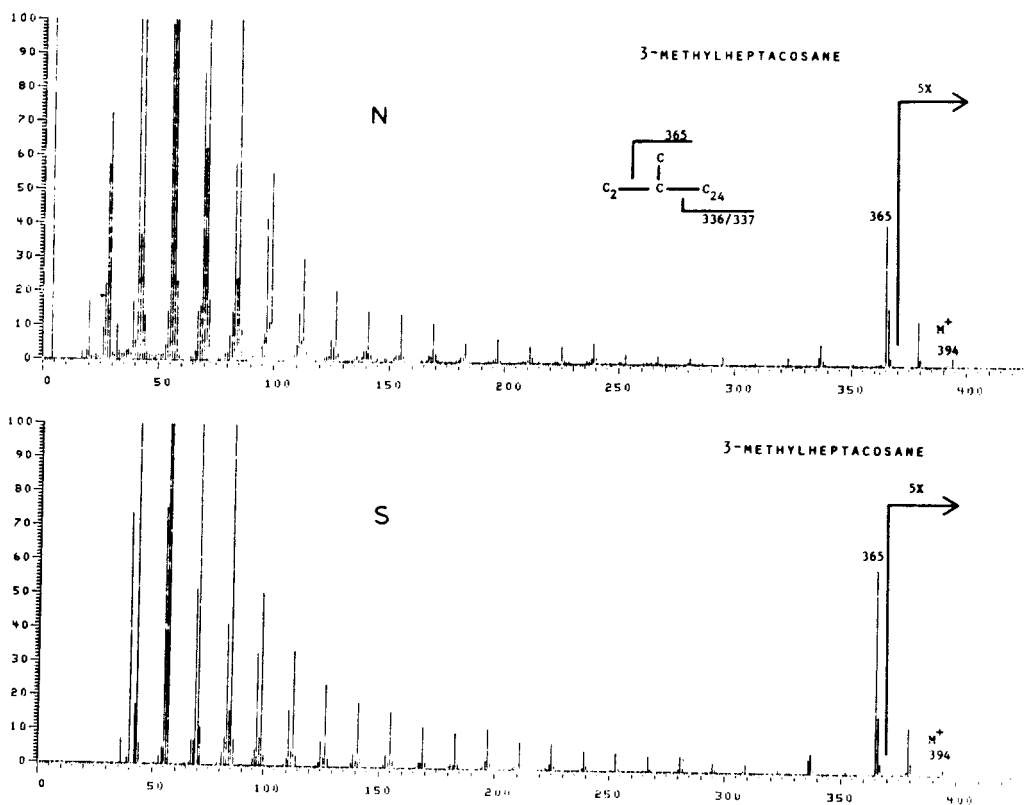


FIG. 7. EI mass spectra of GLC peak 4. (N) 3-Methylheptacosane from PPG of *S. invicta* and (S) synthetic 3-methylheptacosane.

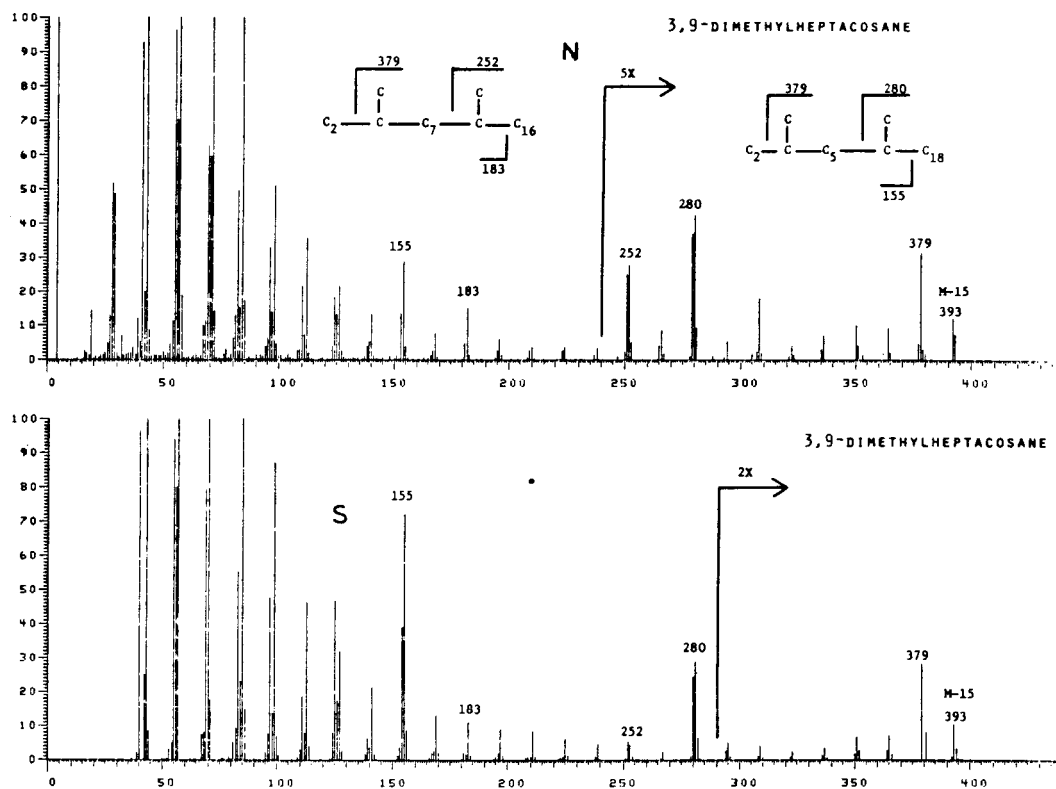


FIG. 8. EI mass spectra of GLC peak 5. (N) 3,9-Dimethylheptacosane and 3,11-dimethylheptacosane from PPG of *S. invicta* and (S) synthetic 3,9-dimethylheptacosane.

EI-MS of the synthetic hydrocarbons are presented in Figures 4-8. The purity of the final products as determined by GLC and argentation TLC was >98% except for 13,15-dimethylheptacosane which was of 90% purity. It contained 10% of the earlier-eluting hydrocarbon.

DISCUSSION

As previously noted, GC-CI-MS is the method of choice for determining the molecular weight of every component in complex alkane mixtures such as those found in insect cuticle (10) and it also provides branch point information (11,12). Thus, the GC-CI-MS (Figs. 2 and 3) readily show the molecular weights and branch points of the components of the peaks 1-5 (Fig. 1), and thus enhance the identification of these hydrocarbons. For example, the molecular weight of 394 and point branch which resulted in the fragment at m/z 365 (Fig. 3, spectrum 4) identified the component of peak 4 as 3-methylheptacosane. In this spectrum, the $(M-1)^+$ peak at m/z 407 indicated the presence of a small amount of an alkane with a molecular weight of 408 that most likely is derived from the component of peak 5. Although EI-MS often provides the best infor-

mation for determining the location of a methyl branch in long chain alkanes, it does not always show the molecular ion. The use of both CI and EI mass spectra greatly facilitated the structural analyses of the hydrocarbons of the PPG.

The results of this study indicate that hydrocarbons represent about 60% of the lipoidal components of the post-pharyngeal glands of queens 1 to 8 weeks after the mating flight. Although repetitive GC-MS scans of peaks 1-5 suggest the presence of several minor hydrocarbons other than those identified, the major hydrocarbons of peaks 1-5 that account for nearly 90% of the hydrocarbons of the PPG of mated queens are 13-methylheptacosane, 13,15-dimethylheptacosane, 3-methylheptacosane and 3,9-dimethylheptacosane. Proof of their structures has been confirmed by synthesis and comparative mass spectral analyses. The results of capillary GC-MS analyses of peaks 1-5 (Fig. 1) suggest that a longer SP-2100 capillary column perhaps would show a physical separation of these minor hydrocarbons, or at least would allow for even better GC-MS analyses and interpretations.

The relative percentages of the hydrocarbons of PPG of queens 2, 3, 4, 5 and 8 weeks after mating remain fairly constant with only a

noticeable change occurring in the relative amounts of 13,15-dimethylheptacosane; it decreased with time. 13-Methylheptacosane remained the predominant hydrocarbon of PPG of queens 2-8 weeks after mating. Interestingly, heptacosane, the smaller peak that precedes the major hydrocarbons of the PPG of mated queens, is a major hydrocarbon in PPG of virgin queens.

We have conducted GC-MS analyses only on the hydrocarbons of the PPG of virgin and mated queens. We have found similar GLC patterns, however, in the hydrocarbon fractions of PPG of repletes, of crops of major workers, and of Dufour's glands of queens 8 weeks after mating, and these results suggest that these hydrocarbons, although minor lipoidal components of crops and Dufour's glands, are present in other organs of the fire ants. In a reanalysis of the cuticular methylalkanes of *S. invicta*, they have been identified as the major hydrocarbons (13).

No interrelationship has been determined or established for the presence of these alkanes in the PPG of mated queens and certain organs or other ant castes. Interestingly, when either 10- or 20- μ g samples of the hydrocarbons in hexane were spotted on filter paper, the hexane evaporated and the paper placed in colonies of fire ants, the ants clustered about the sample and proceeded to masticate the paper in the area that contained the alkanes.

Since the PPG in the virgin queens are much larger than the PPG of other castes (B.M. Glancey, unpublished observations) and since the major hydrocarbons of the PPG are also the major cuticular hydrocarbons of *S. invicta* (13), it is possible that the secretion or contents of these glands may have unique functions in relation to colony organization, caste determination, species and/or case recognition, food exchange or queen brood tending. Work is underway to develop assay systems to determine what biological and physiological func-

tion, if any, the major hydrocarbons from the PPG of queens have in maintaining social organization, caste determination and interaction in colonies of red imported fire ants.

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