Cuticular Lipids of Insects: II. Hydrocarbons of the Cockroaches *Periplaneta australasiae*, *Periplaneta brunnea* and *Periplaneta fuliginosa*

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ABSTRACT

The major cuticular hydrocarbons of the cockroaches *Periplaneta australasiae*, *Periplaneta brunnea* and *Periplaneta fuliginosa* are *n*-tricosane, *cis*-9-tricosene, 3-methyltricosane, 11-methyltricosane and 13-methylpentacosane. There are as yet unexplained quantitative differences between the hydrocarbon compositions of males and females of *P. australasiae* and *P. fuliginosa*, *cis*-9-tricosene being a major hydrocarbon of the males only. A series of mono-methyl internally branched hydrocarbons ranging in chain length from 23 to 26 carbons with the methyl branch on the 13th carbon from one end was observed. Minor quantities of other hydrocarbons have been identified.

INTRODUCTION

In the first paper in this series, we demonstrated that the four principal hydrocarbons in the cuticular lipids of the Madeira cockroach (*Leucophaea maderae*) and the Oriental cockroach (*Blatta orientalis*) are *n*-heptacosane, 11-methylheptacosane, 13-methylheptacosane and 3-methylheptacosane (1). The cuticular hydrocarbons from these two genera of cockroaches are qualitatively identical and quantitatively similar.

The principal hydrocarbons obtained from the hemolymph and the whole carcass of the American cockroach (*Periplaneta americana*) were reported by Baker et al. (2) as *n*-pentacosane, 3-methylpentacosane and *cis,cis*-6,9-heptacosadiene accounting for approximately 12%, 20% and 65% of the total hydrocarbons, respectively. They also observed small quantities of *n*-heptacosane, *n*-nonacosane and C_{41}-C_{43} hydrocarbons. Hydrocarbons represented 0.35% of the hemolymph in males and 0.68% in females. Acree et al. (3,4) reported that the total and the relative quantities of the principal hemolymph hydrocarbons in the American cockroach and in the German cockroach (*Blattella germanica*) are not only sex-dependent but also fluctuate with age and photoperiod.

The present paper reports the identity and quantitative similarity of the cuticular hydrocarbons of three cockroaches in the *Periplaneta* genus.

EXPERIMENTAL PROCEDURES

The Australian cockroaches (*Periplaneta australasiae*, Fabricius), southern brown cockroaches (*Periplaneta brunnea*, Burmeister) and smoky brown cockroaches (*Periplaneta fuliginosa*, Serville) used were all adults. The cockroaches were raised in 30 gal garbage cans affixed with an electric fence near the top to prevent escape. The lid was on the cans except for feeding and watering so the insects were in near darkness most of the time. The insects were fed ad lib with a diet of ground Gravy Train dog food (General Foods Corp., White Plains, N.Y.), 2217 g; vitamin diet fortification mixture in dextrose (Nutritional Biochemical Corporation, Cleveland, Ohio), 200 g; cholesterol, 6.0 g; and zinc chloride, 1.0 g. Water was also available at all times in the form of a 1% agar-99% water gel.

The cockroaches were killed by freezing at -29 C for 20 min. The surface lipids were removed by placing the whole roackes in a Buchner funnel with medium porosity fritted disc and covering the roaches with hexane, allowing the extract to pass through the fritted disc. Fresh hexane was added two more times. The hexane extracts were all combined. The roaches were then extracted with chloroform in the above manner. The solvent was removed under vacuum or under nitrogen sweep. All solvents were glass redistilled, and all samples were stored under nitrogen at 4 C.

The surface lipid extracts were investigated analytically on silica gel coated thin layer plates developed in hexane-diethyl ether-acetic acid (90:10:1 v/v/v) and compared to standards. The hydrocarbons were separated from other surface lipids by column chromatography. The columns were disposable Pasteur pipets stoppered with glass wool and filled with about 10 mg of silica gel per mg of lipid to be separated. The hydrocarbons were eluted with hexane and the other lipids eluted with chloroform. Purity of the fractions was verified using the above thin layer system.

Preparative thin layer chromatography (TLC) of the hydrocarbons on 5% silver

TABLE I

Characteristic Peaks in the Mass Spectra of the Internally Branched Cuticular Hydrocarbons of *P. australasiae*, *P. brunnea* and *P. fuliginosa*

Hydrocarbon	m/e	Fragment	m/e	Fragment
11-Methyltricosane	196(197)	$C_{14}H_{28}(H)$[a]	168(169)	$C_{12}H_{24}(H)$
12-Methyltetracosane	196(197)	$C_{14}H_{28}(H)$	182(183)	$C_{13}H_{26}(H)$
13-Methylpentacosane	196(197)	$C_{14}H_{28}(H)$	196(197)	$C_{14}H_{28}(H)$
13-Methylhexacosane	196(197)	$C_{14}H_{28}(H)$	210(211)	$C_{15}H_{30}(H)$

[a]Parenthesis signifies the presence of a labile hydrogen; the intensity of the peaks in parenthesis ranges from 60% to 100% of the other peak.

nitrate-impregnated silica gel thin layer plates developed in hexane separated the monoene hydrocarbon from the saturated and branched hydrocarbons. The position of the double bond in the monoene hydrocarbon was determined by osmium tetroxide oxidation (5), followed by periodate-permanganate oxidation (6) to form fatty acids. The fatty acids were methylated with diazomethane (7) and gas chromatographed on a 4 ft 2% SE-54 on Gas Chrom Z column comparing the retention times to the NIH Standard Mixture F.

The normal hydrocarbons were separated from the branched hydrocarbons by adding activated molecular seive 5A to a solution of the hydrocarbons in 2,2,4-trimethyl pentane (8). Preparative gas liquid chromatography (GLC) of the branched hydrocarbons on a 10 ft 5% OV-17 on Gas Chrom Z column yielded sufficient quantities of each branched hydrocarbon for mass spectrometry.

Analytical GLC of the hydrocarbons was carried out on a 6 ft 2% SE-54 on Gas Chrom Z column operated at either 200 C isothermal or programmed from 150 to 250 C in 10 min. The flow rate was 40 ml helium per minute. Quantitation was obtained by disc integration of the recorder trace.

Mass spectra were determined with a Hatachi RMU-6A instrument operating at ionizing voltage 75 ev, trap current 80μA. The samples were inserted directly into the ion source.

The infrared spectrum of *cis*-9-tricosene was taken by spreading the compound on a salt plate and taking the spectrum with a Beckman IR-20.

RESULTS AND DISCUSSION

The hexane extracts contained from 1.3 to 2.0 mg of cuticular lipid per cockroach, with the extracts from adult females having slightly more lipid than those from males. Adult females generally weighed on the order of 10% more than did their male counterparts. The hexane extract was composed of approximately 90% hydrocarbon, 7% triglyceride, 2% free fatty acids and 1% sterol (as estimated from preparative TLC). The chloroform extract of the previously hexane-extracted cockroaches contained only up to 0.15 mg per cockroach. Comparative TLC of the hexane extract and the chloroform extract showed that the chloroform extract had more triglyceride and some fatty acid methyl esters that the hexane extract did not have. It appears that chloroform might be penetrating into the internal lipid and extracting more triglyceride. In insects, the fatty acid methyl esters may be real or they may be artifacts of the chloroform extraction as shown by Sloan et al. (9). Since hexane extracts nearly as much lipid and without possible artifacts, only the hexane extract will be discussed further. Also, due to insufficient quantity of triglycerides, free fatty acids and sterols, we will limit our discussion to the cuticular hydrocarbons.

The structural assignment of the normal saturated hydrocarbons was made on the basis of their GLC retention time, their lack of complexing on silver nitrate-impregnated silica gel thin layer chromatography, and their inclusion in molecular seive 5A. Oxidation of the monoene hydrocarbon followed by methylation and gas liquid chromatography gave nonanoic methyl ester and myristic methyl ester in nearly equal quantities. The structure of the normal monoene was assigned as *cis*-9-tricosene. The *cis* assignment was based on the lack of a *trans* peak at 945 cm^{-1} and the presence of a peak at 730 cm^{-1} in the infrared spectrum.

The position of the methyl branch on the hydrocarbons that were not absorbed into molecular seive 5A was made on the basis of the mass spectral data. The mass spectra of the 3-methyl branched hydrocarbons were compared to mass spectra from 3-methyl (anteiso) hydrocarbons from other cockroaches (1,2) and from other sources (10,11). The principal 3-methyl hydrocarbon from these three *Periplaneta* is 3-methyltricosane, which is two car-

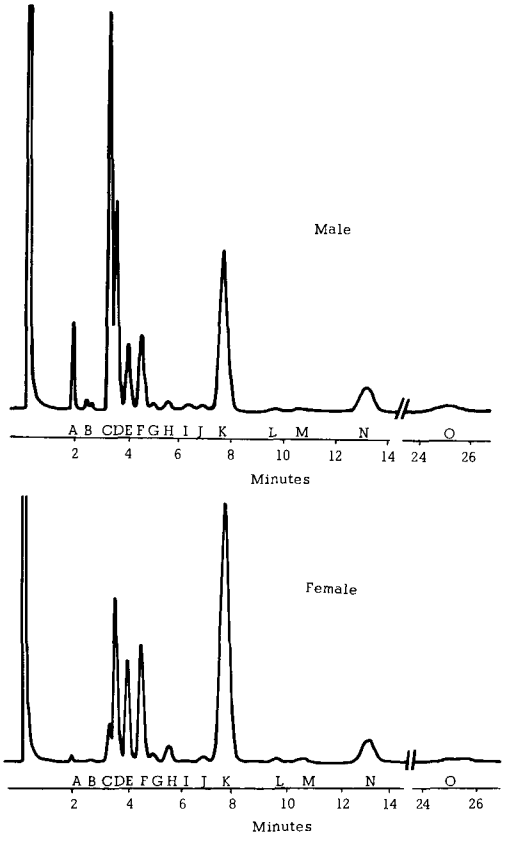

FIG. 1. Gas chromatogram of cuticular hydrocarbons from male and female *P. fuliginosa*. For identification of the hydrocarbons see Table II.

bons shorter than the 3-methylpentacosane from *Periplaneta americana* (2) and four carbons shorter than the 3-methylheptacosane from *L. maderae* and *B. orientalis* (1). The trace quantities of 3-methyltetracosane (a 3-methyl hydrocarbon with an even number of carbons in the chain) on the males of *P. australasiae* and *P. fuliginosa* are interesting and will be studied further, particularly in connection with the biosynthesis of 3-methyl hydrocarbons.

The presence of mono-methyl internally branched hydrocarbons in the cuticular lipids of cockroaches was first observed in *L. maderae* and *B. orientalis* cuticular hydrocarbons (1) and is again observed in the cuticular hydrocarbons of *P. australasiae, P. brunnea* and *P. fuliginosa*. The assignment of 11-methyltricosane, 12-methyltetracosane, 13-methylpentacosane and 13-methylhexacosane to the internally branched hydrocarbons is based on the mass spectral data in Table I. In the mass spectra of all four hydrocarbons there is a fragment of 196(197)m/e, indicating that these are of a homologous series with a characteristic CH_3-$(CH_2)_{11}$-$CH(CH_3)$-$(CH_2)_n$-CH_3 structure where n equals 9,10, 11 and 12. As is characteristic of other natural hydrocarbon isolates, hydrocarbons with an odd number of carbon atoms in the chain predominate, while even chains occur only in traces.

It is generally accepted that acetate units are incorporated into the hydrocarbons of insects (12-16), but beyond that the biosynthetic route to hydrocarbons is unknown. Of the possible biosynthetic routes, only two, the condensation and elongation mechanisms, are being considered seriously at present (for a review see 17). The elongation route consists of the addition of C_2 units derived from acetate to form a fatty acid of an appropriate chain length, then decarboxylation to the hydrocarbon. The condensation route is the condensation of two fatty acids followed by decarboxylation and reduction to the hydrocarbon. In both mechanisms fatty acids of 10 or more carbon atoms are proposed as the precursors to hydrocarbons. If the mono-methyl internally branched hydrocarbons of *P. australasiae, P. brunnea* and *P. fuliginosa* are biosynthesized via either of these routes, one might expect to find some precursor fatty acid with a methyl branch 13 carbons from the methyl end. If the 3-methyl hydrocarbons are also biosynthesized via either of the above routes, one might expect to find some precursor fatty acid with a methyl branch three carbon atoms from the methyl end. So far, the presence of either of these two types (ω-3 or ω-13) of fatty acids has not been observed in insects.

Analytical GLC of the cuticular hydrocarbons demonstrated quantitative differences in the hydrocarbon composition between male and female of *P. australasiae* and *P. fuliginosa* cockroaches (Table II), but not of *P. brunnea*. There are appreciable quantitative differences in the content of *cis*-9-tricosene, 11-methyltricosane, 3-methyltricosane and 13-methylpentacosane. In the major normal saturated hydrocarbon (*n*-tricosane), as well as the *n*-heptacosane and *n*-nonacosane, there is little compositional difference between sexes and among the three *Periplaneta* studied here. There is, however, some difference in the content of *n*-heneicosane, docosene and docosane in the cuticular hydrocarbons of *P. australasiae* and *P. fuliginosa*.

Quantitative differences in hydrocarbon content (2) and composition (3) have been observed in the hemolymph of cockroaches, however, this is the first time that such pronounced compositional differences have been

TABLE II

Cuticular Hydrocarbon Composition of Three *Periplaneta* Species of Cockroaches (Area Per Cent)

	Hydrocarbon	*P. australasiae*		*P. brunnea*		*P. fuliginosa*	
		Female	Male	Female	Male	Female	Male
A	*n*-Heneicosane	Trace[a]	7	ND[b]	ND	Trace	4
B	Docosene and docosane	Trace	3	ND	ND	Trace	1
C	*Cis*-9-tricosene	2	46	ND	ND	4	29
D	*n*-Tricosane	16	14	11	12	14	15
E	11-Methyltricosane	3	2	20	18	10	6
F	3-Methyltricosane	12	3	15	15	13	8
G	*n*-Tetracosane	Trace	Trace	Trace	Trace	Trace	Trace
H	12-Methyltetracosane	Trace	Trace	Trace	Trace	Trace	Trace
I	3-Methyltetracosane	ND	Trace	ND	ND	ND	Trace
J	*n*-Oebtacisabe	Trace	Trace	Trace	Trace	Trace	Trace
K	13-Methylpentacosane	54	9	48	46	45	24
L	*n*-Hexacosane	Trace	Trace	Trace	Trace	Trace	Trace
M	13-Methylhexacosane	Trace	Trace	Trace	Trace	Trace	Trace
N	*n*-Heptacosane	4	5	1	2	6	6
O	*n*-Nonacosane	3	4	2	2	2	2

[a]Trace equals less than 1% but greater than 0.2%.
[b]ND equals not detectable at the 0.2% level.

observed in cuticular hydrocarbons between sexes. The differences might be related to the reproductive processes, be under hormonal control, be related to a precursor supply or be related to a sex-linked compositional control mechanism. We have no evidence to support or refute any of these suggestions.

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