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CUTICULAR HYDROCARBONS OF GREGARIOUS AND SOLITARY LOCUSTS *Locusta migratoria cinerascens*

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Abstract—The cuticular hydrocarbons of *Locusta migratoria cinerascens*—larvae and adults, males and females, gregarious and solitaries—have been investigated by combined gas chromatography–mass spectrometry. The hydrocarbons comprise 52–78% of the cuticular lipids and are divided into *n*-alkanes (28.7–47.3%), 3-, 4-, and 5-methylalkanes (11.3–15.8%), internally branched monomethylalkanes (13.7–19.9%), and internally branched dimethylalkanes (19.8–35.9%) with seven or nine methylenes between the two branch points. While the sexual dimorphism does not seem to be reflected in the cuticular hydrocarbon composition, clear quantitative variations favoring the longest chain alkanes have been observed between gregarious and solitary locusts, thus revealing a new phase character in these insects.

Key Words—Locusts, *Locusta migratoria*, cuticular hydrocarbons, mass spectrometry, phase polymorphism, Orthoptera, Acrididae.

INTRODUCTION

The cuticular lipids play a part in the regulation of water evaporation in insects and also protect them from the penetration of insecticides and microorganisms (Beament, 1964; Ebeling, 1964; David, 1967; Hadley, 1981). These cuticular lipids contain a large proportion of hydrocarbons. Some of these hydrocarbons act as pheromones (Carlson et al., 1978; Blomquist and Jackson, 1979; Howard and Blomquist, 1982; Jallon, 1985) or can be factors of chemotaxonomic differentiation (Blomquist et al., 1976; Lockey, 1976, 1984; Jackson, 1981).

These are the reasons for researchers' sustained interest in the chemical analysis of cuticular hydrocarbons from numerous species (Nelson, 1978;

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Blomquist and Jackson, 1979). Their reviews show that cuticular hydrocarbons are generally homologous series of *n*-alkanes and branched alkanes, mono-, di-, or trimethylated. Monomethylalkanes with terminal (2-, 3-, 4-, 5-methyl) and internal (7- to 23-methyl) branches, have been identified in three species of crickets (Hutchins and Martin, 1968), cockroaches (Tartivita and Jackson, 1970; Jackson, 1972), ants (Lok et al., 1975; Nelson et al., 1980), and bark beetles (Lockey, 1982). The 2-methylalkanes which do not occur in most of the insects already studied but are present in large proportions in three species of crickets (Blomquist et al., 1976) may permit taxonomic assignment. Di- and trimethylalkanes with isoprenoid spacing have been described in various insects (Nelson, 1978). Other polymethylalkanes with 1, 5, 7, 9, or 11 carbon atoms between branch points have also been identified (Nelson et al., 1980, 1981, 1984).

So far, the cuticular hydrocarbons of nine acridids have been studied: *Schistocerca vaga* (Nelson and Sukkestad, 1975), *Schistocerca gregaria* (Lockey, 1976), *Schistocerca americana* (Jackson, 1982), *Melanoplus sanguinipes* and *packardii* (Soliday et al., 1974) reanalyzed along with *differentialis* (Nelson et al., 1984), *Melanoplus bivittatus femurrubrum* and *dawsoni* (Jackson, 1981), and *Locusta migratoria* (Lockey, 1976). In 1976, Lockey suggested that the hydrocarbon compositions of different locusts are closer together if they belong to the same subfamily. Thus, a very fine chemical analysis appears to be the first condition for the use of the hydrocarbon profiles as additional characters in the insects' taxonomic grouping. Since Lockey (1976) has only studied the major components, we undertake here a new study of cuticular alkanes of a subspecies of the migratory locust: *Locusta migratoria cinerascens*.

The migratory locust is well known for its ability to change its kind of life, crowded or gregarious (gregaria) and isolated or solitary (solitaria) "phases." Thus it is interesting to know if the behavioral, morphological, and physiological changes of both phases (Uvarov, 1921, 1966) could lead to changes in the chemical composition of cuticular hydrocarbons.

Chemical stimulations can come into play at different ages of the locust's life (Gillett, 1975; Loher, 1960; Norris, 1970). However, the origin of these chemical pheromones is still under discussion and their nature remains to be specified.

In this study we decided to compare cuticular hydrocarbons at larval and adult ages of both sexes, from gregarious and solitary locusts of the *Locusta migratoria cinerascens* species.

METHODS AND MATERIALS

Insects Used. Last larval instar and mature adult, male and female, gregarious and solitary insects were used. They were raised at the Insect Biology Laboratory. The strain was *Locusta migratoria cinerascens* from Sardinia. Gre-

garious locusts were bred in groups of 200 individuals in cages 40 × 40 × 60 cm. Solitary locusts were maintained from birth in individual 1-liter containers in a separate breeding room. Each room was submitted to a regular change of air 12 times an hour. The photo- and thermoperiods were 12/12 hr with temperatures of 25 ± 1°C (night) and 35 ± 1°C (day). Separate evaluation of characters such as behavior (Nicolas, 1972; Gillett et al., 1972), morphometrics (Nicolas, 1973; Minato et al., 1973), pigmentation, and fecundity (Nicolas, 1972) had shown that, under our laboratory conditions, the locusts isolated from birth are conspicuously different from the crowded ones bred simultaneously.

All locusts were fed on fresh corn shoots and bran every day of the week with no interruption.

The same numbers of the animals of both sexes used were: 23 gregarious, last-instar larvae, two to three days old; 5 gregarious, mature adults, 34 days old; 10 solitary, last-instar larvae, two to three days old; and 21 solitary, mature adults, 21 days old.

All the solitary locusts used were light beige as this color was close to that of the white paper surrounding them to keep them separated. Each insect was killed by freezing at −20°C, then extracted by stirring during 10 min in hexane (5 ml/adult and 3 ml/larva). After evaporation of the solvent, hydrocarbons were separated from the weighed extracted cuticular lipids by thin-layer chromatography (Merck 10 × 20 plates of silica gel 60 F254 with concentration zones) and elution with nanograd hexane. From this weighed fraction of hydrocarbons, branched ones were separated with 5 Å Linde molecular sieves (as described by O'Connor et al., 1962).

Combined gas chromatography–mass spectrometry (GC-MS) was used to identify the components of the different extracts. Mass spectra were obtained on a Nermag R10-10 spectrometer associated with a PDP8 calculator (Digital Equipment Instrument) and coupled to a Girdel 31 chromatograph with a Ros injector. Fractions were temperature programmed from 200 to 300°C at 3°/min with isotherm at 300° on a capillary column either 10 or 25 m long, 0.32 mm wide, coated with CpSil 5 CB Chrompack. The carrier gas was helium and inlet pressure 0.25 bar. Fractions were analyzed by GC-MS either by electronic impact or by positive chemical ionization. In electronic impact, ionization voltage was 70 eV and the temperature of ion source was 110°C for hydrocarbons up to C_{40}. In these conditions, the molecular peak is always present. The temperature of the ion source was 290°C for more condensed hydrocarbons (C_{40} to C_{53}), but then only the ion M-15 is present. Mass scanning was carried out from atomic mass unit (amu) 100 to 600 or 750. In these conditions and without amplification (the sensitivity for the masses examined being better) spectra of branched hydrocarbons often have a base peak which corresponds to a characteristic fragmentation at a methyl branch. Positive chemical ionization was performed using methane generating an internal source pressure of 0.2 torr; ioni-

zation voltage was 90 eV, temperature of ion source 150°C, and mass scanning started at 100 amu.

The mass spectra of alkanes were interpreted according to the criteria proposed by McCarthy et al. (1968), Nelson et al. (1972), Nelson (1978), and Pomonis et al. (1978, 1980). Integration of chromatographic peaks was carried out with a Hewlett Packard integrator coupled to a Varian 3700 chromatograph with the 25-m column mentioned above (see GC-MS), programmed from 40 to 300°C at 4°/min and isothermal at 300° with a flow rate of helium being 18 cm/sec; an "on column" injector was used.

Retention indices (Ettre, 1964) were calculated with the 25-m column, using the *n*-alkanes from C_{22} to C_{37} identified without ambiguity by GC-MS and present in all total hydrocarbonated fractions.

All the bar charts (Figure 14–18) were constructed from the *n*-alkane percentage and the branched alkane percentage columns shown in Table 2 (%/N. and %/B.).

RESULTS

The hydrocarbons comprise 52–78% of all the cuticular lipids (Table 1). The smallest percentages were obtained for the solitary locusts. Our results, qualitatively in agreement with those of Lockey (1976) for the described compounds, exhibit quantitative differences; however, it must be mentioned that Lockey's analyses concern wings rather than total insect extracts. The chromatographic profile of a partial but complex hydrocarbon fraction (up to nonatriacontane) is presented in Figure 1 (25-m column).

Total (A) and corresponding branched (B) fractions (up to tripentacontane) are presented in Figure 2 (10-m column). Table 2 summarizes all the identified alkanes with their percentage for the eight groups of insects studied.

To perform analysis, correlations were carried out with results of electronic impact and chemical ionization in mass spectrometry on the one hand, and difference of retention index (*dI*) in gas chromatography between branched alkanes and *n*-alkanes with the same carbon number, on the other hand. It is known that *dI*s are related to the position of the methyl branch for a monomethylalkane (Mold et al., 1966) and to the number of methyl branches in a polymethylalkane (Nelson and Sukkestad, 1970). These correlations show, in agreement with Lockey (1976), that the fractions studied appear to consist of four classes of alkanes: class A, *n*-alkanes; class B, terminally branched monomethylalkanes; class C, internally branched monomethylalkanes; and class D, dimethylalkanes.

Class A: n-Alkanes. Their mass spectra are characteristic and their presence is in agreement with the comparison between the chromatographic profiles obtained before or after the separation on molecular sieves (Figure 2).

TABLE 1. ALKANE CONSTITUENTS OF CUTICULAR LIPIDS OF *Locusta migratoria cinerascens*[a]

	MGL	FGL	MSL	FSL	MGA	FGA	MSA	FSA
One insect extract weight (mg)	0.3	0.3	0.3	0.3	1.5	1.7	1.3	1.7
Hydrocarbons/cuticular lipids (weight %)	72	77	52	57	78	78	64	66
Relative surfaces (%):								
Class A	45.6	47.3	43.2	39.1	28.7	30.6	38.7	32.8
Class B	12.5	11.3	12.8	12.4	13.7	12.7	15.8	12.9
Class C	17.6	18.8	16.6	19.9	15.2	16.7	13.7	16.6
Class D	22.0	19.8	24.8	25.3	35.9	35.6	26.7	32.8
Unidentified components	2.2	2.7	2.6	3.1	5.8	4.4	5.1	4.9

[a] MGL: male gregarious larvae; FGL: female gregarious larvae; MSL: male solitary larvae; FSL: female solitary larvae; MGA: male gregarious adults; FGA: female gregarious adults; MSA: male solitary adults; FSA: female solitary adults; Class A: *n*-alkanes; class B: terminally branched monomethylalkanes; class C: internally branched monomethylalkanes; class D: dimethylalkanes.

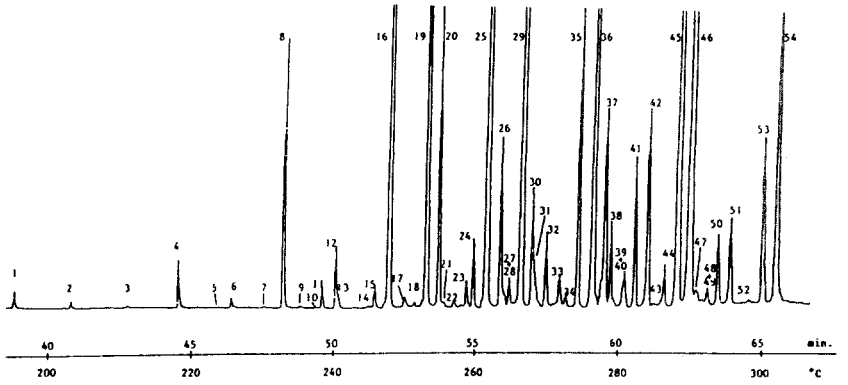


FIG. 1. Partial gas chromatogram of a hydrocarbon fraction of male solitary fifth-instar larvae of *Locusta migratoria cinerascens*. CpSil 5 CB Chrompack capillary column of 25 m × 0.32 mm, temperature programmed from 40 to 300° at 4°/min and held at 300°; "on-column" type injector.

In electronic impact conditions (70 eV, starting mass scanning: 100 amu), the molecular peak $M^{+}\cdot$ is base peak. Sixteen linear alkanes, from C_{22} to C_{37}, have been identified; *n*-nonacosane in gregarious and *n*-hentriacontane in solitary locusts are the most important. The percentage of these total *n*-alkanes decreases from larval to adult ages (Table 1) and more strongly in gregarious locusts.

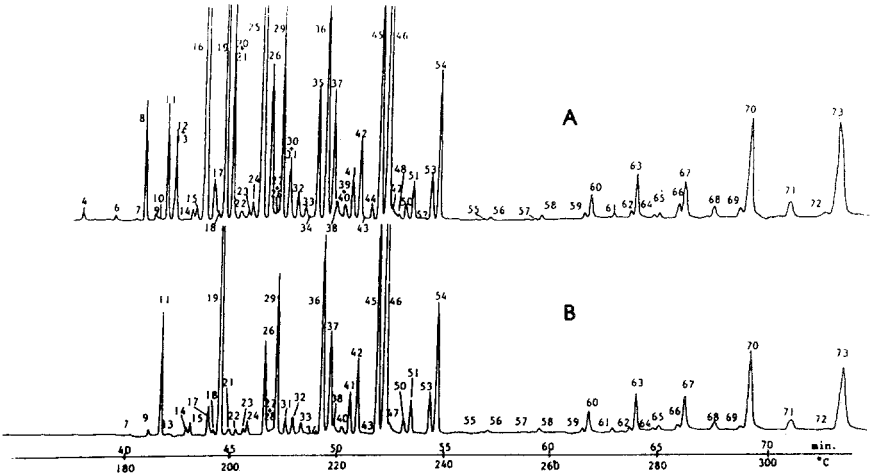


FIG. 2. Gas chromatographic analysis of the total (A) and branched (B) hydrocarbon fractions of male gregarious mature adults, *Locusta migratoria cinerascens*. Same conditions as Figure 1 with a column of 10 m × 0.32 mm.

TABLE 2. CUTICULAR HYDROCARBONS OF *Locusta migratoria cinerascens*

GC Peak No.	Retention indices (I)	MGL[a]			FGL			MSL			FSL		
		%/T[b]	%/N	%/B	%/T	%/N	%/B	%/T	%/N	%/B	%/T	%/N	%/B
1	2200							tr.	tr.		tr.	tr.	
2	2300	tr.	tr.		0.1	0.2		tr.	tr.		tr.	tr.	
3	2400	tr.	tr.		0.1	0.2		tr.	tr.		tr.	tr.	
4	2500	0.3	0.7		1.0	2.1		0.2	0.5		0.3	0.8	
5	2572	tr.		tr.	tr.		tr.						
6	2600	0.2	0.4		0.2	0.4		tr.	tr.		0.1	0.3	
7	2672	tr.		tr.	tr.		tr.						
8	2700	4.5	9.8		4.0	8.5		1.3	3.0		1.2	3.0	
9	2733	0.4		0.7	0.3		0.6						
10	2758	tr.		tr.									
11	2772	2.1		3.9	1.7		3.2	0.2		0.4	0.2		0.3
12	2800	2.0	4.4		1.8	3.8		0.4	0.9		0.4	1.0	
13	2806				0.3		0.6						
14	2858	0.1		0.2	tr.		tr.						
15	2872	0.2		0.4	0.1		0.2	tr.		tr.	tr.		tr.
16	2900	21.3	46.6		22.6	47.7		9.5	22.0		8.7	22.3	
17	2933	1.3		2.4	1.6		3.0	0.2		0.4	0.2		0.3
18	2950	0.1		0.2	0.2		0.4	tr.		tr.			
19	2972	7.3		13.4	6.3		12.0	2.9		5.1	3.6		6.0
20	3000	3.0	6.6		3.1	6.6		2.9	6.7		2.6	6.6	
21	3006	tr.		tr.	tr.		tr.						
22	3033	0.2		0.4	0.3		0.6	tr.		tr.			
23	3058	0.1		0.2	0.1		0.2	0.1		0.2	0.1		0.2
24	3072	0.3		0.6	0.3		0.6	0.4		0.7	0.3		0.5
25	3100	12.4	27.1		12.4	26.2		20.7	47.9		18.1	46.3	
26	3133	3.0		5.5	4.1		7.8	1.2		2.1	1.6		2.6

TABLE 2. Continued

GC Peak No.	Retention indices (I)	MGL[a]			FGL			MSL			FSL		
		%/T[b]	%/N	%/B	%/T	%/N	%/B	%/T	%/N	%/B	%/T	%/N	%/B
27	3150	tr.		tr.	tr.		tr.	0.2		0.4	0.2		0.3
28	3158	tr.		tr.	0.1		0.2						
29	3172	2.1		3.9	1.7		3.2	7.7		13.6	6.9		11.4
30	3200	0.5	1.1		0.4	0.8		1.1	2.5		1.1	2.8	
31	3206	0.2		0.4	0.2		0.4	0.1		0.2	0.2		0.3
32	3233	0.5		0.9	0.5		0.9	0.5		0.9	0.6		1.0
33	3258	0.2		0.4	0.6		1.1	0.2		0.4	0.3		0.5
34	3272	tr.		tr.	0.2		0.4	tr.		tr.	0.1		0.2
35	3300	1.3	2.9		1.4	3.0		5.8	13.4		5.1	13.0	
36	3333	5.3		9.7	5.8		11.0	5.5		9.7	7.1		11.8
37	3360	2.1		3.9	2.0		3.8	2.2		3.9	2.2		3.7
38	3372	tr.		tr.	tr.		tr.	0.7		1.2	0.5		0.8
39	3400	tr.	tr.		tr.	tr.		0.3	0.7		0.3	0.8	
40	3406	tr.		tr.	tr.		tr.	0.2		0.4	0.2		0.3
41	3433	0.8		1.5	0.8		1.5	1.0		1.8	1.1		1.8
42	3460	1.4		2.6	1.1		2.1	1.6		2.8	1.4		2.3
43	3472							0.2		0.4			
44	3500	0.1	0.2		0.2	0.4		0.9	2.1		0.8	2.0	
45	3533	4.8		8.8	4.6		8.7	6.6		11.6	7.8		12.9
46	3560	9.4		17.3	8.2		15.6	10.6		18.7	10.4		17.2
47	3572	tr.		tr.	tr.		tr.	0.2		0.4	0.2		0.3

48	3600	tr.	tr.		tr.	tr.		0.1	0.2		0.1	0.3	
49	3606							tr.		tr.	0.1		0.2
50	3633	0.7		1.3	0.3		0.6	0.6		1.1	0.5		0.8
51	3660	0.5		0.9	0.4		0.8	0.6		1.1	0.5		0.8
52	3700	tr.	tr.		tr.	tr.		tr.	tr.		0.3	0.8	
53	3733	0.6		1.1	0.5		0.9	1.0		1.8	1.2		2.0
54	3760	1.7		3.1	1.6		3.0	2.5		4.4	2.1		3.5
55	>3800	tr.		tr.	tr.		tr.	tr.		tr.	tr.		tr.
56	·	tr.		tr.	tr.		tr.	tr.		tr.	tr.		tr.
57	·	tr.		tr.	tr.		tr.	tr.		tr.	tr.		tr.
58	·	tr.		tr.	tr.		tr.	tr.		tr.	tr.		tr.
59	·	tr.		tr.	tr.		tr.	tr.		tr.	tr.		tr.
60	·	0.2		0.4	0.2		0.4	0.4		0.6	0.4		0.7
61	·	0.2		0.4	0.2		0.4	0.2		0.4	0.2		0.3
62	·							0.2		0.4	0.2		0.3
63	·	0.3		0.6	0.3		0.6	0.4		0.6	0.4		0.7
64	·	tr.		tr.	tr.		tr.	tr.		tr.	tr.		tr.
65	·							0.2		0.4	0.2		0.3
66	·	0.2		0.4	0.3		0.6						
67	·	0.3		0.6	0.2		0.4	0.5		0.8	0.8		1.2
68	·	0.2		0.4	0.2		0.4	0.4		0.6	0.5		0.7
69	·	0.4		0.8	0.6		1.2	0.5		0.8	0.6		0.9
70	·	2.4		4.6	2.1		4.1	2.6		4.5	3.2		5.2
71	·	0.6		1.2	0.4		0.8						
72	·	0.2		0.4	0.3		0.6	0.4		0.6	0.5		0.7
73	·	3.9		7.5	3.7		7.2	3.8		6.6	4.3		7.0

TABLE 2. Continued

GC Peak No.	Retention indices (I)	MGA			FGA			MSA			FSA		
		%/T	%/N	%/B	%/T	%/N	%/B	%/T	%/N	%/B	%/L	%/N	%/B
1	2200	tr.	tr.		tr.	tr.		0.3	0.8		0.2	0.6	
2	2300	tr.	tr.		tr.	tr.		0.1	0.3		tr.	tr.	
3	2400	tr.	tr.		tr.	tr.		0.2	0.5		0.1	0.3	
4	2500	0.1	0.3		0.2	0.7		0.4	1.0		0.2	0.6	
5	2572												
6	2600	tr.	tr.		0.1	0.3		0.4	1.0		0.2	0.6	
7	2672	tr.		tr.	tr.		tr.						
8	2700	1.4	4.9		2.5	8.2		0.9	2.3		0.9	2.7	
9	2733	0.2		0.3	0.3		0.4						
10	2758												
11	2772	1.6		2.2	2.4		3.5	tr.		tr.	0.4		0.6
12	2800	0.9	3.1		1.2	3.9		0.6	1.6		0.5	1.5	
13	2806	0.3		0.4	0.2		0.3						
14	2858	0.2		0.3	0.2		0.3						
15	2872	0.2		0.3	0.2		0.3	tr.		tr.	tr.		tr.
16	2900	12.7	44.3		14.6	47.7		8.4	21.7		8.4	25.6	
17	2933	0.7		1.0	1.0		1.4	0.1		0.2	0.2		0.3
18	2950	0.2		0.3	0.1		0.2	tr.		tr.	tr.		tr.
19	2972	7.4		10.2	6.0		8.6	2.8		4.6	3.4		5.1
20	3000	1.9	6.6		1.8	5.9		2.4	6.2		2.1	6.4	
21	3006	tr.		tr.	0.1		0.2	tr.		tr.	tr.		tr.
22	3033	0.2		0.3	0.2		0.3	tr.		tr.	tr.		tr.
23	3058	0.2		0.3	tr.		tr.	0.2		0.3	0.2		0.3
24	3100	0.3		0.4	0.3		0.4	0.4		0.7	0.3		0.4
25	3100	9.1	31.7		8.1	26.5		15.7	40.6		13.2	40.2	
26	3133	2.0		2.8	2.3		3.3	0.5		0.8	0.9		1.3

27	3150	tr.		tr.	tr.		tr.	0.1		0.2	0.1		0.2
28	3158	tr.		tr.	0.2		0.3						
29	3172	2.8		3.9	2.4		3.5	9.5		15.5	6.7		10.0
30	3200	0.5	1.7		0.4	1.3		1.7	4.4		1.3	4.0	
31	3206	0.4		0.6	0.4		0.6	0.3		0.5	0.4		0.6
32	3233	0.5		0.7	0.5		0.5	0.4		0.7	0.4		0.6
33	3258	0.3		0.4	0.3		0.4	0.4		0.7	0.4		0.6
34	3272	tr.		tr.	tr.		tr.	0.2		0.3	0.2		0.3
35	3300	1.5	5.2		1.2	3.9		5.9	15.2		4.4	13.4	
36	3333	4.2		5.8	4.5		6.5	3.6		5.9	4.7		7.0
37	3360	3.0		4.1	3.5		5.0	2.1		3.4	2.9		4.3
38	3372	0.3		0.4	0.2		0.3	1.7		2.8	0.8		1.2
39	3400	0.2	0.7		0.3	1.0		0.4	1.0		0.2	0.6	
40	3406	0.1		0.2	tr.		tr.	0.3		0.5	0.4		0.6
41	3433	0.8		1.1	0.9		1.3	0.9		1.5	1.2		1.8
42	3460	2.0		2.8	2.2		3.2	1.6		2.6	2.4		3.6
43	3472	tr.		tr.	0.3			tr.		tr.	tr.		tr.
44	3500	0.2	0.7		0.2	0.7		0.9	2.3		0.7	2.1	
45	3533	5.1		7.1	5.2		7.5	6.3		10.3	7.2		10.7
46	3560	12.8		17.7	13.6		19.6	10.5		17.1	14.6		21.7
47	3572	0.2		0.3	0.1		0.2	0.5		0.8	0.4		0.6
48	3600	0.2	0.7		tr.	tr.		0.2	0.5		0.3	0.9	
49	3606							tr.		tr.	tr.		tr.
50	3533	0.5		0.7	0.7		1.0	0.6		1.0	0.6		0.9
51	3660	0.9		1.2	1.0		1.4	0.8		1.3	1.0		1.5
52	3700	tr.	tr.		tr.	tr.		0.2	0.5		0.1	0.3	
53	3733	1.0		1.4	1.1		1.6	1.3		2.1	1.4		2.1
54	3760	3.6		5.0	3.8		5.5	2.6		4.2	3.3		4.9
55	3760	tr.		tr.	tr.		tr.	tr.		tr.	tr.		tr.
56	>3800	tr.		tr.	tr.		tr.	tr.		tr.	tr.		tr.
57	·	tr.		tr.	tr.		tr.	tr.		tr.	tr.		tr.
58	·	0.2		0.3	tr.		tr.	tr.		tr.	tr.		tr.
59	·	0.2		0.3	tr.		tr.	0.2		0.3	tr.		tr.

TABLE 2. Continued

GC Peak No.	Retention indices (I)	MGA			FGA			MSA			FSA		
		%/T	%/N	%/B	%/T	%/N	%/B	%/T	%/N	%/B	%/L	%/N	%/B
60	·	0.8		1.2	0.6		0.9	0.7		1.1	0.4		0.6
61	·	0.2		0.3	0.3		0.4	0.2		0.3			
62	·	0.3		0.5	0.2		0.3	0.3		0.5	0.2		0.3
63	·	1.4		2.2	0.9		1.3	1.0		1.6	0.8		1.2
64	·	tr.		tr.	tr.		tr.	tr.		tr.	tr.		tr.
65	·	0.3		0.5				0.4		0.7	0.2		0.3
66	·	0.6		0.9	0.2		0.3	0.5		0.8	0.5		0.7
67	·	1.4		2.2	1.3		1.9	1.3		2.1	1.0		1.5
68	·	0.6		0.9	1.8		2.6	0.6		1.0	0.5		0.7
69	·	0.5		0.8	0.2		0.3	0.5		0.8	0.8		1.2
70	·	4.4		6.3	2.9		4.1	3.0		4.9	2.3		3.4
71	·	0.8		1.2	0.2		0.3	0.6		1.0	0.8		1.2
72	·	0.5		0.8	0.2		0.3	0.5		0.8	0.7		1.0
73	·	6.4		9.4	6.4		9.1	3.8		6.1	4.5		6.7

GC Peak No.	Retention indices (I)	Carbon No.	Class	Hydrocarbons[c]
1	2200	22	A	*n*-Docosane
2	2300	23	A	*n*-Tricosane
3	2400	24	A	*n*-Tetracosane
4	2500	25	A	*n*-Pentacosane
5	2572	26	B	3-Methylpentacosane
6	2600	26	A	*n*-Hexacosane
7	2672	27	B	3-Methylhexacosane
8	2700	27	A	*n*-Heptacosane
9	2733	28	C	13°- and 15-Methylheptacosanes (1)
10	2758	28	B	5-Methylheptacosane
11	2772	28	B	3-Methylheptacosane
12	2800	28	A	*n*-Octacosane
13	2806	29	?	? (2)
14	2858	29	B	4-Methyloctacosane
15	2872	29	B	3-Methyloctacosane
16	2900	29	A	*n*-Nonacosane
17	2933	30	C	11°-, 13-, and 15-Methylnonacosanes (3)
18	2950	30	B	5-Methylnonacosane
19	2972	30	B	3-Methylnonacosane
20	3000	30	A	*n*-Triacontane
21	3006	31	?	? (2)
22	3033	31	C	11°-, 12-, 13-, 14-, and 15-Methyltriacontanes (4)
23	3058	31	B	4-Methyltriacontane
24	3072	31	B	3-Methyltriacontane
25	3100	31	A	*n*-Hentriacontane
26	3133	32	C	11- and 13°-Methylhentriacontanes

TABLE 2. Continued

GC Peak No.	Retention indices (I)	Carbon No.	Class	Hydrocarbons[c]
27	3150	32	B	5-Methylhentriacontane
28	3158	32	B	Dimethylhentriacontane (5)
29	3172	32	B	3-Methylhentriacontane
30	3200	32	A	*n*-Dotriacontane
31	3206	33	?	? (2)
32	3233	33	C	12-, 13°- and 14-Methyldotriacontanes (6)
33	3258	33	B	4-Methyldotriacontane (7)
34	3272	33	B	3-Methyldotriacontane
35	3300	33	A	*n*-Tritriacontane
36	3333	34	C	11- 13°-, and 15-Methyltritriacontanes
37	3360	35	D	11, 21-, and 13,21-Dimethyltritriacontanes
38	3372	34	B	3-Methyltritriacontane
39	3400	34	A	*n*-Tetratriacontane
40	2406	35	?	? (2)
41	3433	35	C	12-, 13°-, 14-, and 5-Methyltetratriacontanes (8)
42	3460	36	D	12,20-, 12,22-, and 13,21-Dimethyltetratriacontanes
43	3472	35	B	3-Methyltetratriacontane
44	3500	35	A	*n*-Pentatriacontane
45	3533	36	C	13°-, 15-, and 17-Methylpentatriacontanes
46	3560	37	D	13,21-, and 13,23-Dimethylpentatriacontanes
47	3572	36	B	3-Methylpentatriacontane
48	3600	36	A	*n*-Hexatriacontane
49	3606	37	?	? (2)
50	3633	37	C	12-, 13-, 14°-, and 15-Methylhexatriacontanes (9)
51	3660	38	D	12,22- and 13,23-Dimethylhexatriacontanes
52	3700	37	A	*n*-Heptatriacontane
53	3733	38	C	13°-, 15-, 17-, and 19-Methylheptatriacontanes

54	3760	39	D	13,21- and 13,23-Dimethylheptatriacontanes
55	>3800			? (10)
56	·			? (10)
57	·			? (10)
58	·			? (10)
59	·			? (10)
60	·			? (10)
61	·			? (10)
62	·			? (10)
63	·	47	D	13,21-Dimethylpentatetracontane
64	·			? (10)
65	·			? (10)
66	·			? (10)
67	·	49	D	13,21-Dimethylheptatetracontane
68	·			? (10)
69	·			? (10)
70	·	51	D	13,21-Dimethylnonatetracontane
71	·			? (10)
72	·			? (10)
73	·	53	D	13,21-Dimethylhenpentacontane

[a] See footnote to Table 1 for abbreviations.

[b] %/T: percentages (±0.1%), scaled to 100, of total alkanes. %/N: percentages (±0.1%), scaled to 100, of normal alkanes. %/B: percentages (±0.1%), scaled to 100, of branched alkanes. tr.: <0.1%.

[c] °: major isomer. (1) 11- and 13°-methylheptacosanes for MGA and FGA. (2) The retention indice and the carbon number agree with a 3,X-dimethylalkane (Nelson et al., 1980), but the very small percentage of this product did not allow us to get a good spectrum. (3) 11°- and 13-methylnonacosanes for MSL and MGA; 9-, 11°-, and 13-methylnonacosanes for FGA. (4) 10-, 11°-, 12-, and 13-methyltrialontanes for MGA and FGA; 11°-, 12-, 13-, and 14-methyltriacontanes for FGL. (5) Branch points unidentified. (6) 12°-, 13-, and 14-methyldotriacontanes for FGL; 12-, 13°-, and 14-methyldotriacontanes for MSA; 11-, 12°-, 13°-, and 14°-methyldotriacontanes for MGA. (7) + 12,20-dimethyldotriacontane for MGA and MGA; + unidentified dimethyldotriacontane for the other insects. (8) 11-, 12-, 13°-, 14-, 15-, and 16-methyltetratriacontanes for FGL and FSL; 12-, 13°-, 14-, 15-, 16-, and 17-methyltetratriacontanes for MSL. (9) 13°-, 14°-, and 15-methylhexatriacontanes for MGL, FGL, and FSL; 12-, 13°-, 14-, 15-, 16-, 17-, and 18-methylhexatriacontanes for MSL. (10) The small percentage of this component did not allow us to identify it; present on the gas chromatograms after separation on molecular sieves, it is quite probably a branched alkane.

Class B: Terminally Branched Monomethylalkanes. 5-, 4- and 3-methylalkanes have been identified. The *dI*s between these alkanes and the *n*-alkanes with the same carbon number are, respectively, 50, 42, and 28. The first two series account for less than 1% of the different analyzed fractions. According to the biosynthesis of these compounds (Blomquist and Jackson, 1979), 5-methylalkanes are even hydrocarbons (5-methylnonacosane and 5-methylhentriacontane) and 4-methylalkanes are odd hydrocarbons (4-methyloctacosane and 4-methyltriacontane). 3-Methylalkanes are by far the most abundant products of this class: they account for 12% of the total hydrocarbon fraction with a little more for the male solitary adult locusts (15.8%). They range from 26 to 36 carbon numbers; among them, those with an even number of carbons are the most abundant. 3-Methylnonacosane is the major one of the series for gregarious locusts and 3-methylhentriacontane for solitary ones.

Their mass spectra (from 100 amu) give the following characteristic fragments: 5-methylalkanes: M-57 (base peak) and M-85; 4-methylalkanes: M-43 (base peak) and M-71; 3-methylalkanes: M-29 (base peak) and M-57.

Class C: Internally Branched Monomethylalkanes. They account for 13–20% of total hydrocarbon extracts and range from C_{28} to C_{38} (described here but probably up to C_{52}). The *dI* with same number linear hydrocarbons is 67. The predominant branch points are 11, 12, and 13 for odd methylalkanes (the minor ones) and 11 and 13 for even methylalkanes (the major ones).

In fact, each chromatographic peak corresponds to a mixture of odd or even monomethylalkanes, isomers for the position of the branch point. The mass spectrometry of these derivatives has been described by McCarthy et al. (1968) and Nelson et al. (1972). This has enabled us to establish the structures proposed in Table 2.

The mass spectrum of peak 41 of Figure 2 suggests a mixture of 12-, 13-, 14-, and 15-methyltetratriacontanes (Figure 3). In the same way, peak 45 of

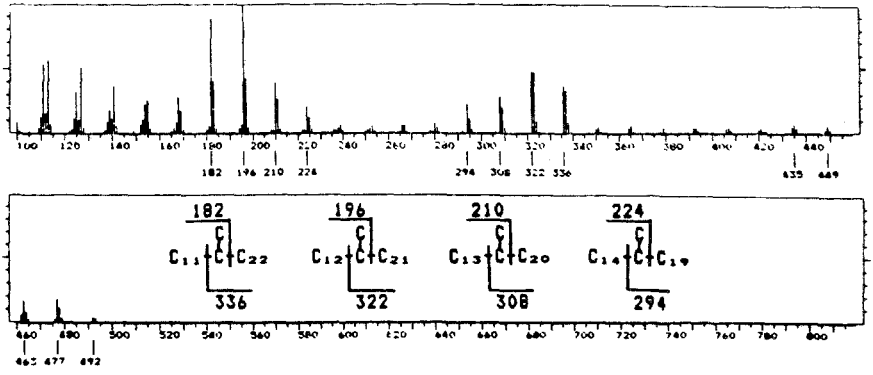


FIG. 3. Mass spectrum of GC peak 41 (Figure 2): 12-, 13-, 14-, and 15-methyltetratriacontanes.

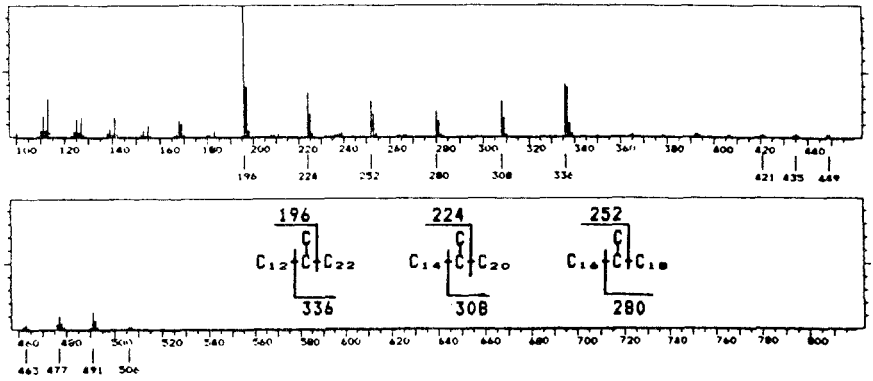


FIG. 4. Mass spectrum of GC peak 45: 13-, 15-, and 17-methylpentatriacontanes.

Figure 1 is interpreted as a mixture of 13-, 15-, and 17-methylpentatriacontanes (Figure 4). In all cases, the base peak of these spectra recorded from 100 amu is the secondary even ion of the weaker molecular mass of the major isomer (*m/z* 196 for 13-methyl isomer in Figures 3 and 4).

Some qualitative differences appear between insect categories. For example, to the isomers identified in peak 41 of Figure 2 (Figure 3), are added 16- and 17-methyltetratriacontanes in peak 41 of Figure 1 (Figure 5). Probably, these qualitative differences come from quantitative variations between monomethylalkanes inside the same chromatographic peak of the various insect categories analyzed.

Class D: Dimethylalkanes. The percentages of these products are close to those of monomethylalkanes (8–19% of total hydrocarbon extracts), and range from C_{33} to C_{53}. After C_{39}, only four odd dimethylalkanes, C_{47} to C_{53}, have been proposed, the others being minor ones. The *dI* of 140 and the molecular

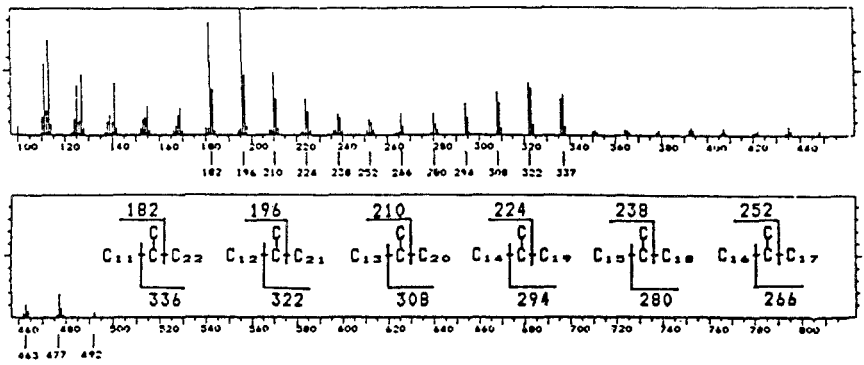
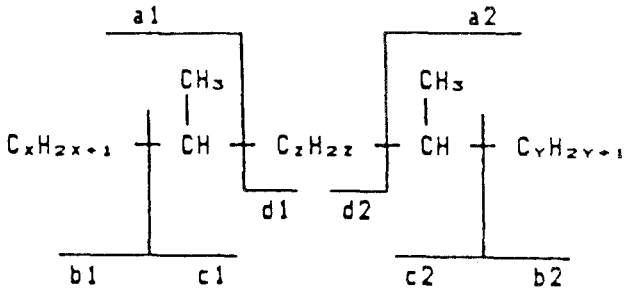


FIG. 5. Mass spectrum of GC peak 41 (Figure 1): 12-, 13-, 14-, 15-, 16-, and 17-methyltetratriacontanes.



SCHEME 1.

masses, determined by chemical ionization, agree with internally branched dimethylalkanes.

The mass spectrometry, more complex than for monomethylalkanes, was used to suggest structures. According to the Nelson school (Nelson et al., 1972; Pomonis et al., 1978, 1980), internally dimethylalkanes undergo two more fragmentations than monomethylalkanes (Scheme 1).

The spectra of 15,19-dimethylpentatriacontane (Sonnet, 1976) (Figure 6) and 13,23-dimethylheptatriacontane (Carlson et al., 1984) (Figure 11B) are presented as examples. Note the relative importance in Figure 6 of m/z 266/267 (even ion major one) which correspond to a pair of ions $d - 1/d$. The other doublet $d - 1/d$ is superimposed with m/z 294/295. Pomonis et al. (1980) mention this rupture with a greater intensity and a major odd ion, $d >> d - 1$, for 9,14-dimethylheptacosane.

As in the case of the monomethylalkanes, each chromatographic peak of the dimethylalkanes corresponds to a mixture of isomers. We present here our suggestions for identifications and the corresponding spectra of some of them (Figures 7–11).

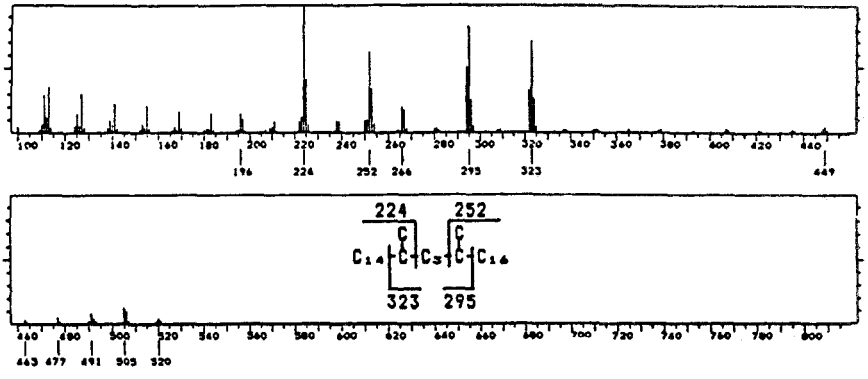


FIG. 6. Mass spectrum of 15,19-dimethylpentatriacontane.

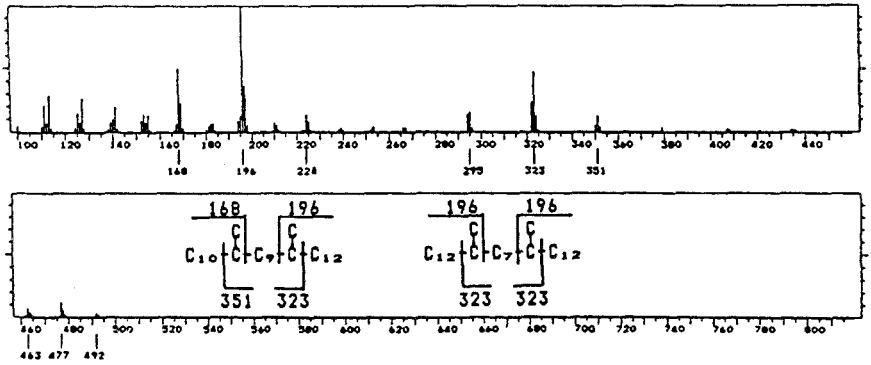


FIG. 7. Mass spectrum of GC peak 37: 11,21- and 13,21-dimethyltritriacontanes.

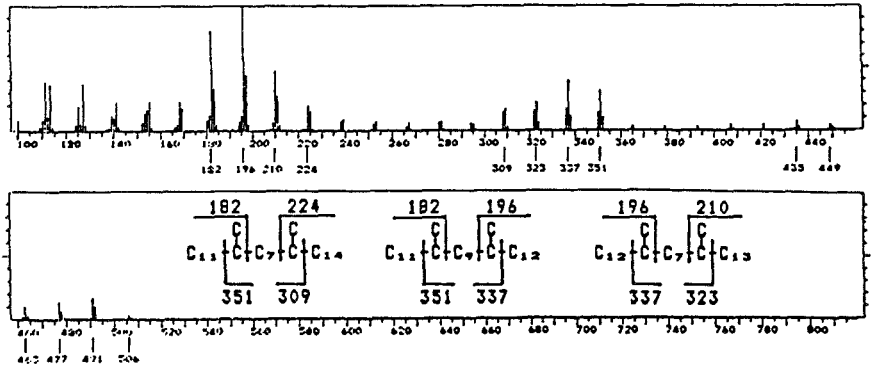


FIG. 8. Mass spectrum of GC peak 42: 12,20-, 12,22-, and 13,21-dimethyltetratriacontanes.

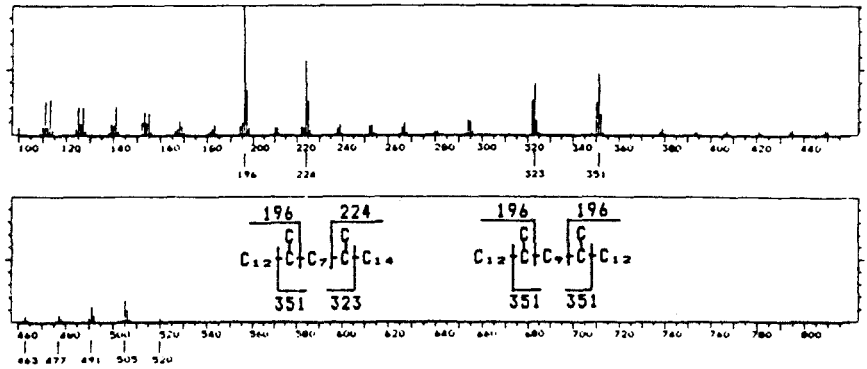


FIG. 9. Mass spectrum of GC peak 46: 13,21- and 13,23-dimethylpentatriacontanes.

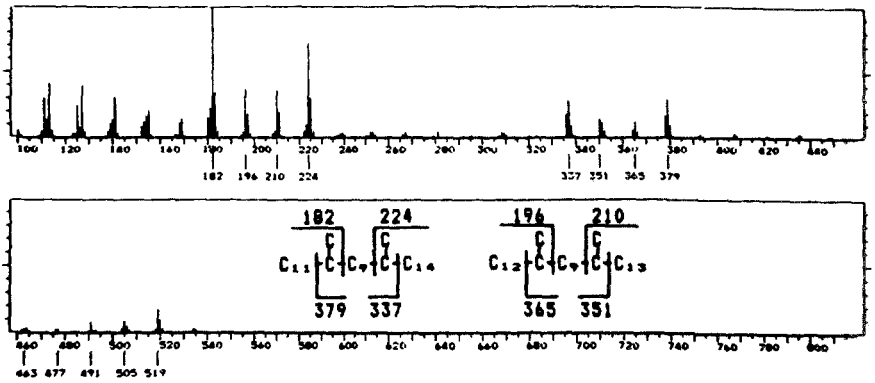


FIG. 10. Mass spectrum of GC peak 51: 12,22- and 13,23-dimethylhexatriacontanes.

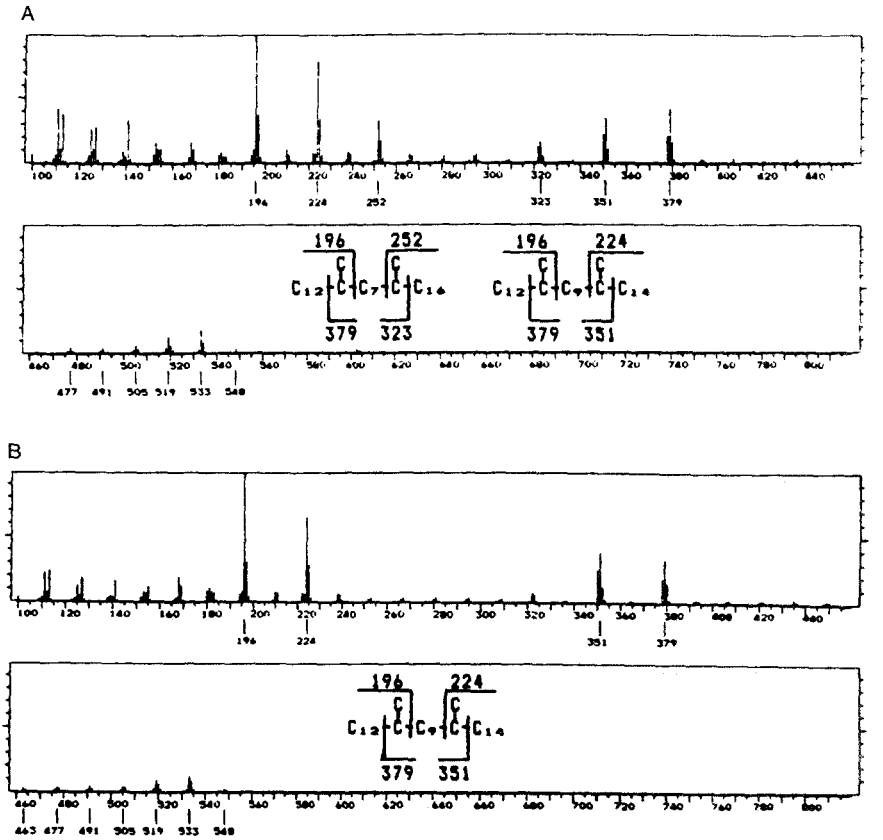


FIG. 11. (A) Mass spectrum of GC peak 54: 13,21- and 13,23-dimethylheptatriacontanes. (B) Mass spectrum of 13,23-dimethylheptatriacontane.

The spectrum of Figure 7 (chromatographic peak 37), corresponds to a mixture of dimethyltritriacontanes (M = 492). According to the pairs of ions **168**/169, **196**/197, 322/**323**, and 350/**351** (major ions in heavy type), we propose 11,21-dimethyltritriacontane. Moreover, the increased intensities of ions at *m/z* **196**/197 and 322/**323** suggest the presence of the symmetric 13,21-dimethyltritriacontane. Finally, the ions at *m/z* **224**/225 and 294/**295** suggest a third isomer with a branch point at carbon 19, but it is difficult to say if it is 11,19- or 13,19-dimethyltritriacontane. The results mentioned further on might be in favor of 11,19 (seven carbons between branch points). We must note that the ion at *m/z* 294 also corresponds to the ion $d - 1$ of the two precedent isomers.

The mass spectrum of Figure 8 (chromatographic peak 42) was interpreted as a mixture of 12,20-, 13,21-, and 12,22-dimethyltetratriacontanes. The two first isomers are sufficient for the attribution of the eight major fragments observed, but the fact that the ratios 351/309 and 337/323 are positive implies the presence of the third isomer.

In the same way, the mass spectrum of Figure 9 (chromatographic peak 46) agrees with the one of 13,21-dimethylpentatriacontane (first proposed by Lockey, 1976) but the ion at *m/z* 351 (>323) suggests the presence of symmetric 13,23-dimethylpentatriacontane (minor one).

For the mass spectrum of Figure 10 (chromatographic peak 51), if we put together the two major even ions at *m/z* 182 and 224 and the two odd ones at *m/z* 337 and 379, we suggest 12,22-dimethylhexatriacontane. The 13,23-dimethylhexatriacontane agrees with other even fragments at *m/z* 196 and 210 and odd fragments at *m/z* 351 and 365. It should be noted that the intensity of the ion at *m/z* 350, greater in this particular case than the ion at *m/z* 351, is partially due to an ion $d - 1$ of the isomer 12,22.

The mass spectrum of peak 54, Figure 11A, in comparison with the spectrum of 13,23-dimethylheptatriacontane (Figure 11B), proves the presence of this hydrocarbon in product 54. But the positive intensity ratio 379/351, on the one hand, and the presence of ions at *m/z* 323 and 252, on the other, can be interpreted as the fragmentation pattern of isomer 13,21.

It should be emphasized that all the dimethylalkane structures suggested here have seven or nine carbons between branch points. Such series are reported, among others, in the hemolymph of the Japanese beetle *Popilla japonica* Newman (Nelson et al., 1975), with seven methylene groups, and in the cuticular hydrocarbons of the house fly *Musca domestica* (Nelson et al., 1981) and of the grasshoppers *Melanoplus differentialis*, *sanguinipes*, and *packardii* (Nelson et al., 1984), with nine methylene groups.

DISCUSSION

The differential bar charts presented in Figures 12 to 16 concern the insect population described in the experimental part. However, similar analyses have

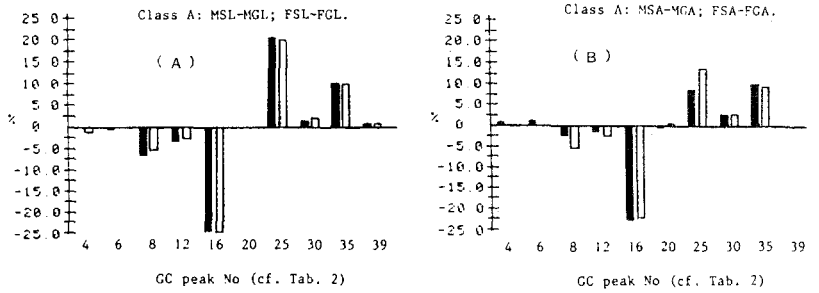


FIG. 12. (A) %MSL − %MGL (black bar chart); %FSL − %FGL (white bar chart). (B) %MSA − %MGA (black bar chart); %FSA − %FGA (white bar chart). MSL: male solitary larvae; MGL: male gregarious larvae; FSL: female solitary larvae; FGL: female gregarious larvae; MSA: male solitary adult; MGA: male gregarious adult; FSA: female solitary adult; FGA; female gregarious adult.

been carried out on at least one other insect population. In all cases, similar typical schemas are observed, even if slight variations in absolute identified hydrocarbon percentages occur from one population to another.

The differential bar charts in Figures 12 to 14 reveal that there is a general tendency for the cuticular hydrocarbons of solitary locusts to be more condensed than those of the gregarious ones. This emerges from the comparison of their hydrocarbon profiles and is valid for both ages and sexes. For the products belonging to classes A, B, and C, we can find a compound representing the variation mean: *n*-triacontane (No. 20) for class A (Figure 12A and B), 3-methyltriacontane (No. 24) for the 3-methylalkanes of class B (Figure 13A and B), and the methyltetratriacontanes (No. 41) for class C (Figure 14A and B). On the other hand, the relative percentages of dimethylalkanes are not modified much by phase changes.

Concerning the evolution of cuticular hydrocarbon composition during the locusts' transformation from larvae to adults, not many changes are observed

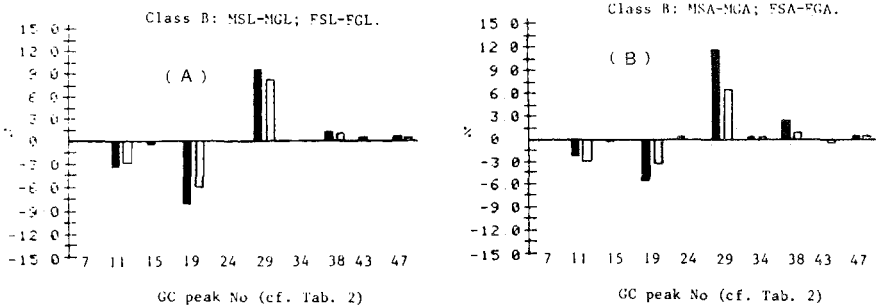


FIG. 13. (A) %MSL − %MGL (black bar chart); %FSL − %FGL (white bar chart). (B) %MSA − %MGA (black bar chart); %FSA − %FGA (white bar chart).

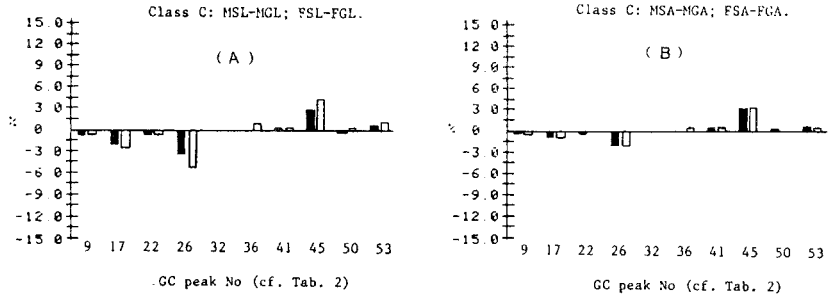


FIG. 14. (A) %MSL − %MGL (black bar chart); %FSL − %FGL (white bar chart). (B) %MSA − %MGA (black bar chart); %FSA − %FGA (white bar chart).

in the *n*-alkanes and the 3-methylalkanes. A decrease in the relative percentages of monomethylalkanes can be observed (Figure 15A and B) in both gregarious (−11.6% for the females and −11.1% for the males) and solitary insects (−8.5% for the females and −6.9% for the males). The variations of dimethylalkanes are presented in Figures 16A and B. They show a clear increase in these compounds in gregarious (+9.4%) and solitary (+8.5%) females. In the males, the tendency seems weaker (+3.0% for gregarious) or reversed (−2.3% for solitary ones). The presence of long-chain branched alkanes, in greater quantities in adults than in larvae, has already been described in *Schistocerca americana* (Jackson, 1982).

The differences between the sexes are slight but seem to be greater in the case of the solitary locusts, especially for dimethylalkanes. This very weak sexual dimorphism leads to the hypothesis of a probable absence of any sexual contact cuticular pheromone of a hydrocarbon nature in these insects.

The differences observed between gregarious and solitary insects show the importance of the role of the cuticle in phase dimorphism; this can be added to the underlying pigmentary tegumental phase dimorphism, which was the only one known until now (Uvarov, 1966; Albrecht, 1967). This new phase char-

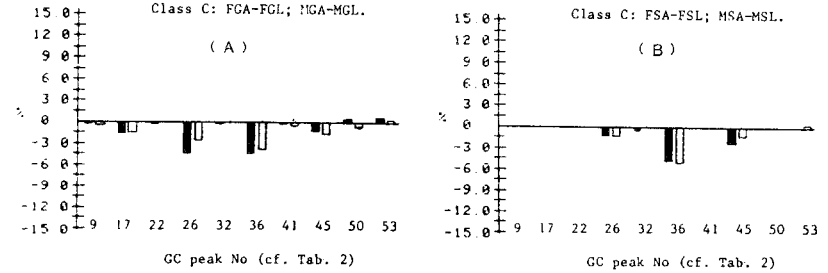


FIG. 15. (A) %FGA − %FGL (black bar chart); %MGA − %MGL (white bar chart). (B) %FSA − %FSL (black bar chart); %MSA − %MSL (white bar chart).

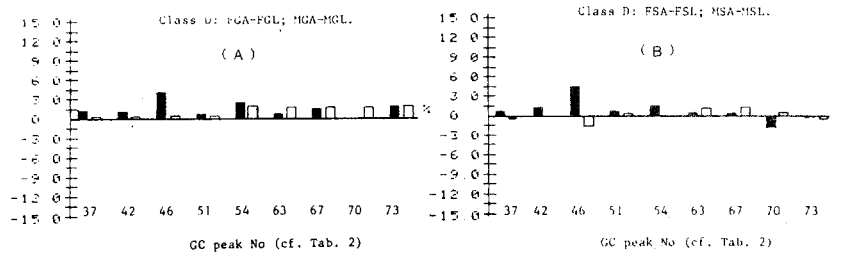


FIG. 16. (A) %FGA − %FGL (black bar chart); %MGA − %MGL (white bar chart). (B) %FSA − %FSL (black bar chart); %MSA − %MSL (white bar chart).

acter is now to be taken into account in biosynthesis problems, on the one hand, and in all taxonomic research with the aim of identifying species according to their hydrocarbon profiles, on the other hand: the kind of life—gregarious or solitary—quantitatively changes the chemical composition of the cuticle. Further research into the possible biological role of these phase change modifications remains to be done.

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