Normal and branched alkanes from cast skins of the grasshopper *Schistocerca vaga* (Scudder)

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Abstract Gas–liquid chromatographic and mass spectral analyses of the hydrocarbons from cast skins of the grasshopper *Schistocerca vaga* (Scudder) demonstrated the presence of four homologous series of alkanes: *n*-alkanes (35%), monomethylalkanes (24%), dimethylalkanes (35%), and trimethylalkanes (3%). The methyl branches were located towards the center of the molecule, and no 2- or 3-methylalkanes were detected by mass spectrometry. The branched alkanes occurred as isomeric mixtures with the methyl group(s) usually located on carbon atom 11, 13, 15, or 17. In the di- and trimethylalkanes, the branch points had isoprenoid spacing. Of the total hydrocarbons, the major component of the *n*-alkane series was nonacosane, 23%; of the monomethylalkanes, it was 11-, 13-, 15-, and 17-methylpentatriacontanes, 13%; of the dimethylalkanes, 9,13-, 11,15-, 13,17-, and 15,19-dimethylpentatriacontanes, 20%; and of the trimethylalkanes, 11,15,19- and 13,17,21-trimethylpentatriacontanes, 1%.

Supplementary key words methyl branched alkanes · mass spectra · gas–liquid chromatography · insects

The occurrence of multiple methyl-branched alkanes (in which the methyl branches have isoprenoid spacing) with at least one long-chain tail in some insects prompted us to look for similar hydrocarbons in other insects. For example, Martin and MacConnell (1) found these alkanes in the ants *Atta colombica* Guerin, *A. sexdens* (L.), and *A. cephalotes isthmicola* Weber; Nelson, Sukkestad, and Terranova (2) in the tobacco hornworm, *Manduca sexta* (L.); Nelson and Sukkestad (3) and Nelson, Sukkestad, and Zaylskie (4) in the eggs of the tobacco hornworm; and Bennett, Kleiman, and Shotwell (5) in the hemolymph of the Japanese beetle, *Popillia japonica* (Newman). The hydrocarbons of a number of other insects did not have multiple methyl-branched alkanes (6–21). Moreover, long-chain multiple methyl-branched alkanes with isoprenoid spacing of the methyl branches have not been found in bacterial hydrocarbons (22) or in plant hydrocarbons (23).

In the present paper, we report the isolation and mass spectral identification of hydrocarbons from cast skins of the grasshopper *Schistocerca vaga* (Scudder). The hydrocarbon fraction is composed of four homologous series of alkanes: the *n*-alkanes and the internally branched mono-, di-, and trimethylalkanes.

MATERIALS AND METHODS[2]

The grasshoppers were reared on commercial lettuce and a synthetic diet (24) containing powdered romaine. The cast skins were collected from all molts and stored at −10°C. Cast skins were used to be certain that only surface alkanes were obtained. The lipid extraction, column, thin-layer, and gas–liquid chromatography, separation of the branched components with molecular sieve (25), and mass spectrometry were carried out as previously described (3, 4). The branched alkanes were separated for analysis by mass spectrometry by repeated GLC.

RESULTS AND DISCUSSION

The synthetic diet and the lettuce fed to the grasshoppers contained 0.013 and 0.004% hydrocarbons by weight, respectively. GLC analysis showed that over 99 and 88% of the diet and lettuce hydrocarbons, respectively, were *n*-alkanes. Bromination of the sample did not change the GLC elution pattern, which indicated that all the components were saturated. Trace amounts of *n*-alkanes with chain lengths as long as 39 carbons in the diet and 37 carbons in the lettuce were observed. The major *n*-alkane in the diet was hentriacontane (36%). No diet hydrocarbons eluted from the gas chromatograph with fractional equivalent chain lengths over 30, which indicated that the major branched alkanes from the grasshopper

Abbreviations: GLC, gas–liquid chromatography.

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[2] Mention of a proprietary product in this paper does not constitute an endorsement of this product by the USDA.

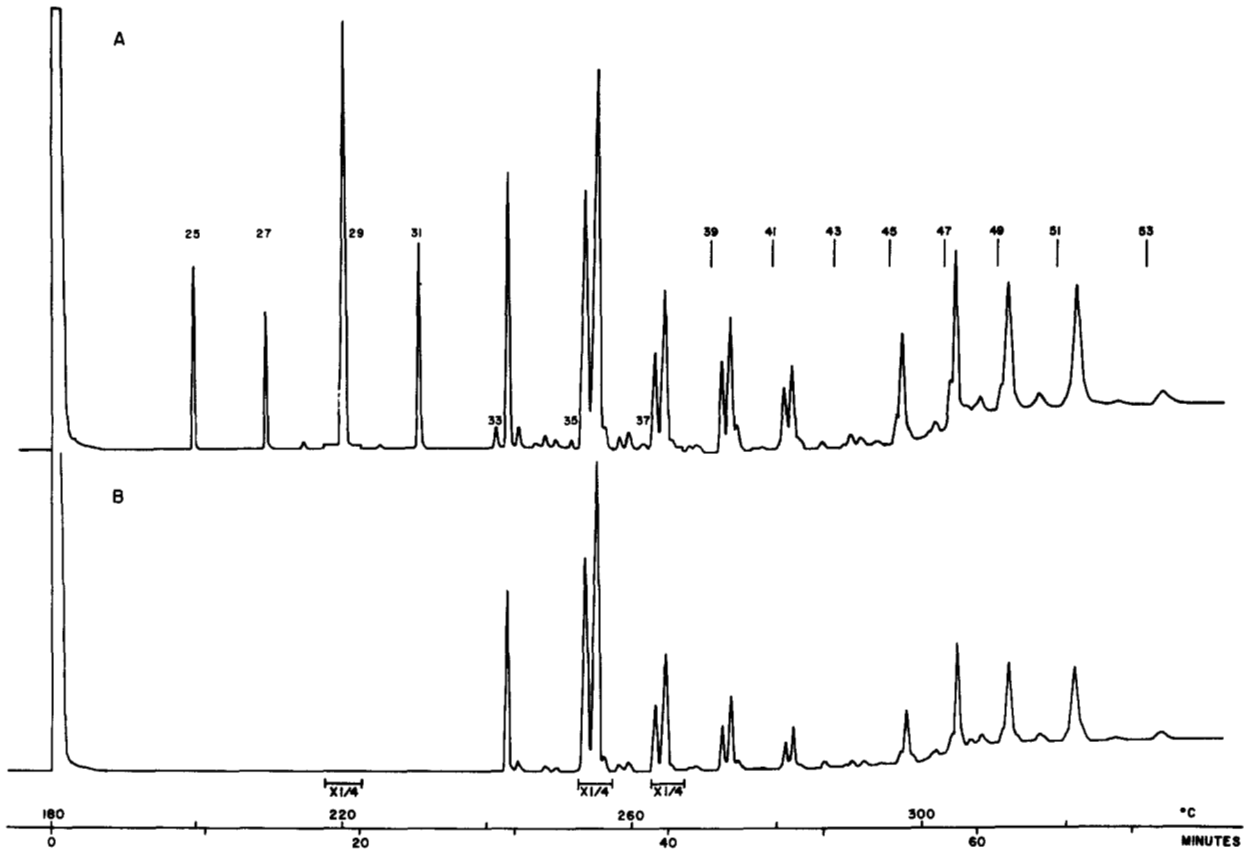

Fig. 1. Gas–liquid chromatographic analysis of the total (*A*) and branched-chain (*B*) hydrocarbon fractions on a 20 ft × 0.125 inch stainless-steel column of 80–100 mesh Diatoport S coated with 1.8% OV-101, temperature programmed from 180 to 310°C in 64 min and held at 310°C. Chart speed was 0.33 inch/min. The branched-chain fraction (*B*) was obtained by the use of 0.0625-inch pellets of Linde molecular sieve 5A. The numbers on the traces refer to the carbon numbers of the corresponding *n*-alkanes; the vertical lines with the numbers above them indicate the position at which an *n*-alkane of that carbon number would elute. The three peaks eluting between the elution points of two adjacent *n*-alkanes were designated by adding *A*, *B*, or *C* to the carbon number of the *n*-alkane after which they eluted, in order of increasing retention time.

cast skins with fractional equivalent chain lengths over 30 were synthesized by the insect.

1 g of *S. vaga* cast skins yielded 44 mg of lipid (4.4% of the cast skin), and column chromatography of this lipid on Florisil yielded a hydrocarbon fraction weighing 30 mg (3% of the cast skin and 68% of the total lipids). The hydrocarbon fraction was analyzed by GLC (**Fig. 1***A*) and found to be composed of over 50 components ranging in chain length from 22 to about 54 carbon atoms (based on retention time; some of the components present in trace amounts are not visible in Fig. 1*A*). The peak number, the carbon number, and the equivalent chain length are the same for the *n*-alkanes. The major hydrocarbons eluting above 33 had fractional equivalent chain lengths, which indicated that they were branched-chain hydrocarbons; they represented 65% of the hydrocarbon fraction.

A fraction containing only branched alkanes was obtained by treating the total hydrocarbon fraction with Linde molecular sieve 5A. Analysis of the straight-chain fraction by GLC showed that some of the branched hydrocarbons were retained by the molecular sieve. However, the branched-chain fraction (Fig. 1*B*) contained only branched hydrocarbons. The absence in Fig. 1*B* of the even- and odd-numbered peaks that are present in Fig. 1*A* verifies the previous conclusion (based on GLC retention times) that the whole-numbered peaks 22 through 35 and 37 are *n*-alkanes. Three branched hydrocarbons eluted between the elution points of any two adjacent *n*-alkanes, and although this was difficult to determine at the higher chain lengths, the branched alkanes eluting with more than 50 carbons were assumed to be composed of three types of hydrocarbons. The three types of branched hydrocarbons were designated by adding *A*, *B*, or *C*, in order of increasing retention time, to the carbon number of the *n*-alkane after which they eluted.

The total, straight-, and branched-chain hydrocarbon fractions showed no change in their GLC traces after bromination, an indication of the absence of unsaturated hydrocarbons. The absence of unsaturation was also confirmed by the mass spectral fragmentation patterns of those hydrocarbons so analyzed.

The presence of four homologous series of hydrocarbons was demonstrated when the logarithms of the isothermal retention distances were plotted vs. the carbon numbers

TABLE 1. Identification and percentage composition of hydrocarbons from grasshopper cast skins

GLC Peak No.	ECL[a]	Mass Spec. Carbon No.[b]	Percentage Composition[c]			Alkane[d]
			Total	Straight-chain	Branched-chain	
22	22		T	T		
23	23		T	1		
24	24		T	T		
25	25	25	3	8		*n*-Pentacosane
26	26		T	T		
27	27	27	2	6		*n*-Heptacosane
28	28		1	3		
29	29	29	23	66		*n*-Nonacosane
30	30		1	2		
31	31	31	4	12		*n*-Hentriacontane
32	32		T	T		
32-A	32.3		T		T	
33	33	33	1	2		
33-A	33.3	34	4		6	11-, 13-,[e] and 15-Methyltritriacontanes
33-B	33.6	35	1		1	9,13-, 11,15-, and 13,17-[e] Dimethyltritriacontanes
34	34		T	T		
34-A	34.3	35	T		1	13-Methyltetratriacontane[e]
34-B	34.6	36	T		1	12,16-, 13,17-, and 14,18-Dimethyltetratriacontanes[e]
35	35		T	T		
35-A	35.3	36	13		20	11-, 13-,[e] 15-, and 17-Methylpentatriacontanes
35-B	35.6	37	20		31	9,13-, 11,15-, 13,17-[e] and 15,19-Dimethylpentatriacontanes
35-C	35.8	38	1		2	11,15,19-,[e] and 13,17,21-Trimethylpentatriacontanes
36-A	36.3	37	T		T	14-Methylhexatriacontane[e]
36-B	36.6	38	T		1	14,18-Dimethylhexatriacontane[e]
37	37		T	T		
37-A	37.3	38	4		6	13-,[e] 15-, 17-, and 19-Methylheptatriacontanes
37-B	37.6	39	8		13	11,15-, 13,17-,[e] 15,19-,[e] and 17,21-Dimethylheptatriacontanes
37-C	37.8	40	1		1	11,15,19-, 13,17,21-,[e] and 15,19,23-Trimethylheptatriacontanes
38-A	38.3	39	T		T	12- and 13-Methyloctatriacontanes[e]
38-B	38.6	40	T		T	11,15- and 14,18-Dimethyloctatriacontanes[e]
39-A	39.3	40	1		2	13-,[e] 15-, 17-, and 19-Methylnonatriacontanes
39-B	39.5	41	3		4	13,17-,[e] 15,19-, and 17,21-Dimethylnonatriacontanes
39-C	39.8	42	T		T	13,17,21-Trimethylnonatriacontanes[e]
40-A	40.3					
40-B	40.5		T		T	
40-C	40.8					
41-A	41.3	42	1		1	13-Methylhentetracontane
41-B	41.5	43	1		2	13,17-[e] and 19,23-Dimethylhentetracontanes
41-C	41.8	44	T		T	13,17,21-Trimethylhentetracontane[e]
42-A	42.3		T		T	
42-B	42.5					
43-A	43.3					
43-B	43.5	45	1		1	13,17-[e] and 19,23-Dimethyltritetracontanes
43-C	43.7					
44-B	44.5		T		T	
45-A	45.3	47	1		1	13,17-Dimethylpentatetracontane[e]
45-B	45.5					
46-B	46.5		T		T	
47-A	47.2	48	1		2	13-Methylheptatetracontane[e]
47-B	47.5	49				13,17-Dimethylheptatetracontane
48-B	48.4		T		T	
49-A	49.2	50	1		1	13-Methylnonatetracontane[e]
49-B	49.4	51				13,17-Dimethylnonatetracontane[e]
50-B	50.4		T		T	
51-B	51.4	53	1		1	13,17-Dimethylhenpentacontane[e]
52-B	52.4		T		T	
53-B	53.4		T		T	

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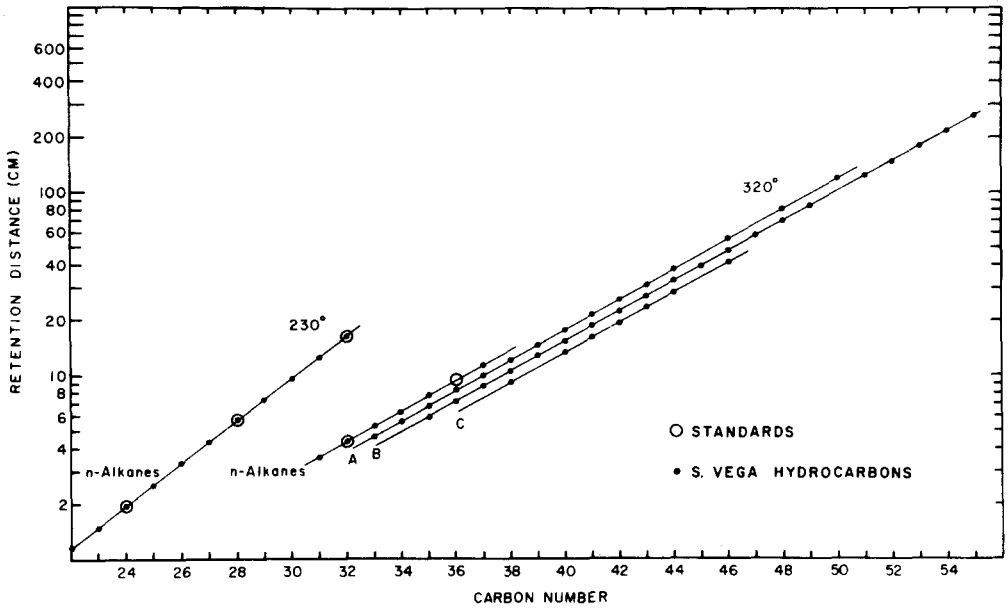

Fig. 2. A semilog plot of the retention distance vs. the carbon number of the individual hydrocarbons in the homologous series of *n*-alkanes and of the individual isomeric mixtures of hydrocarbons in the homologous A, B, and C series of branched alkanes. The data were obtained by GLC of the total hydrocarbon fraction and of the straight-chain and branched-chain hydrocarbon fractions obtained after treatment with molecular sieve. The values plotted for 230 and 320°C are averages of equivalent chain length values obtained by isothermal analysis on a 6 ft × 0.125 inch stainless steel column of 1.8% OV-101 on 80–100 mesh Diatoport S at 230, 250, 280, 300, and 320°C (semilog plot) and by temperature programming from 230 to 310°C in 160 min with a chart speed of 0.5 inch/min. Temperature programming from 230 to 310°C in 160 min gave much better resolution than is observed in Fig. 1 and was used for all other analyses.

(**Fig. 2**). The carbon numbers of the individual hydrocarbons of the *n*-alkane series were determined by a comparison of their retention distances with those of known standards; they were confirmed by trapping those individual hydrocarbons present in sufficient amounts from the gas chromatograph and obtaining their molecular weight by mass spectrometry. The molecular weights were also determined for those branched hydrocarbons present in sufficient amounts that were sufficiently resolved by GLC. The quantitative results of the GLC and mass spectral analyses are tabulated in **Table 1.** The equivalent chain lengths of the *n*-alkane series ranged from 22 to 35 and 37, those of the branched-chain A series ranged from 32.3 to 49.2 (carbon numbers from 33 to 50), those of the branched-chain B series ranged from 33.6 to 53.4 (carbon numbers from 35 to 55), and those of the branched-chain C series ranged from 35.8 to 49.7 (carbon numbers from 38 to 52). Probably all the gas chromatographic peaks resulting from the branched long-chain hydrocarbons were composed of an A, a B, and a C component, though in some cases the small amounts of the A and C components and the incomplete resolution made it difficult to determine whether the A and C components were present along with the B component, which was the major component. If all three components were present, the maximum carbon number for the A series would be 54 and for the C series, 56.

Representative mass spectra of the hydrocarbons for each of the branched-chain series are shown in **Fig. 3.** The molecular ion was of low intensity and, therefore, the characteristic series of peaks (M − 43, M − 29, M − 15) at the high mass end of the spectra that increase in intensity with an increase in atomic weight was used to establish the position of M − 15 and, therefore, the *m/e* of the molecular ion. The homologous series of branched hydrocarbons from *S. vaga* had equivalent chain lengths and mass spectra similar to those found for the hydrocarbons of the tobacco hornworm and were interpreted as pre-

[a] Equivalent chain length. Averages of values obtained from isothermal GLC at 230, 260, 280, 300, and 320°C (semilog plots) and from temperature programming from 200 to 320°C in 160 min (linear plot).

[b] Samples were collected from gas chromatograph in glass tubing. Portion of tubing containing sample was placed in solid sample probe of Varian M-66 and/or MAT CH5-DF mass spectrometer.

[c] Values calculated by triangulation of areas under the GLC peaks. All values were rounded to the nearest percent, and those components present in 0.5% or less are indicated by T.

[d] Structure determined by mass spectrometry.

[e] Major component(s) in a mixture of isomers.

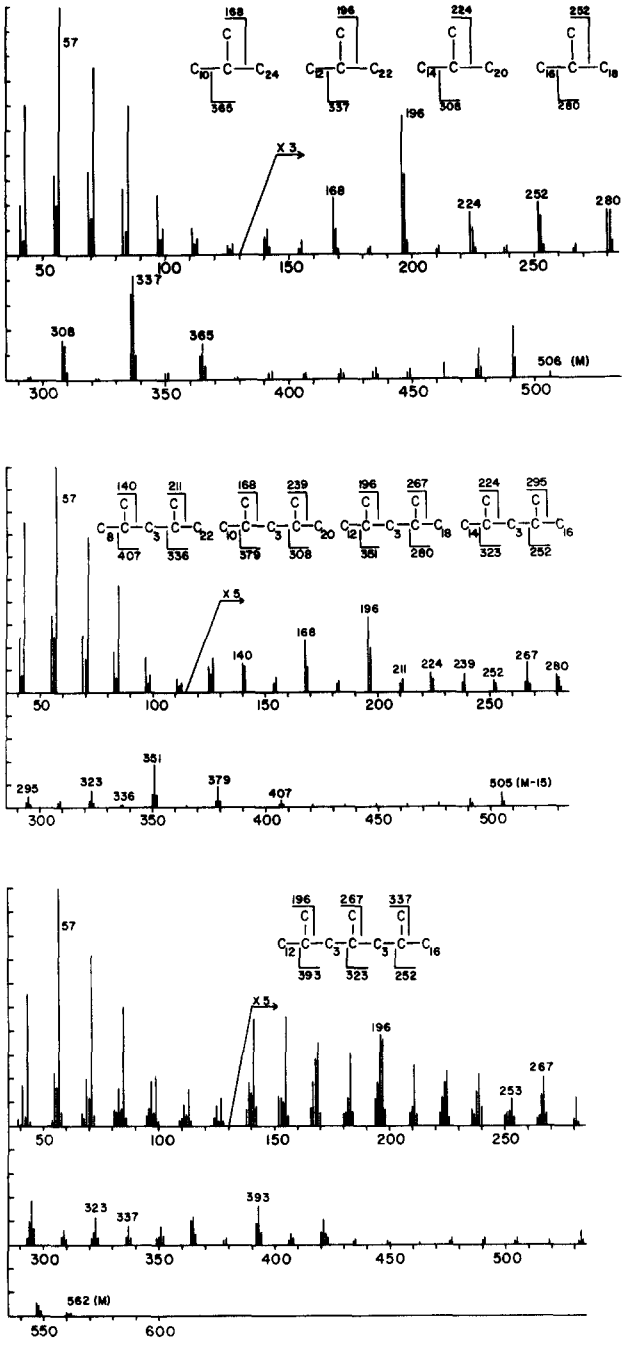

Fig. 3. Mass spectra of *S. vaga* cast skin hydrocarbons trapped from the gas chromatograph and run on a Varian M-66 or MAT CH5-DF mass spectrometer. *Top,* GLC peak 35-A, 11-, 13-, 15-, and 17-methylpentatriacontanes; *middle,* GLC peak 35-B, 9,13-, 11,15-, 13,17-, and 15,19-dimethylpentatriacontanes; *bottom,* GLC peak 37-C, 11,15,19-, 13,17,21-, and 15,19,23-trimethylheptatriacontanes. The structure of the major isomer only is shown.

viously described (3, 4). The A series was composed of monomethylalkanes, the B series of dimethylalkanes, and the C series of trimethylalkanes.

The mass spectrum of GLC peak 35-A of the homologous A series, with an equivalent chain length of 35.3 and a carbon number of 36, is shown in Fig. 3, *top*. The difference of 0.7 between the equivalent chain length and the carbon number was consistent with the presence of a single methyl branch towards the center of the molecule (3, 4, 26). The presence of only major pairs of fragmentation peaks with the even mass peak larger or almost as large as the adjacent odd mass peak showed that the spectrum was from a mixture of isomers of internally branched monomethylalkanes. The mass spectrum of GLC peak 35-A had eight characteristic pairs of peaks with large even masses at *m/e* 168, 196, 224, 252, 280, 308, 336, and 364, demonstrating the presence of four monomethylalkane isomers: 11-, 13-, 15-, and 17-methylpentatriacontanes. The major secondary ion fragments at *m/e* 336 and 364 were slightly smaller than the fragments at *m/e* 337 and 365 because of the relatively greater dissymmetry of 11- and 13-methylpentatriacontanes with respect to the position of the methyl branch (3, 4). A comparison of the relative intensities of the secondary ion fragments at *m/e* 168, 196, 224, and 252 showed that 11-, 15-, and 17-methylpentatriacontanes were present in about equal amounts and that about twice as much of the 13-methylpentatriacontane was present.

The mass spectrum of a branched alkane of the homologous B series with an equivalent chain length of 35.6 and a carbon number of 37 is shown in Fig. 3, *middle.* The difference of 1.4 to 1.5 between the equivalent chain length and the carbon number was consistent with the presence of two methyl branches towards the center of the molecule, with each methyl branch decreasing the GLC retention time the equivalent of 0.7 carbon atom (3, 4). The presence of groups of peaks with a predominant even mass peak and of groups of peaks with a predominant odd mass peak showed that the spectrum was of an internally branched dimethylalkane.

The mass spectrum of GLC peak 35-B (Fig. 3, *middle*) had eight characteristic odd mass peaks, which showed that it was a mixture of four isomers. The large peaks at *m/e* 196 and 351 showed that the major component of the mixture had the first branch point at C-13, and the smaller peaks at 280 and 267 established this component as 13,17-dimethylpentatriacontane. The isomers present in lesser amounts were 9,13-, 11,15-, and 15,19-dimethylpentatriacontanes.

GLC peak 37-C of the homologous C series of branched alkanes had an equivalent chain length of 37.8 and a carbon number of 40. The difference of 2.2 between the equivalent chain length and the carbon number was consistent with the presence of three internal methyl branches, with each methyl branch decreasing the GLC retention time the equivalent of 0.7 carbon atom (3, 4). The presence of the large even mass peak in the mass spectrum (Fig. 3, *bottom*) at *m/e* 196 from a secondary ion fragment without a second branch and of the large odd mass peak at *m/e* 393 from a secondary ion fragment with multiple branching established the first branch point of the major isomer as being at C-13. The presence of significant odd mass peaks at *m/e* 267 and 323 from cleavage

at the middle branch point, and of an odd mass peak at *m/e* 337 from cleavage external to the third branch point, established the major isomer as 13,17,21-trimethylheptatriacontane (the structure of this isomer only is shown in Fig. 3, *bottom*). The peak at *m/e* 253 was larger than that at 252 because cleavage internal to the third branch point is a relatively minor mode of fragmentation and because other isomers were present that contributed to the intensity of *m/e* 253. The peaks in the mass spectrum at *m/e* 421 and 365 showed that 11,15,19-trimethylheptatriacontane and 15,19,21-trimethylheptatriacontane, respectively, were also present.

Thus, the combination of GLC retention times and mass spectral fragmentation patterns established that four homologous series of alkanes were present in the surface lipids of the grasshopper. The *n*-alkane series accounted for 35% of the total hydrocarbons, with the major components being nonacosane (23%) and hentriacontane (4%); the major even-numbered *n*-alkanes were octacosane (1%) and triacontane (1%) (Table 1). In the branched-chain series, the A series accounted for 24%, the B series for 35%, and the C series for 3% of the total hydrocarbons. The major component of the A series was GLC peak 35-A (13%), consisting of a mixture of 11-, 13-, 15-, and 17-methylpentatriacontanes in which the 13-methyl isomer was the major isomer. The major component of the B series was GLC peak 35-B (20%), consisting of a mixture of 9,13-, 11,15-, 13,17-, and 15,19-dimethylpentatriacontanes in which the 13,17-dimethyl isomer was the major isomer. The smallest branched-chain series was the C series, whose major component was GLC peak 35-C (1%), consisting of a mixture of 11,15,19- and 13,17,21-trimethylpentatriacontanes in which the 11,15,19-trimethyl isomer was the major isomer. It is interesting to note that in the major isomers of the branched alkanes from the grasshopper, the first methyl branch occurred on carbon atom 11 or 13; however, in the branched alkanes from the tobacco hornworm (3, 4) the first methyl branch usually occurred on carbon atom 15 or 17. In the total hydrocarbon fraction, over 66% by weight of the hydrocarbons had an odd number of carbon atoms. In the individual series of hydrocarbons, the *n*-alkane series had about 94% odd-numbered hydrocarbons, the A series had an estimated 5%, and the B series had about 97%. The C series could not be estimated, but the majority of the components were even-numbered.

Although hydrocarbons have been identified in meteorites (27) (no alkanes were found in lunar samples [28] or in parts of graphite–troilite nodules of iron meteorites not exposed to the earth's atmosphere [27]), in petroleum (29), in plants (23), in a mollusc (30), in a diplopod (31), and in insects (1–21), the internally branched trimethylalkanes have been identified from only two sources, both insects, the tobacco hornworm, *M. sexta* (3, 4), and the grasshopper *S. vaga*. The internally branched dimethylalkanes have been identified in *M. sexta* (3, 4), in the Japanese beetle, *P. japonica* (5), and in *S. vaga*. However, the internally branched monomethylalkanes have been identified in a number of insects (3–7, 9, 11–14, 16–18, 21, 32), in plants (algae [33–35], lichen [36], and walnut tree [37]), in wool wax (26), and in meteorites (27).

It is likely that in the future many more branched alkanes similar to the internally branched mono-, di-, and trimethylalkanes will be identified; for example, unidentified internally branched monomethylalkanes were found in *Rosmarinus officinalis* L. (38). Unidentified branched alkanes (other than 2- and 3-methylalkanes and the isoprenoid paraffins squalane, pristane, and phytane) were reported in the millipede *Graphidostreptus tumuliporus* (31), the banded wood snail, *Cepaea nemoralis* (L.) (30), the tsetse fly *Glossina morsitans* Westwood (39), the silkworm *Bombyx mori* L. (40), the pea aphid, *Acyrthosiphon pisum* (Harris) (19), plant waxes (41–44), olive oil (45), bitumens (46), shale (47), and the alga *Scenedesmus quadricauda* (Turp.) Bréb. (48).

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