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Comparative Biochemistry and Physiology Part B 134 (2003) 447–466

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Hydrocarbons in the surface wax of eggs and adults of the Colorado potato beetle, *Leptinotarsa decemlineata*

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Received 11 September 2002; received in revised form 28 November 2002; accepted 28 November 2002

Abstract

The major components of the egg hydrocarbons were dimethylalkanes (40%) and trimethylalkanes (24%) in which the first methyl branch was on carbon 2. The major dimethylalkanes were an approximately 2:1 mixture of 2,10- and 2,6-dimethyloctacosanes in females and eggs. The major trimethylalkanes were a mixture of 2,10,16- and 2,10,18-trimethyloctacosanes. 2,*x*- and 2,*x,y*-methyl-branched alkanes with an odd-numbered carbon backbone were proposed to have an even number of carbon atoms between the first and second methyl branch points indicating that their biosynthesis started with a primer derived from leucine. 13,17,21,25-Tetramethylheptatriacontane was the only tetramethylalkane identified. Females and eggs had more hydrocarbons with a 2-methyl branch point than did the males. The eggs had the lowest amount of internally-branched dimethylalkanes but the largest amount of 2,*x*-dimethylalkanes in their surface hydrocarbons. Only trace amounts of *n*-alkanes and alkenes were detected in the surface hydrocarbons of adult males and females, larvae and eggs, of the Colorado potato beetle.

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Keywords: Insects; Hydrocarbons; Methylalkanes; Cuticular surface lipids; Eggs; Adults; Colorado potato beetle; *Leptinotarsa decemlineata*; Mass spectrometry

1. Introduction

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is the major defoliator of potatoes worldwide (Hare, 1990) and of staked tomatoes in northeastern North America (Schalk and Stoner, 1979). It can quickly develop resistance to insecticides. Host plant resistance is one method of control and a novel mechanism has been identified (Balbyshev and Lorenzen, 1997). The leaves of a *Solanum* spp. hybrid are hypersensitive to the egg masses of the Colorado potato beetle. The presence of an egg mass results in

detachment of a necrotic zone containing the egg mass, which falls to the ground before the eggs hatch, exposing the eggs and subsequent larvae to predators. The material causing the necrosis was shown to be associated with the egg itself. Another control method is biological control. *Perillus bioculatus* has been effective in controlling the beetle in augmentative release programs (Biever and Chauvir, 1992), and *P. bioculatus* and *Beauvaria* have both been demonstrated to be successful on potatoes grown in the Red River Valley of the North. The role of the surface chemistry of the host in predation and in fungal penetration remains to be determined.

Methyl-branched hydrocarbons have been identified in many species of insects (Blomquist et al., 1987; Lockey, 1988; Nelson, 1993; Nelson and

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Blomquist, 1995). In order to characterize components of the egg surface lipids and clarify the identity of adult cuticular hydrocarbons, we obtained surface hydrocarbons from both eggs and adults of the Colorado potato beetle. The results of our characterization of the individual components are presented herein. We have used three criteria in the interpretation of the data: (1) the retention index (RI) (van den Dool and Kratz, 1963) (analogous to the Kováts Indices (Kováts, 1965) or equivalent chain lengths (ECL) (Miwa, 1963)) must be the same as would be expected from the proposed number of methyl branches and their positions along the carbon backbone of the molecule (Mold et al., 1966; Nelson, 1978, 1993; Kissin et al., 1986; Blomquist et al., 1987; Carlson et al., 1998); (2) the proposed structure must be biosynthetically feasible (Nelson, 1993; Nelson and Blomquist, 1995); and (3) the relative intensity of adjacent even- and odd-mass fragment ions in the mass spectra must correspond to that expected for fragment ions with or without methyl branches (Nelson, 1978, 1993; Blomquist et al., 1987).

The composition of cuticular hydrocarbons and internal methyl-branched hydrocarbons from adult Colorado potato beetles has been described (Dubis et al., 1987) and qualitatively, the composition was the same for male and female beetles. They reported some novel methyl-branched hydrocarbons and later, gas chromatography–mass spectrometry (GC–MS) data was used to propose structures for several of the methyl-branched hydrocarbons (Maliński et al., 1986; Szafranek et al., 1994). However, our examination of the published data indicated that some structures proposed were neither supported by the published mass spectra, nor were compatible with the observed retention times (Kováts Indices), and/or were not biosynthetically feasible. The authors also proposed the presence of a tetramethylalkane which was not supported by their published data.

In this report, we describe the surface hydrocarbons from eggs, and adult male and female Colorado potato beetles. Structures were deduced from GC–MS and the electron impact mass spectra were correlated with the gas chromatographic retention time of the individual peaks, or of the relative location of components within a single peak.

2. Methods

2.1. Insects

Eggs, larvae and adults were obtained from laboratory-reared colonies that had originated from field collections in Fargo, ND, in 1995. The colony was reared in the laboratory on Luther Burbank potatoes at 80 °F, 70% RH, and a photoperiod consisting of dim lights from 05.00 h to 07.00 h and 20.00 h to 22.00 h, and high pressure sodium lights from 07.00 h to 20.00 h. Eggs were collected within 24 h of oviposition and adults were 7–10 days old. Analyses were replicated 3–4 times on eggs (40–60 eggs/sample) and adults (2–12 adults/sample).

2.2. Analysis

The insects and eggs were slurried in 5 ml hexane for 2 min followed by 5 ml of chloroform for 45 s to remove the surface lipids. Both samples were analyzed by capillary GC–MS. If hydrocarbons were found in the chloroform it was combined with the hexane. The chloroform rinse of the eggs contained excessive amounts of diacylglycerols and was not combined with the hexane. Because the hexane sample from the eggs also contained some diacylglycerols, it was chromatographed on a 0.5 mm×9.5 cm column of silica gel in a champagne funnel and the hydrocarbons eluted with 8 ml hexane.

Lipid classes were separated by thin-layer chromatography (TLC) on high-performance silica gel plates with hexane/diethyl ether/formic acid (80:20:1 v/v) as the developing solvent. The location of lipid bands was visualized by charring plates sprayed with a solution of 5% conc. sulfuric acid in 95% ethanol and heating at 140 °C for 10 min then increasing the heat to 240 °C for an additional 10–30 min as needed.

Samples were analyzed by GC–MS on a Hewlett-Packard quadrupole system equipped with an autoinjector and a temperature and pressure programmable cool on-column injection port (Nelson, 2001). The injection port was connected to a 1 m retention gap connected to a 12 m×0.2 mm capillary column of cross-linked dimethyl silicone Ultra 1. The column temperature was held at 150 °C for 4 min following sample injection and then was programmed from 150 to 320° at 4°/min and

held at 320° for approximately 30 min or until all components had eluted. Mass spectra of the hydrocarbons were interpreted as previously described (Nelson, 1978, 1993; Blomquist et al., 1987; Bernier et al., 1998; Carlson et al., 1998; Schulz, 2001).

RI were determined by comparing the retention times of the hydrocarbon peaks to those of known *n*-alkanes and the *n*-alkanes of a paraffin wax standard obtained using linear temperature programs (the indices are frequently referred to as Kováts Indices or ECL although the latter two were obtained with isothermal temperature programs). The size of the sample injected for both the beetle hydrocarbons and the standards was kept as low as possible to minimize errors in the RI due to peak shape and peak width. Wherever possible, the RI were calculated using the retention time of the leading edge or center of the eluting peak instead of the retention time of the highest point of the peak, which in our experience tends to minimize the effect of peak shape on the calculated RI value. This technique has a further advantage in that RI of unresolved isomers or types of methylalkanes can be estimated by determining the time at which they begin to elute by examining sequential scans for the first appearance of ions due to their presence.

The GC–MS total ion current data were analyzed using a computer spreadsheet program (LOTUS® or EXCEL®) in which the dose response was adjusted using a 3 component standard curve prepared using acetate ester, wax ester and *n*-alkane standard mixture. The dose response from 0 to 3.1 ng was linear, polynomial from 3.1 to 100 ng, and again linear over 100 ng. The formula in the spreadsheet selected the proper dose response formula to be used based on the total ion current peak area for each GC–MS peak for quantitation as nanograms. The amount of each class of hydrocarbon in overlapping GC–MS peaks was estimated by examining sequential mass spectral scans to locate the point of separation, then manually integrating the two portions of the peak where possible (Nelson et al., 2001).

3. Results

3.1. Thin-layer chromatography

The surface lipids of adult males and females consisted almost exclusively of one TLC fraction,

the hydrocarbons, 90 ± 2 and $94 \pm 2\%$, respectively. The majority of the remaining material was at the origin. The major materials in the eggs were found at the origin and in a band corresponding in relative mobility to diacylglycerols, but were not characterized further. Egg hydrocarbons were estimated at 11–18% of the total material in the sample.

3.2. Gas-chromatography–mass spectrometry

A short-hand designation was used to describe the methylalkanes: the number indicates the number of carbon atoms in the backbone of the molecule, the letters A, B, C and D indicate 1, 2, 3 or 4 methyl branches, respectively, and the prime symbol indicates one of the methyl branches is on carbon 2 or 3 of the backbone. The hydrocarbon fraction from the surface of adult males (167 ± 26 $\mu\text{g}/\text{insect}$) and females (179 ± 4 $\mu\text{g}/\text{insect}$) consisted mainly of a series of methyl-branched hydrocarbons from about GC–MS peak 25 (pentacosane; not shown) to peak 53C (trimethyltripentacontane) (Fig. 1a and b; Table 1). The hydrocarbons from eggs ranged from peak 25 (pentacosane) to peak 39C (trimethylnonatriacontane) (Fig. 1c; Table 1). In all analyses, *n*-alkanes were trace components which eluted with the B' components and were only detected by the presence of a molecular ion. Trace amounts of the alkene, hentriacontene, were detected by its molecular ion on the tailing edge of peak 30A', 2-methyltriacontane. The major hydrocarbon classes were the internally branched dimethylalkanes in males and the 2,*x*-dimethylalkanes in females and eggs (Table 2). The Colorado potato beetle was unique in that a majority of the hydrocarbons had a methyl branch on carbon 2: In the eggs they were 71.9% of the total hydrocarbons vs. 52.9% in the adult males and 65.1% in the adult females.

Each insect stage had two or three methylalkane components constituting 10% or more of the hydrocarbons: GC–MS peak 34C', was a mixture of 2,10,16- and 2,10,18-trimethyltetracontanes (10–11%) in males, females and eggs; GC–MS peak 30A', 2-methyltriacontane was 11–12% in males and females, but only 5% in the eggs; GC–MS peak 30B', 2,6-dimethyltriacontane, was 11% in eggs, and GC–MS peak 35B, a mixture of dimethylpentatriacontanes, was 9.9% in adult males (Table 1). GC–MS peak 28B', 2,10-dime-

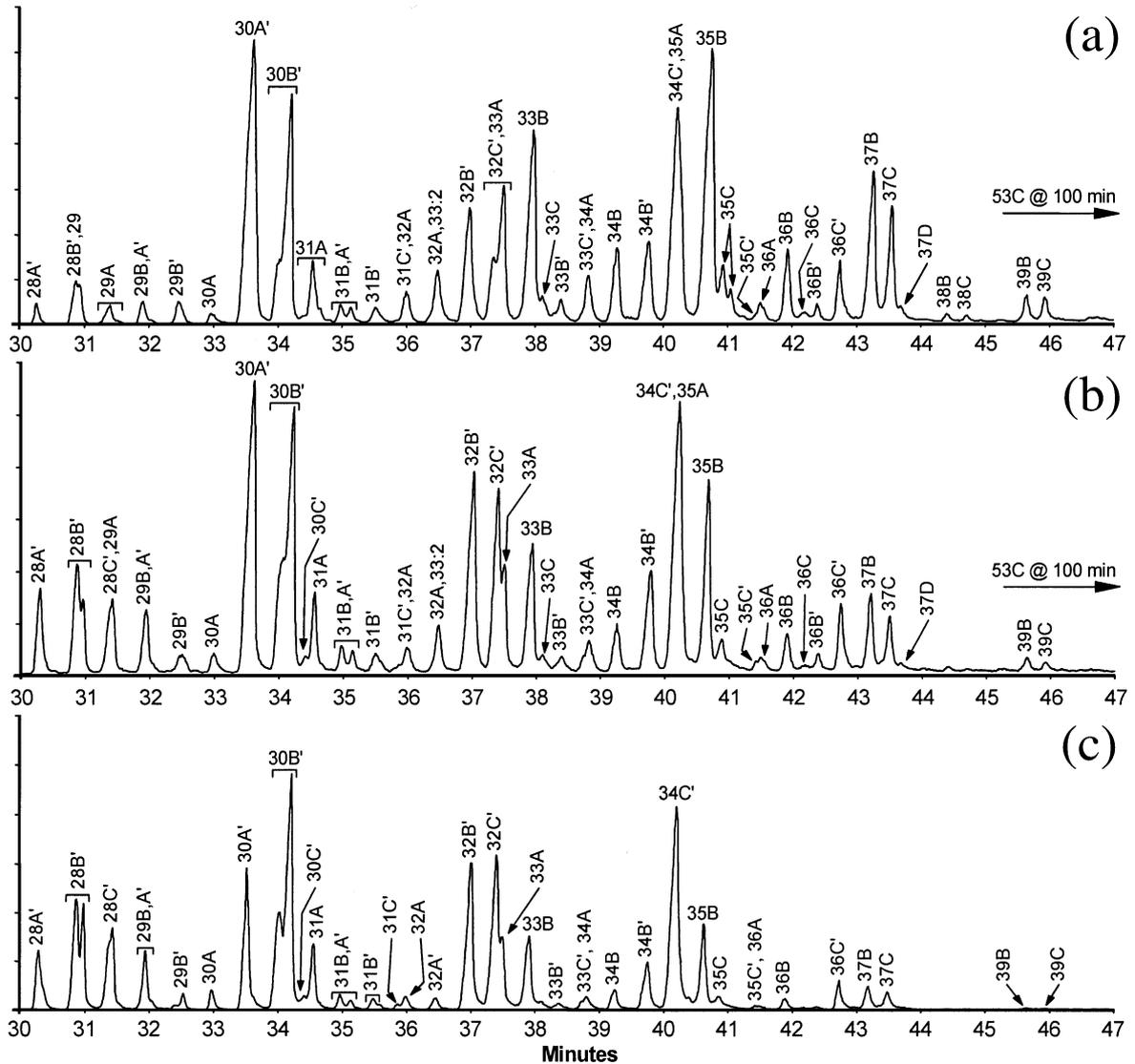


Fig. 1. GC-MS total ion current trace of the hydrocarbons from Colorado potato beetles: (a) adult males; (b) adult females; (c) eggs. Components eluting earlier and later are not shown (Table 1). Only peak 53C was visible in this trace. Other peaks beyond 39B were only visible in overload analyses. The numbers labeling the peaks indicate the number of carbon atoms in the backbone of the molecule; the letters indicate the number of methyl branches on the carbon backbone: A, B, C and D indicate 1, 2, 3 or 4 methyl branches, respectively. A letter with a prime symbol indicates that the first methyl branch is on a carbon near the end of the carbon chain backbone, i.e. on carbons 2 or 3.

thyoctacosane, went from 0.7% in males to 2.9% in females and 5.8% in eggs. Its isomer, 2,6-dimethyloctacosane, went from 0.9% in males to 1.1% in females to 2.9% in eggs. This change from males to eggs was paralleled by 2,10,18-trimethyloctacosane which increased from 0.4% in males to 1.4% in females to 2.4% in eggs. This pattern of increase from males to females to eggs also was observed for other B' peaks.

The elution times of individual components in overlapping peaks made discovery and identification of individual isomers difficult. The C' hydrocarbons (2,x,y-trimethylalkanes) eluted just ahead of the A hydrocarbons (internally branched monomethylalkanes) but the peaks were not resolved. However, when the methyl branches were all closer to the end of the carbon chain, the C' component eluted on the tailing edge of the A

Table 1
Percent composition of the hydrocarbons in the cuticular lipids of the Colorado potato beetle

GC-MS peak number ^a	Total carbons	Kováts Indices	Males		Females		Eggs		Hydrocarbon ^b
			Average % composition	Standard deviation	Average % composition	Standard deviation	Average % composition	Standard deviation	
25	25	2500	<i>t</i>						<i>n</i> -Pentacosane
26	26	2600	<i>t</i>						<i>n</i> -Hexacosane
26A'	27	2663							2-Methylhexacosane
26B									?
27	27	2700	<i>t</i>		<i>t</i>		0.2		<i>n</i> -Heptacosane
26B'	28	2702	<i>t</i>		0.1		<i>t</i>		2,8-Dimethylhexacosane
26B'	28	2709	<i>t</i>		0.1	0.1	1.0	0.1	2,6-Dimethylhexacosane
27A	29	2732	<i>t</i>		<i>t</i>		0.2	0.1	11- and 9-Methylheptacosanes
27B	29	2770					0.1		11,17- and 9,17-Dimethylheptacosanes
27B'	29	2797	0.1		0.1		0.3	0.2	2,17 ^{f,e} - and 2,21-Dimethylheptacosanes
28	28	2800					<i>t</i>		<i>n</i> -Octacosane
28A	29	2831	<i>t</i>		0.1	0.1	0.5	0.2	11- and 10*-Methyloctacosanes
28A'	29	2862	0.5	0.1	1.4	0.2	1.9	1.2	2-Methyloctacosane
28B	30	2873	0.1		0.5	0.1	<i>t</i>		10,18-Dimethyloctacosane
29:1							0.4	0.1	<i>n</i> -Nonacosane
28B'	30	2899	0.7		2.9	0.4	5.8	2.5	2,10-Dimethyloctacosane
29	29	2900	0.4						<i>n</i> -Nonacosane
28B'	30	2905	0.9	0.2	1.1	0.2	2.9	0.4	2,6*- and 2,4-Dimethyloctacosanes
28C'	31	2925	0.4	0.1	1.4	0.3	2.4	0.5	2,10,18*- , 2,10,16 ^e - and 2,10,18 ^e -Trimethyloctacosanes
29A	30	2932	0.5	0.1	1.0	0.3	2.3	0.9	11-Methylnonacosane
29A	30	2946	0.1		0.1		0.3	0.1	7-Methylnonacosane
29A'	30	2958	<i>t</i>		<i>t</i>				2-Methylnonacosane
29B	31	2963	0.8	0.1	1.6	0.1	1.9	0.1	11,19*- and 7,11 ^e -Dimethylnonacosanes
29A'	30	2970	0.1		0.2	0.1	0.5	0.2	3-Methylnonacosane
29B'	31	2997	0.4	0.1	0.3		0.3	0.2	2,17 ^e - and 2,19 ^{f,e} -Dimethylnonacosanes
30	30	3000			0.1				<i>n</i> -Triacontane
29B'	31	3008	0.4		0.4		0.7	0.1	2,23-Dimethylnonacosane
30A	31	3030	0.4		0.6	0.1	0.9	0.1	12-Methyltriacontane
30A'	31	3063	11.5	0.9	10.9	1.6	5.0	1.1	2-Methyltriacontane
31:2		3072	0.8	0.4	0.4	0.1	0.6	0.1	Hentriacontadiene
30B'	32	3095	2.0	0.5	4.5	0.5	4.9	0.8	2,12*- and 2,10 ^{f,e} -Dimethyltriacontanes
30B'	32	3104	7.4	0.6	7.5	0.5	11.0	0.9	2,6*- and 2,4-Dimethyltriacontanes
30C'	33	3123	0.3		0.4		0.8	0.1	2,12,18*- and/or 2,10,18 ^{e,f} - , 2,10,20 ^{e,f} - and 2,12,20 ^{e,f} -Trimethyltriacontanes
31A	32	3132	1.5	0.1	1.7	0.1	2.3	0.2	13*- , 11- and 9-Methylhentriacontane
30C'	33	3145			0.1		0.1		2,6,12-Trimethyltriacontane
31A	32	3152	0.2	0.1	0.1		0.1		7-Methylhentriacontanes
31B	33	3160	0.4	0.2	0.7		0.8	0.1	11,17 ^f - , 11,19*- and 7,11-Dimethylhentriacontanes
31A'	32	3172	0.4		0.5		0.6	0.1	3-Methylhentriacontane
31B'	33	3198	0.4		0.5		0.7		2,15-, 2,19- and 2,21*-Dimethylhentriacontanes
32	32	3200	0.1		<i>t</i>				<i>n</i> -Dotriacontane
31B'	33	3204	0.1		0.1		0.4	0.1	2,25-Dimethylhentriacontane
31C'	34	3219	0.2	0.1	0.2	0.1	0.3	0.1	2,15,21-Trimethylhentriacontane

Table 1 (Continued)

GC-MS peak number ^a	Total carbons	Kováts Indices	Males		Females		Eggs		Hydrocarbon ^b
			Average % composition	Standard deviation	Average % composition	Standard deviation	Average % composition	Standard deviation	
32A	33	3232	0.7		0.6		0.7	0.1	16-, 14-, 13-, 12*- and 10*-Methyldotriacontanes
32B	34	3261	<i>t</i>		0.2	0.4	0.6	0.2	12,16-, 12,18- and 10,16 ^e -Dimethyldotriacontanes
32A'	33	3262	1.4	0.1	0.9	0.5	0.4	0.1	2-Methyldotriacontane
33:2	33	3274	0.2	0.1	0.1	0.1			Tritriacontadiene
32B'	34	3298	5.1	0.6	7.6	0.4	8.1	0.6	2,16-, 2,14-, 2,12-, 2,10*- and 2,6-Dimethyldotriacontanes
32C'	35	3324	2.7	0.8	6.2	0.4	7.3	0.7	2,10,16 ^{*e} - and 2,12,16 ^{m,f} -Trimethyldotriacontanes
33A	34	3332	3.5	0.7	2.0	0.2	2.2	0.1	17-, 15-, 13- and 11 ^{*e} -Methyltritriacontanes
33B	35	3362	6.0	1.0	4.4	0.2	3.1	0.4	11,17- and 11,21*-Dimethyltritriacontanes
33C	36	3377	0.6		0.5		0.7	0.1	13,17,21*-Trimethyltritriacontane, and etc. ?
33B'	35	3398	0.7	0.1	0.6		0.6		2,17- and 2,23*-Dimethyltritriacontanes
33C'	36	3426	0.3		0.4		0.5	0.1	2,17,23*- and 2,19,23-Trimethyltritriacontanes
34A	35	3430	1.1	0.1	0.8		0.7	0.1	17*- , 12- and 10-Methyltetracontanes
34D'	38	3445					<i>t</i>		2,10,14,18-Tetramethyltetracontane
34B	36	3461	1.9	0.2	1.3	0.1	1.0	0.2	12,18- and 10,18*-Dimethyltetracontanes
34C			0.2	0.1	0.1	0.1			?
34B'	36	3497	3.4	0.6	3.3	0.2	2.3	0.5	2,16- and 2,10*-Dimethyltetracontanes
34C'	37	3524	10.2	1.4	10.5	0.7	11.0	1.9	2,10,16- and 2,10,18*-Trimethyltetracontanes
35A	36	3526	2.5	0.3	2.2	0.1	0.7	0.1	17*- , 15-, 13- and 11-Methylpentatriacontanes
35B	37	3560	9.9	1.2	6.0	0.4	3.0	0.6	13,19-, 11,17- and 11,23-Dimethylpentatriacontanes
35C	38	3580	1.5		1.0	0.1	1.1	0.2	13,17,23*- and 11,15,23-Trimethylpentatriacontanes
35C'	38	3593	0.6	0.2	0.3	0.2	0.5	0.1	9,13,23-Trimethylpentatriacontane ^c
35B'			0.2		0.1		?		?
35C'	38	3623	0.3		0.2		<i>t</i>		2,17,25-Trimethylpentatriacontane
36A	37	3631	0.5		0.4		<i>t</i>		18-, 17-, 15- and 13-Methylhexatriacontanes
36B	38	3666	1.7	0.1	1.0	0.1	0.7	0.1	12,16-, 12,18-, 12,20-, 13,??- and 10,18-Dimethylhexatriacontanes
36C	39	3680	0.4		0.3	0.1	0.2		?
36B'	38	3700	0.6	0.1	0.5		0.2		2,20-, 2,18- and 2,10*-Dimethylhexatriacontanes
36C'	39	3722	1.9	0.3	1.8	0.3	1.3	0.3	2,10,18-Trimethylhexatriacontane
37A	38	3733	0.2	0.1	0.2	0.1	0.1	0.1	19-, 17-, 15- and 13-Methylheptatriacontanes
37B	39	3758	4.2	0.6	2.1	0.3	1.1	0.1	13,17-, 13,19-, 13,21-, 13,23-, 13,25- and 11,23-Dimethylheptatriacontanes
37C	40	3782	3.0	0.1	1.6	0.2	1.0	0.1	13,17,25*- and 11,15,23-Trimethylheptatriacontanes
37D	41	3807	0.5		0.4		0.3	0.2	13,17,21,25-Tetramethylheptatriacontane
?			<i>t</i>		<i>t</i>				?
38B	40	3854	0.2	0.1	0.2	0.1	<i>t</i>		12,24 ^f -, 13,25 ^f -, 13,20 ^m - and 12,19 ^m -Dimethyloctatriacontanes
38C	41	3878	0.1	0.1	0.1				13,17,21-Trimethyloctatriacontane
38C'			0.1		0.1				?
39B	41	3956	0.6	0.1	0.4		0.2		13,19-, 13,23- and 13,25*-Dimethylnonatriacontanes
39C	42	3977	0.5	0.1	0.4	0.1	0.1		13,19,25- and 13,17,25*-Trimethylnonatriacontanes
39B'			0.1		0.1				?
39C'			0.2	0.2	0.1	0.1			?
41B			<i>t</i>		<i>t</i>				?
51B	53	5144	0.1		0.1				13,21- and 13,23*-Dimethylpenpentacontanes
51C	54	5164	0.1		0.2	0.1			13,21,25-Trimethylpenpentacontane

Table 1 (Continued)

GC–MS peak number ^a	Total carbons	Kováts Indices	Males		Females		Eggs		Hydrocarbon ^b
			Average % composition	Standard deviation	Average % composition	Standard deviation	Average % composition	Standard deviation	
51D					t				?
52B			0.1		0.1				?
52C			0.1		0.1				?
53B	55	5341	0.1		0.2	0.1			13,21-Dimethyltripentacontane
53C	56	5363	0.7	0.1	0.6	0.1			13,21,25- and 13,23,27-Trimethyltripentacontanes
55B			t		t				?
55C	58		t		t				13,21,27-Trimethylpentapentacontane

Values are the averages and standard deviations of 3 samples of males, 4 samples of females, and 4 samples of eggs determined from the total ion current plots from GC–MS analysis. A 't' means the peak was present at less than 0.05%. Where no standard deviation is listed, the value was less than 0.05%.

^a The GC–MS peaks correspond to those marked in Fig. 1. The number is the number of carbon atoms in the backbone of the molecule. The letters A, B, C, and D indicate 1, 2, 3, and 4 methyl branches, respectively. A letter with a prime symbol means that one of the methyl branches is near the end of the molecule, i.e. on carbon atom 2, 3, or 4. Therefore, it is possible to have two or three peaks in sequence marked with a prime symbol, e.g. an eluting sequence of 4-, 2- and 3-methylalkanes. Where a peak is multi-component, the compounds are listed in their order of elution as determined by examining individual scans throughout the peak. If the major component could be estimated it is marked with an asterisk.

^b The individual hydrocarbons were determined from their electron impact mass spectra and their estimated ECL. If the major isomer could be determined, it is marked with an asterisk. If the major isomer was different between samples, the major isomer in each sample is marked with a *m (=males), *f (=females), or *e (=eggs). If an isomer was present in one sample, that isomer is marked with a m (=males), f (=females) and e (=eggs). A '?' indicates the identity could not be determined from the mass spectra.

^c There may be some 35D on the tailing edge of the 35C. This seemed to be present in the females and the eggs, but it cannot be determined definitively.

Table 2
Percent distribution of the hydrocarbon classes in males, females and eggs of the Colorado potato beetle

Hydrocarbon Class	Males	Females	Eggs
<i>n</i> -Alkanes	0.5	0.2	0.3
Monomethylalkanes*	11.2	9.8	11.0
Dimethylalkanes*	26.0	18.7	12.2
Trimethylalkanes*	7.1	4.7	3.0
Tetramethylalkanes*	0.5	0.4	0.3
2-Methylalkanes	13.4	13.1	7.3
3-Methylalkanes	0.5	0.7	1.2
2, <i>x</i> -Dimethylalkanes	22.7	29.9	39.8
2, <i>x,y</i> -Trimethylalkanes	16.3	21.4	23.6
Alkenes	1.0	0.5	1.0
Unknown	0.8	0.5	0.3
Total with a methyl group on carbon 2	52.9	65.1	71.9

* These methylalkanes were internally methyl-branched, i.e. there were no methyl branches on carbons 1, 2, 3 or 4.

hydrocarbons. This is seen in Table 1 where 30C' (2,10,16-trimethyltriacontane) eluted on the leading edge of 31A (monomethylhentriacontanes). However, the 30C' isomer with the methyl branches near the end of the chain, 2,6,12-trimethyltriacontane, eluted on the tailing shoulder of the 7-methylhentriacontane isomer in the 31A portion of the peak. The RI was estimated by examining sequential mass spectral scans. Tetramethylalkanes were trace components, eluted on the tailing shoulder of trimethylalkanes, and only one was identified, 13,17,21,25-tetramethylheptatriacontane.

3.3. Mass spectra

Unresolved peaks, and the range of branching configurations which affect GC–MS retention times, made interpretation of the data complicated. The B' components eluted with retention times corresponding to *n*-alkanes. In some cases it was necessary to find a definitive mass spectra of one of the components of a homologous series in order to determine the branching positions. One could then return to the mass spectra of other peaks which were composed of mixtures, e.g. of C' and A components, to determine which ions came from the C' component and which came from the A component. In some cases, the same ions originated from both, but knowing that the A components eluted later than the C' components allowed ions to be matched with both components. It was always necessary to evaluate whether a structure proposed from the mass spectra would have the observed retention times and if it was biosynthetically feasible.

3.3.1. Mass spectra of B' hydrocarbons

Representative mass spectra of the B' series of hydrocarbons with an even number of carbon atoms in the backbone are presented in Fig. 2. Interpretation of the mass spectrum is complicated by the fact that it could be one of two compounds or pairs with similar RI's (Schulz, 2001): e.g. in our case 2,12-C30 could be paired with 2,18-C30, and 2,10-C30 with 2,20-C30, and so on. We have interpreted the mass spectra assuming that the correct structure of a possible pair is that with the fewest methylenes between the methyl branch points. This approach is consistent with our general understanding of biosynthesis of insect methylalkanes and resulted in structures with expected RI's in agreement with the observed RI's.

The first isomers of GC–MS peak 30B' to elute were 2,14- and 2,12-dimethyltriacontanes (Fig. 2a). Seven scans later both the 2,12- and 2,10-isomers were eluting (Fig. 2b). The ion pair at *m/z* 364:365 show that the 2,6-isomer is beginning to elute. Nine scans later, the 2,6-isomer was the only isomer eluting (Fig. 2c). A component tentatively identified as the 2,4-isomer was not resolved from the 2,6-isomer and was only detectable in a single scan (occasionally in two scans) at the end of the tailing shoulder of the peak (Fig. 2d) (the only other 2,4-component found under similar circumstances was 2,4-dimethyloctacosane). The 2,4-isomer was characterized by the appearance of an additional diagnostically significant fragmentation apparently formed by α -cleavage internal to the methyl branch on carbon 2 forming a 29-carbon branched primary fragment ion at *m/z* 407. Both the retention time and order

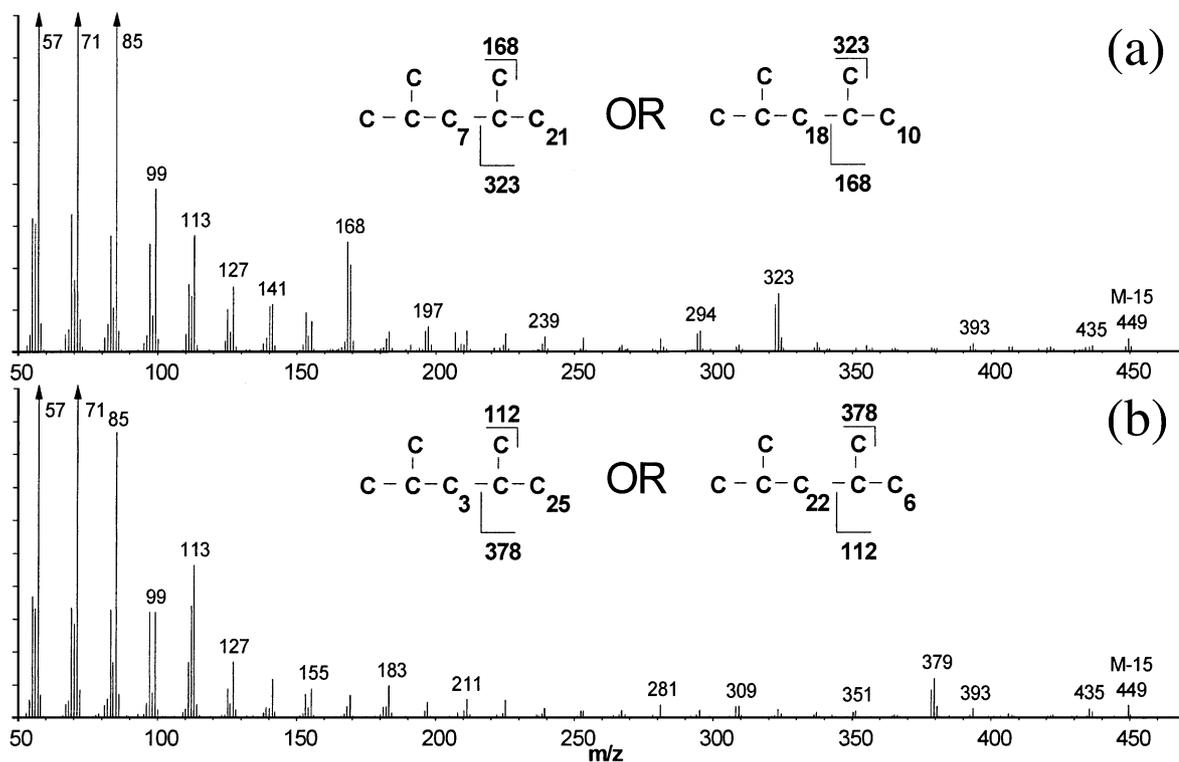


Fig. 3. GC–MS mass spectra of peak 31B', an example of 2,*x*-dimethyl branching on an odd carbon-numbered backbone: (a) 2,21-dimethylhentriacontane; and (b) 2,25-dimethylhentriacontane. The *m/z* value above a pair of ions is that of the most intense ion.

of elution was that expected for this series of isomers. The only 2,8-branching sequence was found in GC–MS peak 26B'. A branching sequence of 2,16 was found in GC–MS peaks 32B' and 34B', and a sequence of 2,14 in peak 32B'. These structures were compatible with the mass spectra, the retention times, and are biosynthetically feasible.

Interpretation of the branching sequence for 2,*x*-dimethylalkanes with an odd number of carbon atoms in the straight-chain portion (backbone) of the molecule required a different approach. Interpreting the mass spectra as described above for peak 30B', resulted in structures for GC–MS peaks 27B', 29B', 31B' and 33B' with an odd-numbered straight-chain tail after the last methyl branch point. However, our current understanding of carbon-chain elongation and reductive decarboxylation does not allow for the reduced carbon-chain to terminate with an odd number of carbon atoms after the last methyl branch point. An exception might be if the last elongating unit was propionate and if it could undergo a reductive decarboxylation (or reductive decarbonylation) there would then

be an odd number of carbon atoms beyond the last methyl branch point. Or, if the carboxyl of the propionate could be reduced, elongation could terminate with one carbon beyond the last methyl branch point. It is not known if propionate can undergo such reactions. Therefore, the structures assigned to the mass spectra had to be designed to be compatible with the mass spectra, the RI, and with the known feasibility of biosynthesis.

For example, the mass spectra for GC–MS peak 31B' showed the presence of two isomers (Fig. 3a and b). Assuming these are part of a homologous series with similar branch points, they could be assigned structures of 2,10- and 2,6-dimethylhentriacontanes, respectively. However, both of these structures have an odd number of carbon atoms in the carbon chain after the second (last) methyl branch point. The structures with branching sequences of 2,21 (Fig. 3a) and 2,25 (Fig. 3b) have an even number of carbon atoms in the carbon-chain after the last methyl branch point. The biosynthesis of 2,21- and 2,25-dimethylhentriacontanes is feasible if synthesis begins with a primer derived from the amino acid leucine. Thus,

the proposed methyl branch sequences of 2,21 and 2,25 are compatible with the mass spectra, the RI, and the feasibility of biosynthesis.

3.3.2. Mass spectra of methylalkanes in the series from 35C' to 37D

The terminally branched trimethylalkanes were the major components of the unresolved C',A peaks. They eluted just ahead of the internally branched monomethylalkanes but were not resolved from the minor amounts of the monomethylalkanes. The first in the series of GC–MS peaks presented herein is the unresolved peak 35C',A. (Fig. 4a). The initial structure drawn for the first component to elute was that of 2,10,16-trimethylpentatriacontane. However, this structure for 35C' has an odd number of carbon atoms in the backbone of the molecule and has an odd number of carbon atoms (19) in the straight-chain tail of the molecule beyond the last methyl branch point. As presented above for the B' components with an odd number of carbon atoms in the terminal tail of the molecule, a structure must be proposed that is biosynthetically feasible. Such a structure was 2,17,25-trimethylpentatriacontane (Fig. 4a). This structure is compatible with the RI and is biosynthetically feasible starting with a primer derived from leucine. Also, this structure accounts for the high intensity of the ion pair at m/z 266:267 much better than does the 2,10,16 branching sequence. In the 2,10,16 structure, the presence of the methyl branch on carbon 10 would be expected to reduce the rearrangement of a hydrogen during formation of the secondary fragment ion formed by cleavage of the 19-carbon tail at the methyl branch on carbon 16. Such a fragmentation would have resulted in the ion at m/z 267 being much larger than that at m/z 266. The observed intensity of these ions in all scans showed that they were of comparable intensity. This would be expected if the only methyl branch in the secondary fragment ion was on carbon 2. A methyl branch on carbon 2 does not suppress the rearrangement of a hydrogen during formation of the secondary fragment ion and would result in an ion pair where both ions would be of comparable intensity, which was observed for the pair at m/z 266:267. Thus, the correct structure was concluded to be 2,17,25-trimethylpentatriacontane.

The next components of the 36C',A peak, internally-branched monomethylalkanes, eluted on the tailing shoulder of the C' series. The first isomers

of 36A to elute on the tailing shoulder of 35C' were a mixture of the 18- and 17-methylhexatriacontanes (Fig. 4b) with lesser amounts of the 15- and 13-methyl isomers (data not shown). The next peak to elute after peak 36C',A was peak 36B, which was a mixture of 12,20- and 12,18-dimethylhexatriacontanes (Fig. 4c), and these isomers were followed 5 scans later by the 10,18-dimethyl isomer (Fig. 4d).

GC–MS peak 36B was followed by putative peak 36C (too minor a component for definitive identification) and then by GC–MS peak 36B' (Fig. 1). GC–MS peak 36B' was composed of three isomers. The first two isomers eluted together and were identified as 2,20- and 2,18-dimethylhexatriacontanes (Fig. 5a). Five scans later the 2,10-isomer was eluting (Fig. 5b). These structures are supported by the relative intensities of the even- and odd-mass ion pairs in the mass spectra, the RI, and are biosynthetically feasible starting with a primer derived from the amino acid valine. The next peak to elute was GC–MS peak 36C' (Fig. 5c). The mass spectra were definitive for a structure of 2,10,18-trimethylhexatriacontane. The secondary fragment ions with a second methyl branch were of odd mass as expected from the structure. Those secondary fragment ions without another methyl branch, or with a methyl branch on carbon 2, had a large even-mass ion as expected. The structure had the expected RI and is biosynthetically feasible.

The next three GC–MS peaks to elute in the series being presented herein were all of internally branched methylalkanes, GC–MS peaks 37B, 37C and 37D (Fig. 1). Peak 37B was a mixture of isomers with the major isomer in eggs being 13,25-dimethylheptatriacontane (Fig. 6a). The intensity of the ions at m/z 407 and 224 show that a minor amount of the 11,23-isomer also was present. By examining each scan through the peak, the 13,17-, 13,19-, 13,21-, and 13,23-isomers may also be present but definitive mass spectra could not be found. GC–MS peak 37C was mainly one isomer, 13,17,25-trimethylheptatriacontane (Fig. 6b). The presence of a possible 11,15,23-trimethylheptatriacontane was indicated only on the tailing shoulder of the peak by a small increase in the intensity of the ion at m/z 421 (data not shown). Mass spectral data for the last component presented herein, is that for peak 37D, 13,17,21,25-tetramethylheptatriacontane (Fig. 6c). Only trace amounts of peak 37D were present in cuticular

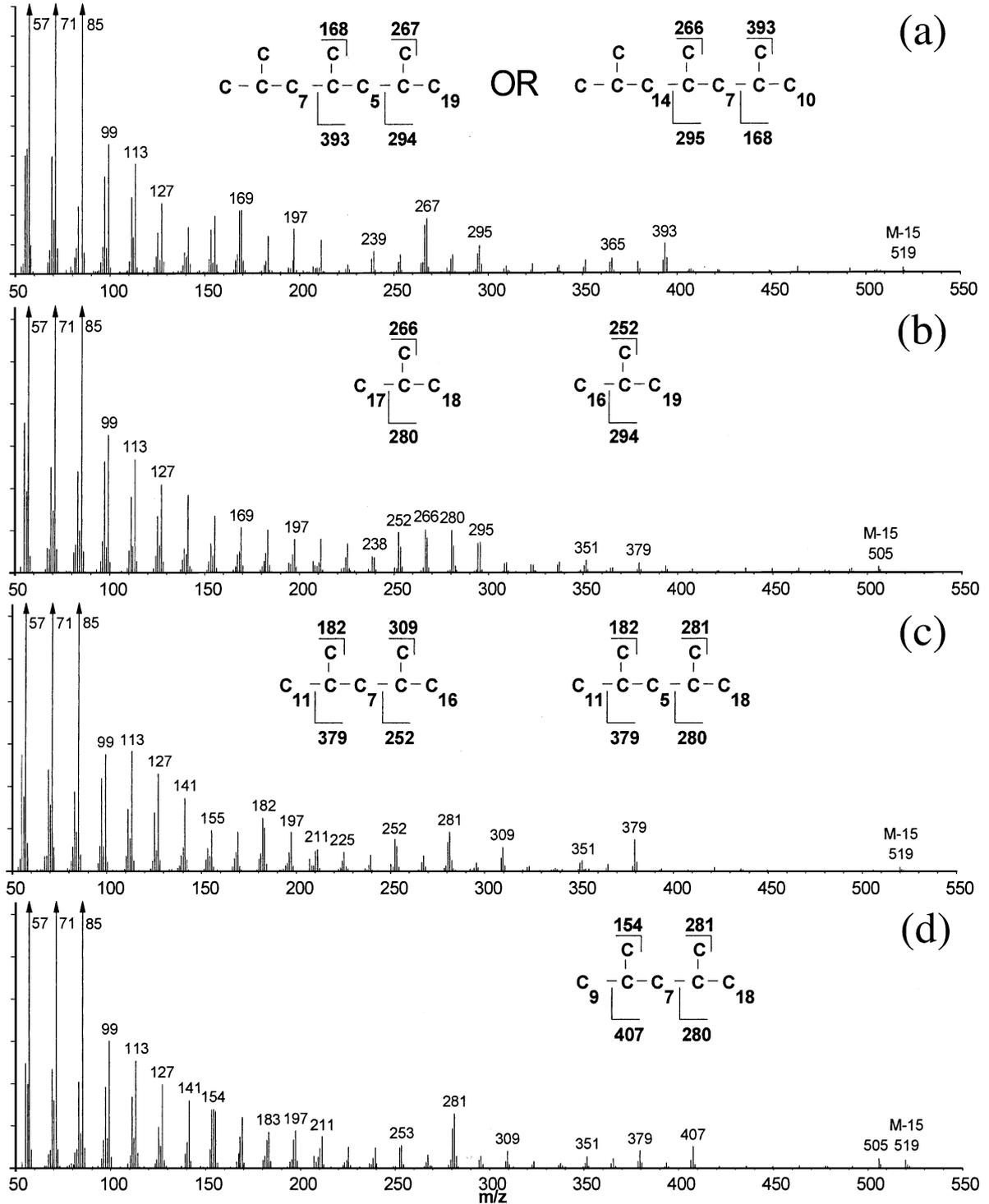


Fig. 4. GC-MS mass spectra of peak 35C' plus 36A, and peak 36B: (a) 2,17,25-trimethylpentatriacontane (35C'); (b) 18- and 17-methylhexatriacontanes (36A); and (c and d) 12,20- and 12,18-dimethylhexatriacontanes and 10,18-dimethylhexatriacontane (36B). The m/z value above a pair of ions is that of the most intense ion.

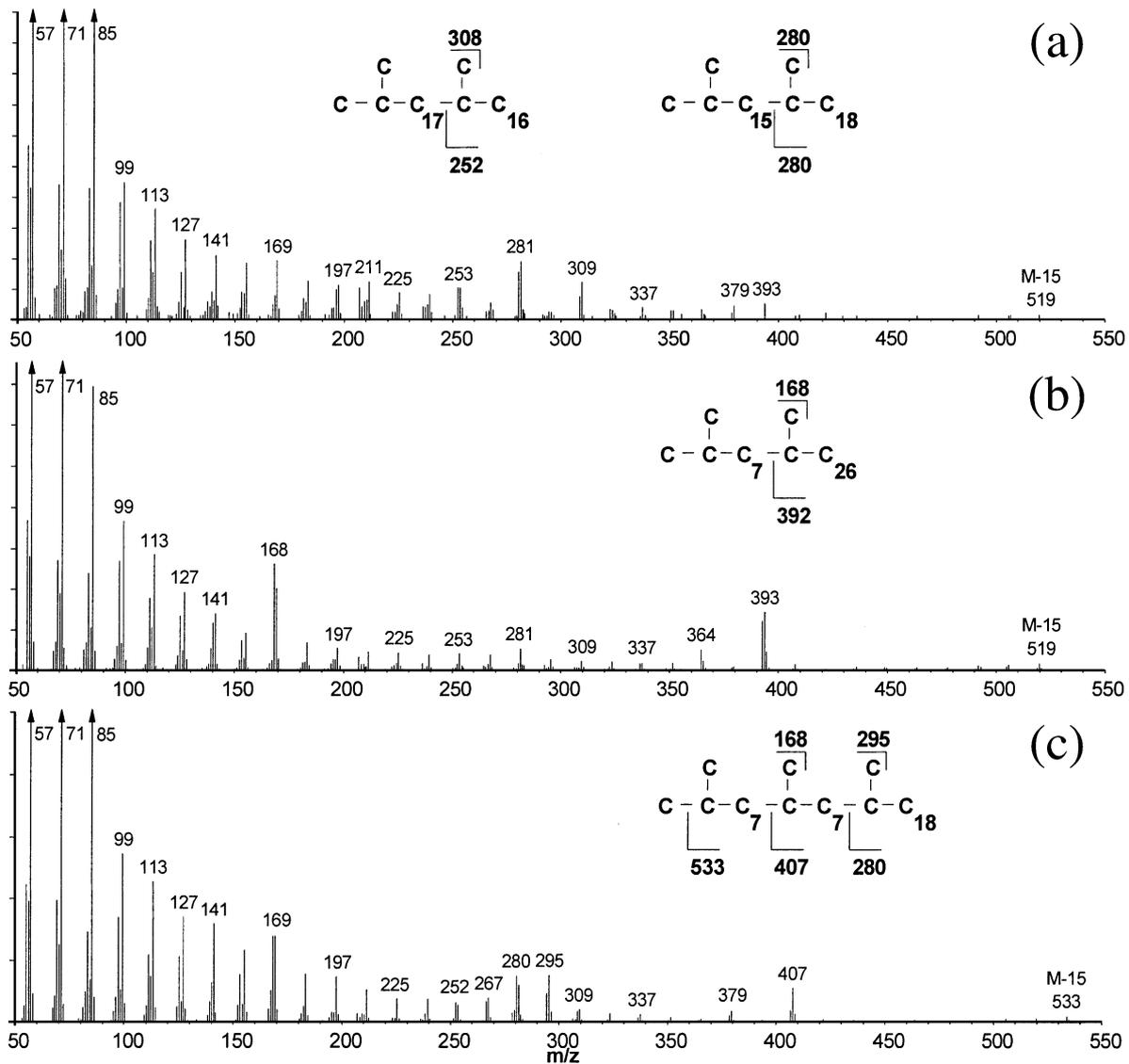


Fig. 5. GC–MS mass spectra of peaks 36B' and 36C': (a) 2,20- and 2,18-dimethylhexatriacontane; (b) 2,10-dimethylhexatriacontane; and (c) 2,10,18-trimethylhexatriacontane (36C'). The m/z value above a pair of ions is that of the most intense ion.

hydrocarbons of the adults and it was not identified in hydrocarbons from the eggs. This compound is novel in that it is symmetrically branched on its 37-carbon backbone. Also, note that for this sequence of peaks with a 37-carbon backbone, 37B, 37C, and 37D, that the first and last methyl branch points were on the same carbon of the backbone for the major isomers, i.e. on carbons 13 and 25.

4. Discussion

In the presentation of the mass spectral data, we have attempted to present mass spectra for methyl-branched alkanes which have not been published previously for either the Colorado potato beetle or for other species. We used a short-hand designation to describe the methylalkanes: the number indicates the number of carbon atoms in the backbone

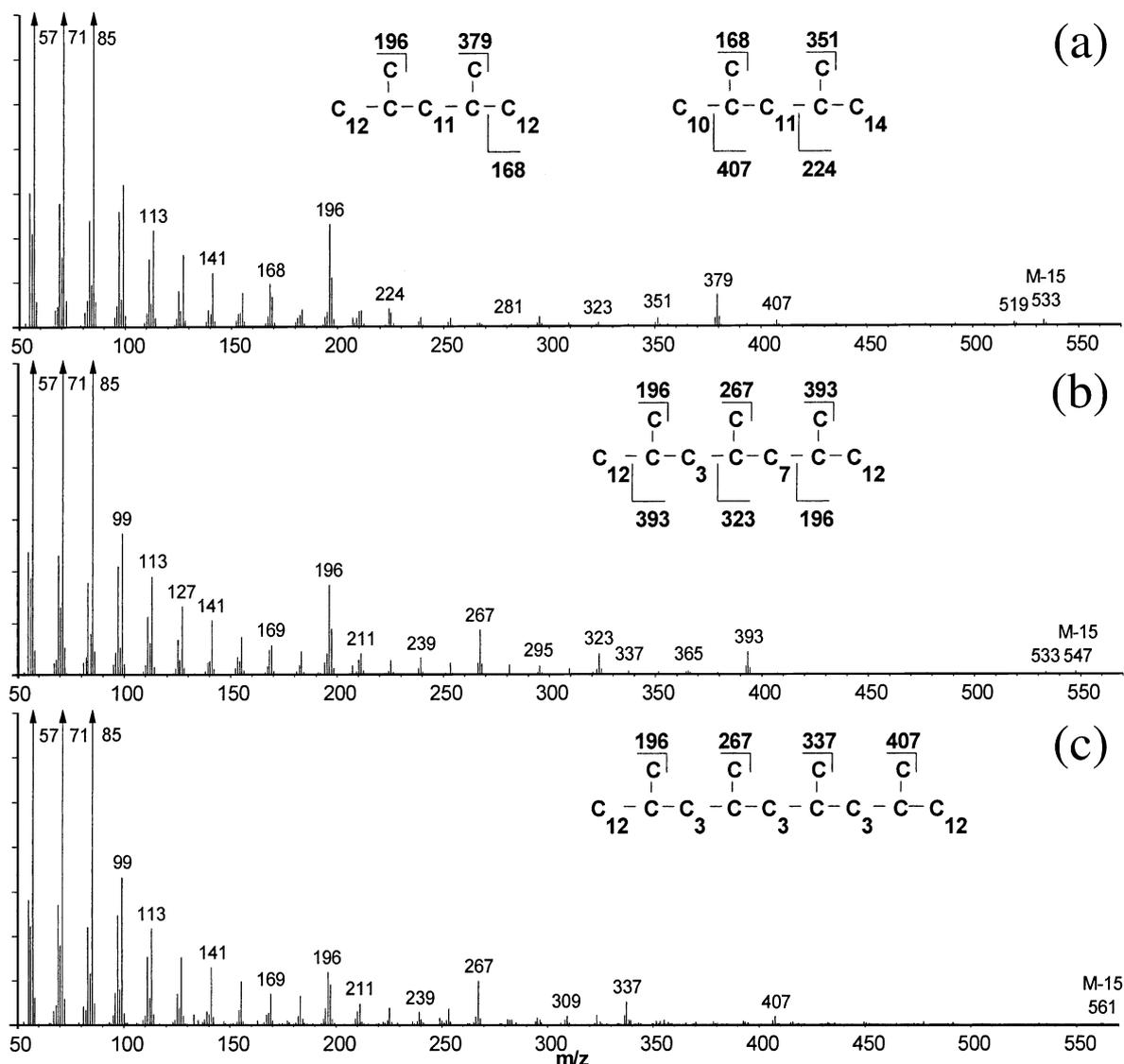


Fig. 6. GC–MS mass spectra of peaks 37B, 37C and 37D: (a) 13,25-dimethylheptatriacontane (37B) and some 11,13-dimethylheptatriacontane; (b) 13,17,25-trimethylheptatriacontane (37C); and (c) 13,17,21,25-tetramethylheptatriacontane (37D). The m/z value above a pair of ions is that of the most intense ion.

of the molecule, the letters A, B, C and D indicate 1, 2, 3 or 4 methyl branches, respectively, and the prime symbol indicates one of the methyl branches is on carbon 2 or 3 of the backbone; e.g. 28B' indicates a dimethylalkane with a 28-carbon backbone and one of the methyl groups is on carbon 2 or 3.

Hydrocarbons of the Colorado potato beetle have been reported as a major component of the surface lipids (Maliński et al., 1986; Dubis et al., 1987; Szafranek et al., 1994). Some of their

published mass spectra were not compatible with their proposed structures or KI (read RI) values. Valid mass spectra and KI's were presented for 28B', 2,10- (or 2,18-) and 2,6-dimethyloctacosanes (Maliński et al., 1986), and for 28B', 2,6-dimethyloctacosane, 28C', 2,10,18-trimethyloctacosane, and 30B', 2,6-dimethyltriacontane (Szafranek et al., 1994). Qualitatively, the composition of surface hydrocarbons was reported to be similar for male and female beetles and was similar to the composition of the internal hydrocarbons. Methyl-

branched alkanes were the major component, along with phospholipids, of the lipids from lipophorin although no mass spectral data were published (Katagiri and de Kort, 1991). The major components identified in both epicuticular and internal hydrocarbons were 2,6-dimethyloctacosane, 2-methyltriacontane and a tetramethyltrtriacontane (Dubis et al., 1987). The major hydrocarbon in the surface wax of potato leaves was hentriacontane (C31) followed by 2-methyltriacontane (Dubis et al., 1987). Although 2-methyltriacontane was a major component of the beetle surface lipids as well as of potato leaves, only trace amounts of *n*-alkanes, including C31, were detected based on their molecular ions. Thus, either the beetle provides its 2-methyltriacontane by *de novo* synthesis, or has a very selective process for determining which dietary hydrocarbons are transported to the cuticular surface. Taken together, the data, either mass spectral or gas chromatographic or both, did not support some of their structural assignments and no data were presented for tetramethylalkanes.

Methyl-branched hydrocarbons as well as *n*-alkanes have been identified in many species of insects (Blomquist et al., 1987; Lockey, 1988; Nelson, 1993; Nelson and Blomquist, 1995). Although very infrequent, tetramethylalkanes have been identified in the surface hydrocarbons of some insects: tsetse flies (Nelson et al., 1988), *Onymacris bicolor* (Lockey, 1991), *Epiphysa* spp. (Lockey, 1992), *Ips* spp. (Page et al., 1997) and *Lymantria dispar* (Jurenka and Subchev, 2000).

Three concepts are involved in determining structures for methyl-branched hydrocarbons in insects, and the proposed structure must be compatible with all three: (1) the influence (Nelson and Sukkestad, 1970; Nelson, 1978; Carlson et al., 1998; Schulz, 2001) of the number of methyl branches and their positions on gas chromatographic retention as measured by RI (van den Dool and Kratz, 1963), Kováts Indices (Kováts, 1965) or the similar 'ECL' (Miwa, 1963); (2) the influence of the number and position of methyl branches on the mass spectral fragmentation patterns (McCarthy et al., 1968; Nelson and Sukkestad, 1970; Nelson, 1978, 1993; Pomonis et al., 1980, 1989; Blomquist et al., 1987; Nelson and Blomquist, 1995; Schulz, 2001); and (3) the proposed structures must be biosynthetically feasible (Blomquist et al., 1987; Nelson and Blomquist, 1995; Schulz, 2001).

For internally branched methylalkanes, the first two digits of the RI, KI or ECL indicate the number of carbon atoms in the backbone of the molecule. A RI of 3120–3140 indicates a monomethylhentriacontane, and a RI of 3150–3160 indicates a dimethylhentriacontane, both with 31 carbon atoms in the backbone of the molecule. This use of the first two digits may not be valid for all methylalkanes with more than one methyl branch if the first methyl branch point is on carbon 2, 3 or 4. Such methylalkanes may have a carbon-chain backbone one carbon less than indicated by the first two digits of the RI depending on the position of the second methyl branch point and the size of the molecule. A 2,*x*-dimethylhentriacontane with 31 carbon atoms in the backbone can elute with a RI of approximately 3195–3205 depending on the position of the second methyl branch point. A summary of the effect of methyl branches and their positions on gas chromatographic retention times has been published (Carlson et al., 1998).

The terminal methyl group, i.e. the methyl group on carbon 2, on 2,*x*-dimethylalkanes is not readily evident in their mass spectra (Pomonis, 1989; Pomonis et al., 1989; Schulz, 2001). This fact results in a mass spectrum that may look like that of an internally branched monomethylalkane. Schulz (2001) has shown that 2,6-dimethyloctacosane, the alternate structure 2,22-dimethyloctacosane, and 7-methylnonacosane have similar mass spectra. Very likely in practice they could not be reliably differentiated on the basis of their mass spectra alone. However, the 2,*x*-dimethyloctacosanes can be distinguished from 7-methylnonacosane based on differences in the RI's. Also, the 2,*x*-dimethylalkanes (as well as 3,*x*- and 4,*x*-dimethylalkanes) may have RI's similar to those of *n*-alkanes one carbon number smaller and may be masked by the *n*-alkanes (Nelson and Blomquist, 1995; Carlson et al., 1998).

The effect of the methyl groups on reducing the retention time is approximately additive. Thus, 2-methylnonacosane will have an RI of approximately 2960, 9-methylnonacosane approximately 2940, and 2,9-dimethylnonacosane with 31 carbon atoms will have a RI of approximately 3000 vs. that for a 31-carbon *n*-alkane of 3100. We do not know of any reports of 2,2'-dimethylalkanes, i.e. a methyl group on carbon 2 from each end of the molecule. However, there are three reports of 3,3'-dimethylalkanes: a series of 3,3'-dimethylalkanes

in the silverfish, *Lepisma saccharina* (Jacob et al., 1997), 3,24-dimethylhexacosane in the yellow-headed spruce sawfly, *Pikonema alaskensis* (Bartelt et al., 1984), and 3,26-dimethyloctacosane and 3,28-dimethyltriacontane in the moth, *Scoliopteryx libatrix* (Subchev and Jurenka, 2001). These last three compounds from insects had KI's of 2734, 2934 and 3134, respectively. Mass spectra were not published for *S. libatrix* but the listing of diagnostic ions had m/z 56 > 55 which indicates a methyl branch on the third carbon from the end of the chain.

We reviewed the published data on the methylalkanes of the Colorado potato beetle (Maliński et al., 1986) and have the following comments:

(1) The proposed 11,14-dimethylhexacosane is untenable on two counts: (a) it is not biosynthetically feasible since an internally branched dimethylalkane cannot have an even number of methylenes between the branch points; and (b) the expected KI would be approximately 2660, not the observed KI of approximately 2700.

(2) Gas chromatographic peak 5 appears to be 2,18-dimethyloctacosane. The KI of approximately 2900 and the mass spectrum support this structure. The KI and mass spectrum do not support the proposed alternate structure of an 11,18-isomer nor is this structure biosynthetically feasible. This component was later reported to be either 2,10- or 2,18-dimethyloctacosane, both of which form similar mass spectra (Szafranek et al., 1994).

(3) A similar argument as that used for peak 5 applies to peak 6, reported as 7,22- and/or 2,6-dimethyloctacosane. Mass spectra supporting the 2,6-dimethyloctacosane structure were published (Szafranek et al., 1994). Although no mass spectra were published, similar arguments also would apply to peak 13, 11,18- and/or 2,18-dimethyltriacontane, peak 14, 7,24- and/or 2,6-dimethyltriacontane, and peak 17, 11,22- and/or 2,22-dimethyldotriacontane, and 13,20- and/or 2,22-dimethyldotriacontane. Mass spectra were later published supporting the structure of 2,6-dimethyltriacontane and peak 17 was identified as 2,10-dimethyldotriacontane (Szafranek et al., 1994).

(4) Peak 10 represents the special case where the 2,x-dimethylalkane has an odd-number of carbon atoms in the backbone. The observed KI was approximately 2960 which is more compatible with that of an internally branched dimethylalkane. The peak was later identified as 11,19-dimethylnonacosane (Szafranek et al., 1994). Szafranek et

al. (1994) also reported 2,6-dimethylnonacosane. However, the 2,6-structure has an odd number of carbon atoms in the tail after the last methyl branch point so is not biosynthetically feasible. An appropriate structure would be that of 2,23-dimethylnonacosane which could be synthesized starting with a primer derived from leucine.

(5) Peak 8 was proposed to be an internally branched trimethylalkane but the structure would not have the observed KI and is not biosynthetically feasible.

(6) Peak 18 was proposed to be 3,10,16,21-tetramethylhentriacontane (Maliński et al., 1986). Such a structure is not biosynthetically feasible and would be expected to have a KI of approximately 3280. The observed KI of 3310–3324 indicates a carbon-chain backbone of 32 or 33 carbon atoms, not of 31 carbon atoms. The proposed homologue of peak 18, peak 22, 3,10,16,23-tetramethyltriacontane, also is not supported by the feasibility of biosynthesis, the KI, or the mass spectrum. The expected KI would be approximately 3480 which is a reduction from the carbon number (37) of approximately 30 KI for the 3-methyl group plus approximately 60–70 KI for each of the internal methyl branches. The reported KI was approximately 3520 which indicates a compound with a carbon-chain backbone of 34 or 35 carbon atoms, not 33 carbon atoms. Thus, the compound was more likely that of a terminally branched trimethylalkane or internally branched monomethylalkane although the mass spectrum did not appear to support such structures. In a later report, no tetramethylalkanes were indicated (Szafranek et al., 1994).

(7) The proposed structures for peak 23, 2,10,18- and/or 2,12,18-trimethyltetracontanes, would be expected to have a KI of approximately 3520, not the observed KI of 3560 (Maliński et al., 1986). Although the spectrum does not appear to be that of a single component, the major component might be the dimethylalkane, 11,19-dimethylpentatriacontane. Trimethylalkanes with a KI of 3510 have been reported as 2,10,16- and 2,16,24-trimethyltetracontanes but no mass spectra were published (Szafranek et al., 1994). We found 2,10,16- and 2,10,18-trimethyltetracontanes in this study (Table 1).

The biosynthesis of methyl-branched hydrocarbons results from a process like that for fatty acid biosynthesis in which a primer, usually acetate, is elongated by the sequential addition of 2-carbon

units derived from malonyl-CoA (Nelson and Blomquist, 1995). When a methyl branch is to be added to the elongating chain, propionate, as a 3-carbon unit derived from methylmalonyl-CoA is added. This biosynthetic process results in an odd number of carbon atoms, e.g. 3, 5, 7, etc., between the methyl branch points of methylalkanes with two or more methyl groups. The final chain elongation occurs by the addition of 2-carbon units. Then, because in insects the hydrocarbons are formed in the final step by a reductive decarboxylation (Nelson and Blomquist, 1995), there is an even number of carbon atoms in the straight-chain tail beyond the last methyl branch point. The possible exception to this would be if propionate was the last unit to be added during chain elongation. In this case, the loss of the carboxyl group would result in an odd number of carbon atoms beyond the last methyl branch point. Or, as pointed out earlier, if the carboxyl group of the propionate could be reduced, there also would be an odd number of carbons, in this case one carbon atom, beyond the last methyl branch point. There are no known reports of propionate being added as the last elongating unit. However, it is a possibility that may be deserving of investigation.

However, methylalkanes with a methyl group on carbon 2 may have an even number of carbon atoms between the methyl branch points. 2-Methylalkanes are formed starting with a primer derived from the amino acids leucine (Blailock et al., 1976; Charlton and Roelofs, 1991) or valine (Blailock et al., 1976). Starting with valine makes it possible to have one methylene between the first and second methyl branch points if the first elongating unit is propionate (Fig. 7). Starting with leucine makes it possible to have two methylenes between the first and second methyl branch points. Thus, starting with a primer derived from leucine and adding 2-carbon units, it is possible to have an even number of carbon atoms (2, 4, 6, etc.) between the first two methyl branch points. If the primer is derived from leucine, and propionate is the first elongating unit, the resulting addition product would have a 2,5-dimethyl branching sequence counting from the alkyl end. A sex pheromone component of the eastern hemlock looper, *Lambdina fiscellaria fiscellaria* (Gries et al., 1991) and the western hemlock looper, *L. f. lugubrosa*, has been identified as 2,5-dimethylheptadecane (Gries et al., 1993). The addition of acetate units for carbon-chain elongation before

the propionate is incorporated into the elongating carbon chain would allow the biosynthesis of compounds with a larger even-number of methylenes between the branch points, e.g. 2, 7, 2, 9, etc., sequences of methyl branching.

The possible primers for methylalkane biosynthesis and the resulting odd- or even-numbered carbon-chain backbones are summarized in Fig. 7. The effect of the addition of either acetate or propionate at various times during chain elongation on the number of carbon atoms (methylene groups) possible between methyl branch points also is summarized. When alkanes with multiple methyl-branches were first described in insects the adjacent methyl branch points were separated by 3 carbon atoms (i.e. were referred to as an isoprenoid-type of structure). Subsequent studies over the past 32 years have demonstrated the presence of both odd- and even-numbers of carbon atoms between adjacent methyl branch points ranging from 1 to over 20. For example, methylalkanes with one carbon between adjacent methyl branch points have been identified in several species: 13,15-dimethylheptacosane in fire ants, *Solenopsis invicta* (Nelson et al., 1980), 13,15-dimethylnonacosane (Haverty et al., 1990) and 13,15,17-trimethylnonacosane in the subterranean termite, *Coptotermes formosanus* (Haverty et al., 1996), 13,15,19-trimethylhentriacontane and 13,15,21-trimethyltrtriacontane in the red harvester ant, *Pogonomyrmex barbatus* (Nelson et al., 2001), and 2,18,20-trimethyltetratriacontane, 2,18,20-trimethylhexatriacontane and 2,24,26-trimethyldotetracontane in the corn earworm, *Helicoverpa zea* (Nelson, 2001).

An even number of methylenes between methyl branch points has also been found in methyl-branched alcohols. Alcohols characterized with an even number of methylenes separating the branch points are 6,10,13-trimethyltetradecanol (a 2,5,9-branching sequence counting from the alkyl end, the direction of biosynthesis) from the predatory stink bugs *Mineus strigipes* (Aldrich and Lusby, 1986) and *Stiretrus anchorago* (Aldrich et al., 1986), and as the isovalerate ester in *Oplomus dichrous* (Aldrich and Lusby, 1986), *O. severus* and *P. bioculatus* (Aldrich et al., 1986).

There are a seemingly endless number of structures possible for the methyl-branched hydrocarbons found in insects and possibly in arthropods in general. This makes it essential that care be taken in interpreting the data so that all three

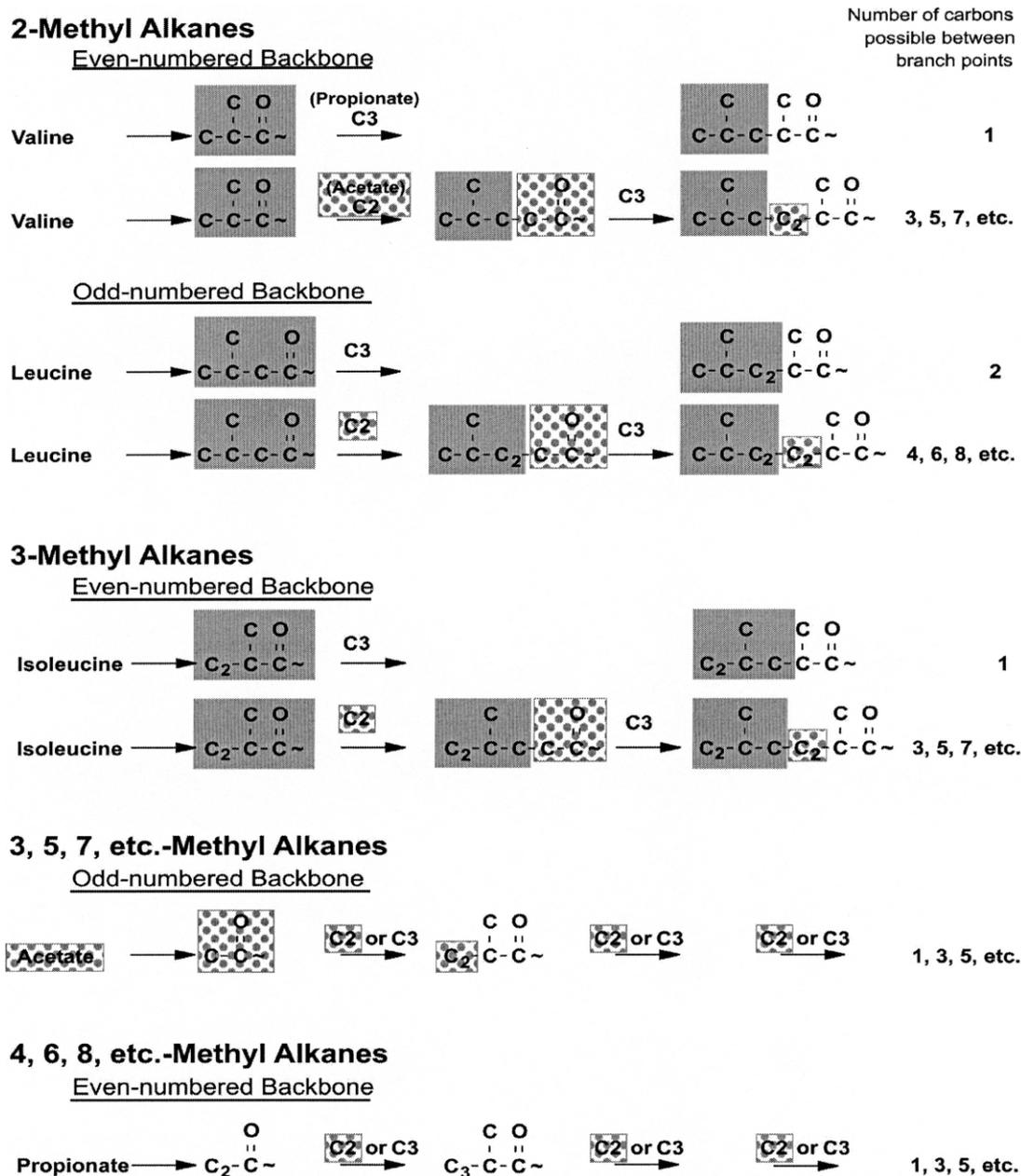


Fig. 7. Summary of the possible sources of primers for the initiation of biosynthesis of methylalkanes with an odd- or even-numbered backbone; and if multiple methyl branches, the number of carbons (methylene groups) possible between adjacent methyl branch points. Isoleucine is a primer for 3-methylalkanes in plants.

criteria described above for a valid structure are met. The feasibility of biosynthesis is a major consideration when assigning structures to methyl-branched alkanes with an odd-numbered carbon chain in insects. However, biosynthesis as described herein is not universal. Schulz (2001) has pointed out that in some other organisms, e.g.

cyanobacteria (Köster et al., 1999), that other routes of biosynthesis are operative.

Acknowledgments

We are grateful to Bonnie Muhl, Computer Assistant, for data processing and preparation of

graphics. Special thanks to Lori Nelson, Russell Jurenka, Ralph Howard, Coby Schal and Stefan Schulz (who suggested the possibility of propionate as the final elongating unit) for insightful comments during the preparation of this work.

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