

Mass spectra of methyl-branched hydrocarbons from eggs of the tobacco hornworm

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Abstract Hydrocarbons from three homologous series of branched alkanes from the eggs of the tobacco hornworm, *Manduca sexta* (L.), were identified by mass spectrometry. Gas-liquid chromatography (GLC) peaks 37-A (equivalent chain length of 37.2) and 39-A (equivalent chain length of 39.2) were mixtures of 13-, 15-, 17-, and 19-methylheptatriacontane and 13-, 15-, 17-, and 19-methylnonatriacontane, respectively. GLC peaks 33-B, 37-B, and 39-B with equivalent chain lengths of 33.4, 37.4, and 39.4, respectively, were mixtures of 13,17- and 15,19-dimethyltritiacontane, 13,17-, 15,19-, and 17,21-dimethylheptatriacontane, and 13,17-, 15,19-, and 17,21-dimethylnonatriacontane, respectively. GLC peak 37-C (equivalent chain length of 37.6) was a mixture of 11,15,19-, 13,17,21-, and 15,19,23-trimethylheptatriacontane.

Supplementary key words methyl-branched alkanes
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THE PRESENCE OF methyl-branched aliphatic hydrocarbons in insects has been reported by a number of investigators. A 3-methylalkane is present in the American cockroach, *Periplaneta americana* (L.) (1). 3-Methylalkanes and internally branched monomethylalkanes are in the big stonefly, *Pteronarcys californica* Newport (2), in the Madeira cockroach, *Leucophaea maderae* (F.) (3), in the oriental cockroach, *Blatta orientalis* L. (3), in the Australian cockroach, *Periplaneta australasiae* (F.) (4), in the brown cockroach, *P. brunnea* Burmeister (4), and in the smokybrown cockroach, *P. fuliginosa* (Serville) (4).

Also, 2-methylalkanes and internally branched monomethylalkanes are in the common house cricket, *Acheta domesticus* (L.) (5). Internally branched monomethylalkanes and 2- or 3-methylalkanes, or both, were reported in the honey bee, *Apis mellifera* L. (6); unidentified branched hydrocarbons were reported in the house fly, *Musca domestica* L. (7), and in the so-called Australian sheep blowfly, *Phaenicia cuprina* (Wiedemann) (8); and unidentified methyl-branched hydrocarbons were reported in the tsetse fly, *Glossina moritans* Westwood (9) and in the house cricket (5). *n*-Alkanes are present in the hydrocarbon fraction of all these insects, and some had alkenes (1, 2, 4-7) and cycloalkanes (7).

Recently, several new homologous series of methyl-branched alkanes were found in insects. Martin and MacConnell (10) found two homologous series of trimethylalkanes in the ants *Atta colombica* Guerin and *A. sexdens* (L.) and identified them as the 3,7,11-trimethylalkanes and the 4,8,12-trimethylalkanes; in *A. cephalotes isthmicola* Weber, only the 3,7,11 series was present. Nelson and Sukkestad (11) identified two new homologous series from the eggs of the tobacco hornworm, *Manduca sexta* (L.), one of dimethylalkanes and the other of trimethylalkanes, with the methyl branches located internally on the molecule and with isoprenoid spacing. In addition, an *n*-alkane series was present in the ant hydrocarbons, and an *n*-alkane and an internally branched monomethylalkane series were present in the tobacco hornworm hydrocarbons.

In the present paper, we report the mass spectral characterization of some additional methyl-branched hydrocarbons from tobacco hornworm eggs. They were obtained in sufficient quantity and purity by GLC to permit an assignment of structure based on their equivalent chain lengths (11) and mass spectral fragmentation patterns.

Abbreviations: GLC, gas-liquid chromatography.

(C_xH_{2x}) relative to the intensities of the odd mass peaks (C_xH_{2x+1}) are summarized in Table 1.

RESULTS AND DISCUSSION

Four homologous series of alkanes were previously identified in the hydrocarbon fraction from tobacco hornworm eggs: the *n*-alkanes, the internally branched monomethylalkanes, the internally branched dimethylalkanes, and the internally branched trimethylalkanes (11). The GLC peaks were numbered so that for the *n*-alkanes, the peak number was identical with the carbon number. The branched alkanes were eluted between the *n*-alkanes; they were designated by adding A, B, or C to the carbon number of the *n*-alkane after which they eluted. Thus the three branched alkanes that were eluted with equivalent chain lengths greater than 37 but less than 38 were designated 37-A, 37-B, and 37-C, in order of increasing retention time. The homologous A series was composed of monomethylalkanes, the B series of dimethylalkanes, and the C series of trimethylalkanes; the branches were located internally in the molecule (11).

GLC was used to separate and trap six additional branched alkanes from tobacco hornworm eggs in order to obtain their mass spectra. The mass spectra of two branched alkanes from the homologous A series (GLC peaks 37-A and 39-A) with equivalent chain lengths of 37.2 and 39.2 and with carbon numbers of 38 and 40, respectively, are shown in Figs. 1 and 2, respectively. The difference of 0.8 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 in the hydrocarbons of GLC peaks 37-A and 39-A (11, 13). The first point to be made concerning the mass spectra of 37-A was that although the molecular ion was not readily apparent, its value was established by the intense peaks at $M - 15$ and $M - 29$. The second point was that no characteristic odd mass peaks (C_xH_{2x+1}) were present that were not also associated with an even mass peak (C_xH_{2x}) of about the same or greater intensity. This is an indication that only internally branched monosubstituted alkanes were present which could fragment to give secondary ion fragments with a long straight-chain tail and with at least seven carbons. The third point was that seven major secondary ion fragments were present with even mass peaks at m/e 196, 224, 252, 280, 308, 336, and 364 with intensities greater than those of the corresponding odd mass peaks (the slightly greater intensity of the odd mass peaks at m/e 337 and 365 than of the even mass peaks at m/e 336 and 364 was because of the large dissymmetry of two of the alkanes). Therefore, because seven major secondary ion fragments appear but an internally branched monomethylalkane gives only two major characteristic secondary ion fragments, four isomers must be present, and one must be symmetrical.

The position of the branching in the first isomer of the mixture was established as being on carbon 13 by the characteristic peaks at m/e 196 and 197 (ions $a - 1$ and a , respectively; $x = 12$, scheme 1) and by the small pair of peaks at m/e 168 and 169 (ions $d - 1$ and d , respectively;

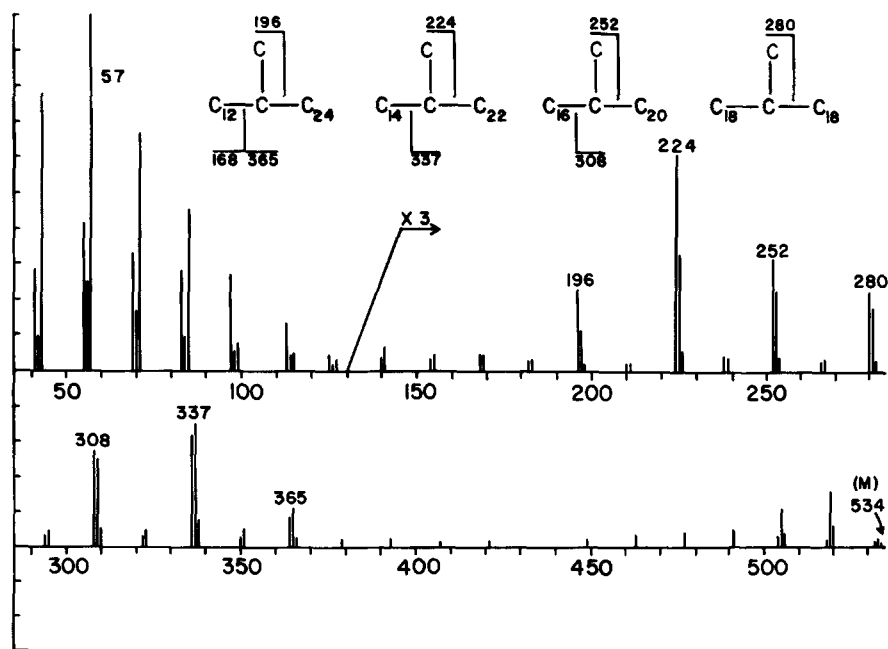


FIG. 1. Mass spectrum of GLC peak 37-A: 13-, 15-, 17-, and 19-methylheptatriacontane. Ordinate, relative intensity; abscissa, m/e .

TABLE 1. Characteristic ion fragments of long-chain internally branched methylalkanes

| Alkane | Characteristic Ion Fragments ^a | |
|--|--|--|
| | Secondary Ions ^b | Primary Ions |
| $\begin{array}{c} \text{CH}_3 \\ \\ \text{C}_x\text{H}_{2x+1}-\text{C}-\text{C}_y\text{H}_{2y+1} \\ \\ \text{H} \end{array}$ | $\begin{array}{c} \text{CH}_3 \\ \\ \text{C}_x\text{H}_{2x+1}-\text{C}^+ \\ \\ \text{H} \end{array} \quad E > O$ | $\text{C}_x\text{H}^+_{2x+1} \quad E \geq O$ |
| | $\begin{array}{c} \text{CH}_3 \\ \\ \text{C}^+-\text{C}_y\text{H}_{2y+1} \\ \\ \text{H} \end{array} \quad E > O$ | $\text{C}_y\text{H}^+_{2y+1} \quad E \geq O$ |
| $\begin{array}{c} \text{CH}_3 \qquad \text{CH}_3 \\ \qquad \quad \\ \text{C}_x\text{H}_{2x+1}-\text{C}-(\text{CH}_2)_s-\text{C}-\text{C}_y\text{H}_{2y+1} \\ \qquad \quad \\ \text{H} \qquad \quad \text{H} \end{array}$ | $\begin{array}{c} \text{CH}_3 \\ \\ \text{C}_x\text{H}_{2x+1}-\text{C}^+ \\ \\ \text{H} \end{array} \quad E > O$ | $\text{C}_x\text{H}^+_{2x+1} \quad E \geq O$ |
| | $\begin{array}{c} \text{CH}_3 \qquad \text{CH}_3 \\ \qquad \quad \\ \text{C}_x\text{H}_{2x+1}-\text{C}-(\text{CH}_2)_s-\text{C}^+ \\ \qquad \quad \\ \text{H} \qquad \quad \text{H} \end{array} \quad E < O$ | |
| | $\begin{array}{c} \text{CH}_3 \\ \\ \text{C}^+-\text{C}_y\text{H}_{2y+1} \\ \\ \text{H} \end{array} \quad E > O$ | $\text{C}_y\text{H}^+_{2y+1} \quad E \geq O$ |
| | $\begin{array}{c} \text{CH}_3 \qquad \text{CH}_3 \\ \qquad \quad \\ \text{C}^+-\text{C}-(\text{CH}_2)_s-\text{C}_y\text{H}_{2y+1} \\ \qquad \quad \\ \text{H} \qquad \quad \text{H} \end{array} \quad E < O$ | |
| $\begin{array}{c} \text{CH}_3 \qquad \text{CH}_3 \qquad \text{CH}_3 \\ \qquad \quad \qquad \quad \\ \text{C}_x\text{H}_{2x+1}-\text{C}-(\text{CH}_2)_s-\text{C}-(\text{CH}_2)_s-\text{C}-\text{C}_y\text{H}_{2y+1} \\ \qquad \quad \qquad \quad \\ \text{H} \qquad \quad \text{H} \qquad \quad \text{H} \end{array}$ | $\begin{array}{c} \text{CH}_3 \\ \\ \text{C}_x\text{H}_{2x+1}-\text{C}^+ \\ \\ \text{H} \end{array} \quad E > O$ | $\text{C}_x\text{H}^+_{2x+1} \quad E \geq O$ |
| | $\begin{array}{c} \text{CH}_3 \qquad \text{CH}_3 \\ \qquad \quad \\ \text{C}_x\text{H}_{2x+1}-\text{C}-(\text{CH}_2)_s-\text{C}^+ \\ \qquad \quad \\ \text{H} \qquad \quad \text{H} \end{array} \quad E < O$ | |
| | $\begin{array}{c} \text{CH}_3 \qquad \text{CH}_3 \qquad \text{CH}_3 \\ \qquad \quad \qquad \quad \\ \text{C}_x\text{H}_{2x+1}-\text{C}-(\text{CH}_2)_s-\text{C}-(\text{CH}_2)_s-\text{C}^+ \\ \qquad \quad \qquad \quad \\ \text{H} \qquad \quad \text{H} \qquad \quad \text{H} \end{array} \quad E < O$ | |
| | $\begin{array}{c} \text{CH}_3 \\ \\ \text{C}^+-\text{C}_y\text{H}_{2y+1} \\ \\ \text{H} \end{array} \quad E > O$ | $\text{C}_y\text{H}^+_{2y+1} \quad E \geq O$ |
| | $\begin{array}{c} \text{CH}_3 \qquad \text{CH}_3 \\ \qquad \quad \\ \text{C}^+-\text{C}-(\text{CH}_2)_s-\text{C}_y\text{H}_{2y+1} \\ \qquad \quad \\ \text{H} \qquad \quad \text{H} \end{array} \quad E < O$ | |
| | $\begin{array}{c} \text{CH}_3 \qquad \text{CH}_3 \qquad \text{CH}_3 \\ \qquad \quad \qquad \quad \\ \text{C}^+-\text{C}-(\text{CH}_2)_s-\text{C}-(\text{CH}_2)_s-\text{C}_y\text{H}_{2y+1} \\ \qquad \quad \qquad \quad \\ \text{H} \qquad \quad \text{H} \qquad \quad \text{H} \end{array} \quad E < O$ | |

^a E is the even mass ion fragment at C_xH_{2x} ; O is the odd mass ion fragment at $\text{C}_x\text{H}_{2x+1}$. The intensity of the primary ion fragment is small compared with that of the secondary ion fragment. Peaks at $M - 15$, $M - 29$, and $M - 43$, in order of decreasing intensity, will also be present and aid in establishing the position of the molecular ion.

^b If the molecule is very unsymmetrical with respect to the branch point, the secondary ion fragment formed will have the even mass peak equal to or less than the odd mass peak. If the secondary ion fragment contains a second branch, the even mass peak will be less than the odd mass peak.

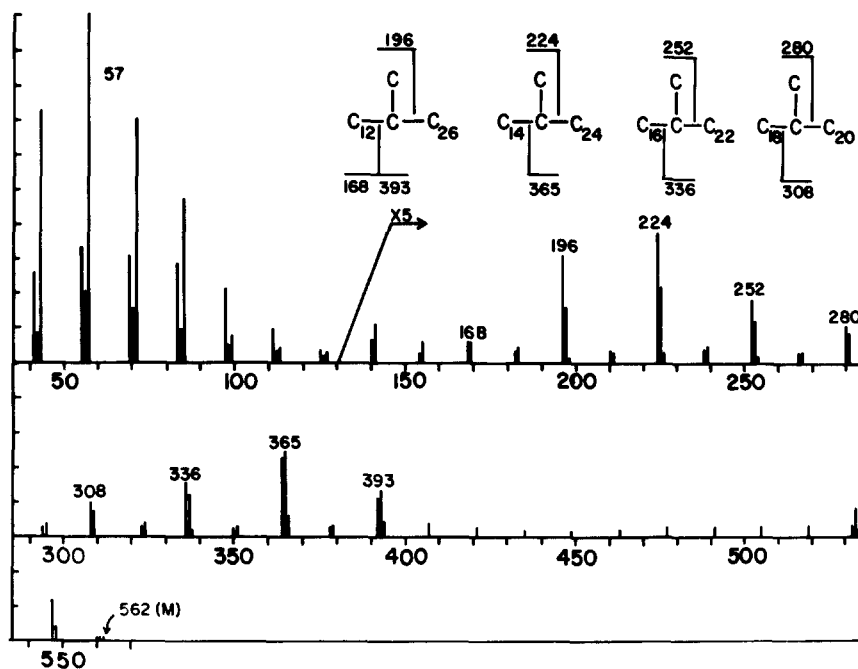


FIG. 2. Mass spectrum of GLC peak 39-A: 13-, 15-, 17-, and 19-methylnonatriacontane. Ordinate, relative intensity; abscissa, m/e .

$x = 12$, scheme 2) which were from the 12-carbon primary ion fragment (Fig. 1). Cleavage on the other side of the branch point of the 13-methyl isomer would give a pair of peaks at m/e 364 and 365 (m/e 365 was slightly greater than m/e 364 because of the large dissymmetry of the molecule). The small pair of peaks expected at m/e 336 and 337 for the 24-carbon primary ion fragment was not considered because of the large contribution to these masses from the secondary ion fragment from the 15-methyl isomer. By the same token, the primary ion fragments from the other isomer were not considered further. The 15-methyl isomer of the mixture (Fig. 1) would be expected to cleave on either side of the branch point to give peaks at m/e 224 and 225 and at m/e 336 and 337 (m/e 337 was slightly greater than m/e 336 because of the large dissymmetry of the molecule). The 17-methyl isomer would cleave on either side of the branch point to give secondary ion fragments with peaks at m/e 252 and 253 and at m/e 308 and 309; the symmetrical 19-methyl isomer would cleave to give a secondary ion fragment with peaks at m/e 280 and 281. Because the expected peaks for the proposed mixture of monomethylalkanes were all present in the mass spectrum, because all major peaks in the mass spectrum were accounted for, and because the mixture had a GLC retention time compatible with that expected for an internally branched monomethylalkane, we concluded that the mixture of alkanes in GLC peak 37-A was composed of 13-, 15-, 17-, and 19-methylheptatriacontane.

A comparison of the relative intensities of the peaks of the secondary ion fragments at m/e 196, 224, 252, and 280 showed that 13- and 19-methylheptatriacontane were minor components (the relative intensity of the peak at 280 was taken as one-half because the 19-methyl isomer was symmetrical; the peak at 196 was slightly increased because of the 14-carbon primary ion fragment from the 15-methyl isomer, which was the major isomer). About two times as much of the 17-methyl isomer was present as of the 13- and 19-methyl isomers, and about two times as much of the 15-methyl isomer was present as of the 17-methyl isomer.

The mass spectrum (Fig. 2) of GLC peak 39-A had peaks at m/e 547 ($M - 15$) and 533 ($M - 29$); this confirmed the presence of the molecular ion at m/e 562 ($C_{40}H_{82}$). The eight characteristic pairs of peaks with large even masses at m/e 196, 224, 252, 280, 308, 336, 364, and 392 for secondary ion fragments indicated that four monomethylalkane isomers were present. The first major peak of significance in the spectrum occurred at m/e 196, corresponding to a 14-carbon secondary ion fragment. This conclusion was supported by the presence of a pair of small peaks that occurred at m/e 168 and 169 from the 12-carbon primary ion fragment of the 13-methyl isomer. A secondary ion fragment of 28 carbons formed by cleavage on the other side of the branch point was expected at m/e 393 with a peak at m/e 392 of about the same intensity. The peaks at m/e 392 and 393 were present in the mass spectrum and confirmed that the

methyl branching occurred at carbon 13. By reasoning similar to that used to interpret the mass spectrum of the hydrocarbons of GLC peak 37-A, the structures of the other isomers present in GLC peak 39-A were deduced, and it was concluded that GLC peak 39-A was a mixture of 13-, 15-, 17-, and 19-methylnonatriacontane.

A comparison of the relative intensities of the secondary ion fragments in the mass spectrum for 39-A (Fig. 2) showed that the 15-methyl isomer was the major component, that there was about three-fourths and one-half as much of the 13- and 17-methyl isomers, respectively, and that the 19-methyl isomer was the minor component of the mixture.

The mass spectra of three branched alkanes of the B series (GLC peaks 33-B, 37-B, and 39-B) are shown in Figs. 3, 4, and 5, respectively. GLC peak 33-B had an equivalent chain length of 33.4 and a carbon number of 35; GLC peak 37-B had an equivalent chain length of 37.4 and a carbon number of 39; and GLC peak 39-B had an equivalent chain length of 39.4 and a carbon number of 41. The difference (1.6) between the equivalent chain length and carbon number was consistent with the presence of two internal methyl branches on the molecule (11, 13). Also, the mass spectrum of 33-B (Fig. 3) had three major groups of peaks with odd mass peaks (C_xH_{2x+1}) at m/e 267, 295, and 323 which were much greater than the corresponding even mass peaks (C_xH_{2x}) at m/e 266, 294, and 322, an indication that the hydrocarbons were not internally branched monomethylalkanes (11, 12, 14). The three groups of peaks in the

mass spectrum (Fig. 3) with predominant even mass peaks at m/e 196, 224, and 252 showed that the branch points were so positioned in the molecule that secondary ion fragments with a long straight-chain tail could be formed. The presence in the mass spectrum of three major groups of peaks with a predominant odd mass peak and an equal number of groups of peaks with a predominant even mass peak indicated that only two branches were present in the molecule; this was in agreement with the conclusion reached from the GLC retention times. The uneven number of major groups of peaks with a predominant even mass peak and of groups of peaks with a predominant odd mass peak indicated that 33-B was a mixture of two dimethylalkanes and that one of the isomers was symmetrical. The mass spectrum (Fig. 3) was compatible with that expected for a mixture of 13,17- and 15,19-dimethyltritiacontane. The 13,17-dimethyl isomer would cleave internally to the two branch points to give secondary ion fragments with a predominant even mass peak at m/e 196 and 252 and would cleave externally to the two branch points to give double-branched secondary ion fragments with a predominant odd mass peak at m/e 267 and 323. The other component, the 15,19-dimethyl isomer, was a symmetrical molecule and would cleave internally to the branch points to give a secondary ion fragment with a predominant even mass at m/e 224; it would also cleave externally to the branch point to give a double-branched secondary ion fragment at m/e 295. A comparison of the relative intensities of the peaks in the mass spectrum

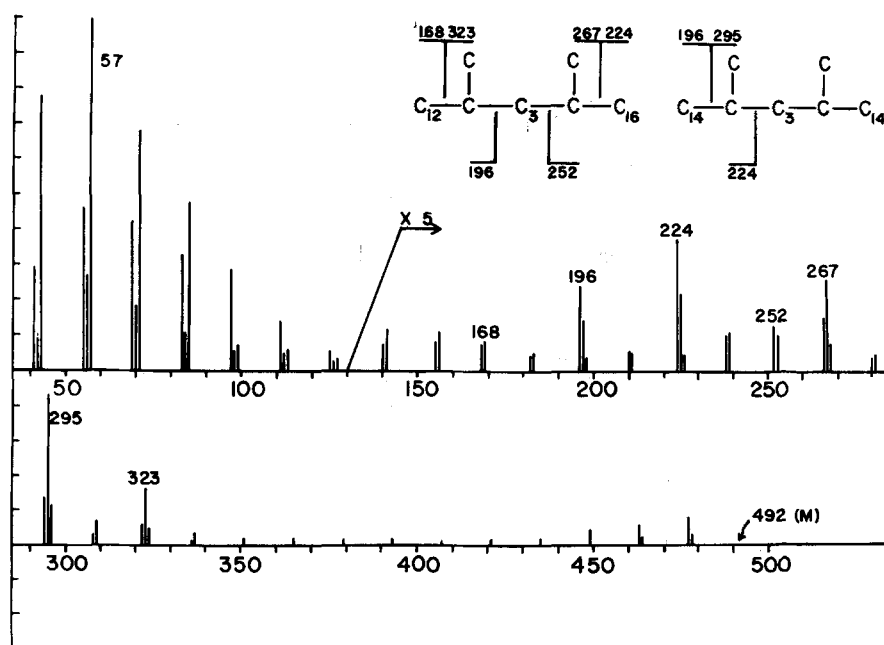


FIG. 3. Mass spectrum of GLC peak 33-B: 13,17- and 15,19-dimethyltritiacontane. Ordinate, relative intensity; abscissa, m/e .

(taking into consideration that the 15,19-dimethyl isomer had two identical modes of fragmentation) indicated that the amount of 13,17-dimethyltrtriacontane was slightly less than the amount of 15,19-dimethyltrtriacontane.

The mass spectrum of GLC peak 37-B (Fig. 4) had five major groups of peaks with a predominant even mass peak at m/e 196, 224, 252, 280, and 308, and five major groups of peaks with a predominant odd mass peak at m/e 267, 295, 323, 351, and 379, an indication that more than two dimethylalkanes were present and that one was symmetrical. The presence of the first branch point on carbon atom 13 was shown by the peak at m/e 196, and this conclusion was supported by the pair of small peaks at m/e 168 and 169 from the 12-carbon primary ion fragment. The expected fragmentation pattern for a mixture of 13,17-, 15,19-, and 17,21-dimethylheptatriacontane was compatible with the mass spectrum.

When the relative intensities of the peaks in the mass spectrum were compared, account was taken of the fact that the 17,21-dimethyl isomer had two equal modes of fragmentation and that the peak at m/e 196 for the 13,17-dimethyl isomer would be slightly larger because of a contribution from the 14-carbon primary ion fragment from the 15,19-dimethyl isomer, the major component of the mixture. The comparison showed that 13,17-dimethylheptatriacontane and 17,21-dimethylheptatriacontane were present in about equal amounts and that about

twice as much 15,19-dimethylheptatriacontane was present.

The mass spectrum of GLC peak 39-B (Fig. 5) had six major groups of peaks with a predominant even mass peak and six major groups of peaks with a predominant odd mass peak; this pattern indicated a mixture of three dimethylalkanes, none of which were symmetrical. The absence of a distinctive pair of peaks at m/e 140 and 141 and the low intensity of the pairs of peaks at m/e 168 and 169 did not allow us to determine if the peaks at m/e 168 and 169 were from a primary ion fragment or from a secondary ion fragment without a second branch. However, the peak at m/e 407 was the result of the formation of a double-branched secondary ion fragment of 29 carbon atoms and a neutral radical of 12 carbons, and established the first branch point at carbon 13. Therefore, the pair of peaks at m/e 168 and 169 were from the 12-carbon primary ion fragment. A mixture of 13,17-, 15,19-, and 17,21-dimethylnonatriacontane would be expected to give the major fragmentation peaks found in the mass spectrum. A comparison of the major fragmentation peaks in the mass spectrum showed that the three isomers were present in about equal proportions.

A component of the homologous C series of branched hydrocarbons, GLC peak 37-C, had an equivalent chain length of 37.6 and a carbon number of 40. The difference of 2.4 between the equivalent chain length and the carbon number was consistent with the presence of three

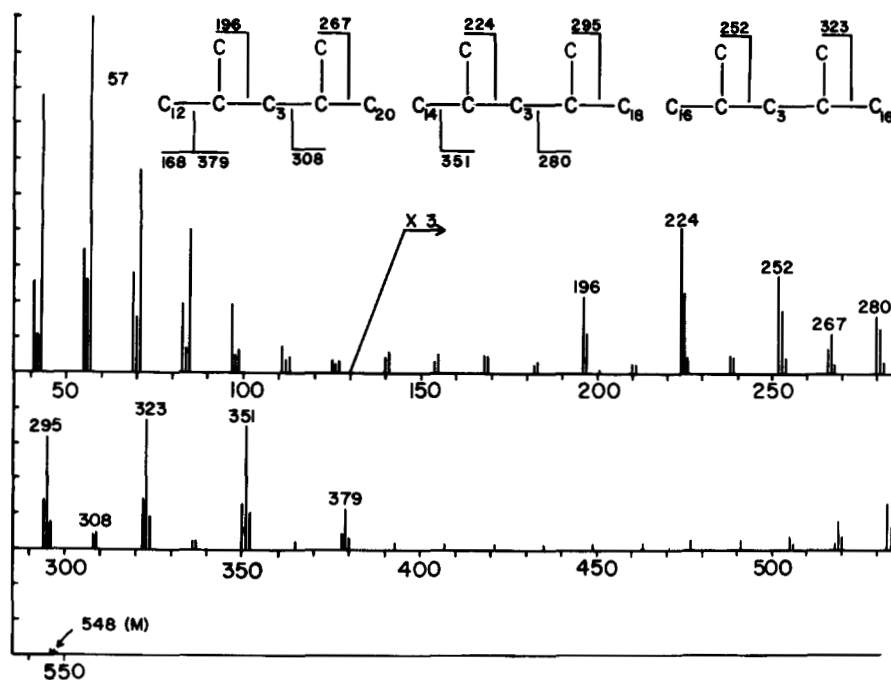


FIG. 4. Mass spectrum of GLC peak 37-B: 13,17-, 15,19- and 17,21-dimethylheptatriacontane. Ordinate, relative intensity; abscissa, m/e .

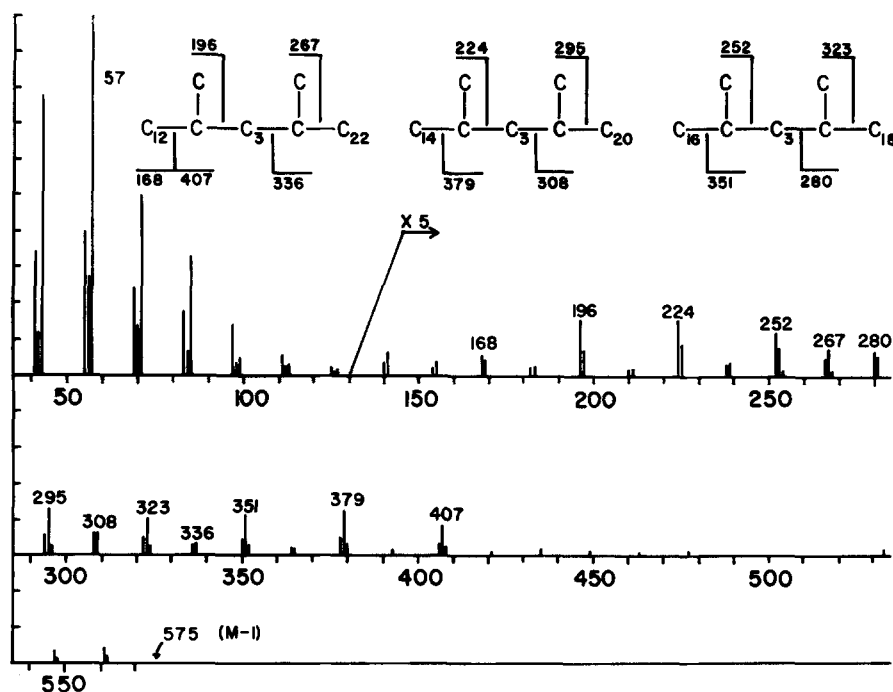


FIG. 5. Mass spectrum of GLC peak 39-B: 13,17-, 15,19-, and 17,21-dimethylnonatriacontane. Ordinate, relative intensity; abscissa, m/e .

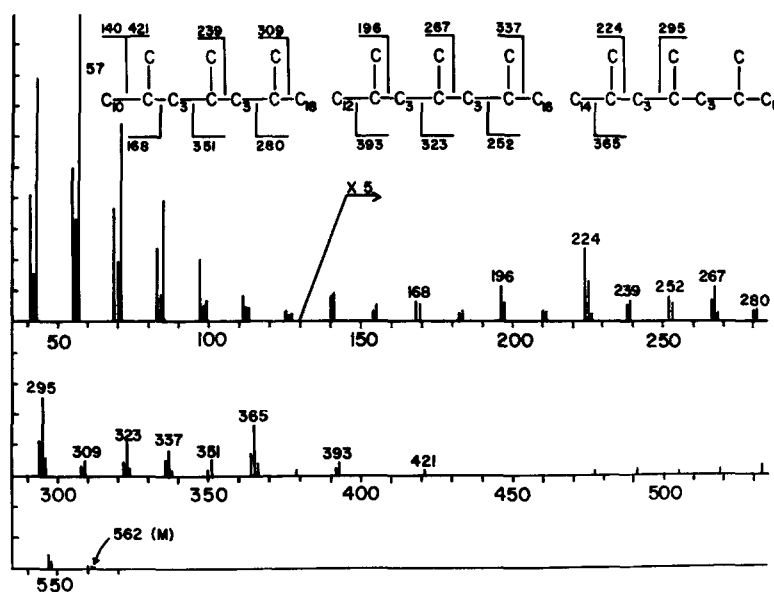


FIG. 6. Mass spectrum of GLC peak 37-C: 11,15,19-, 13,17,21-, and 15,19,23-trimethylheptatriacontane. Ordinate, relative intensity; abscissa, m/e .

internal methyl branches (11). The large number of groups of characteristic fragmentation peaks in the spectrum that had either a predominant even or odd mass peak (Fig. 6) was compatible with multiple branching and with a mixture of isomers; the presence of the small peak in the mass spectrum at m/e 421 from a multiple-branched secondary ion fragment and the pair of peaks of about equal intensity at m/e 140 and 141 from a pri-

mary ion fragment showed that the first branch point was at carbon 11. A mixture of 11,15,19-, 13,17,21-, and 15,19,23-trimethylheptatriacontane would have cleavage points compatible with the mass spectrum shown in Fig. 6. A comparison of the relative intensities of the peaks in the mass spectrum indicated that the smallest component was the 11,15,19-trimethyl isomer and that 1.5 to 2 times as much of the 13,17,21-trimethyl

isomer was present. There was slightly more of the 15,19-, 23 isomer than of the 13,17,21 isomer.

Six additional branched alkanes were identified from tobacco hornworm eggs. Two GLC peaks from the homologous monomethylalkane series (37-A and 39-A) were identified as mixtures of 13-, 15-, 17-, and 19-methylheptatriacontane and of 13-, 15-, 17-, and 19-methylnonatriacontane, respectively. Three GLC peaks from the homologous dimethylalkane series (33-B, 37-B, and 39-B) were mixtures of dimethylalkanes. GLC peak 33-B was a mixture of 13,17- and 15,19-dimethyltriacontane; GLC peak 37-B was a mixture of 13,17-, 15,19-, and 17,21-dimethylheptatriacontane, and GLC peak 39-B was a mixture of 13,17-, 15,19-, and 17,21-dimethylnonatriacontane. The only additional GLC peak (37-C) from the homologous trimethylalkane series that has been obtained in sufficient quantity and purity for mass spectral analysis was identified as a mixture of 11,15,19-, 13,17,21-, and 15,19,23-trimethylheptatriacontane.

We previously showed that GLC peak 35-A was a mixture of 15- and 17-methylpentatriacontane; GLC peak 35-B was a mixture of 13,17- and 15,19-dimethylpentatriacontane; and GLC peak 35-C was identified as 13,17,21-trimethylpentatriacontane (11). A shorter-chain dimethylalkane (GLC peak 27-B) was identified as 9,13-dimethylheptacosane (11), and this structure was confirmed by synthesis (14). The internally branched methylalkanes and *n*-alkanes were found in the integument, fat body, and hemolymph of the tobacco hornworm, but the internally branched methylalkanes were not found in the diet (15).

Although the hydrocarbons in a number of insects have been determined, none had solely internal multiple-branched methylalkanes with isoprenoid spacing. Martin and MacConnell (10) found multiple-branched methylalkanes with isoprenoid spacing in several species of ants (as noted), but the first methyl branch occurred on either the third or fourth carbon atom of C₃₄ to C₃₉ trimethylalkanes. Also, a low molecular weight, multiple-branched methylalkane (2,6,10-trimethyltetradecane) has been found in shale (16). We have analyzed other insects for the presence of internal multiple-branched methylalkanes and have found that the grasshopper, *Schistocerca vaga* (Scud.), has hydrocarbons very similar to those of the tobacco hornworm. The grasshopper hydrocarbons are presently being identified.

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