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ELUTION PATTERNS FROM CAPILLARY GC FOR METHYL-BRANCHED ALKANES

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Abstract—A common and confusing problem in analyses of insect hydrocarbons is in making sense of complicated gas chromatograms and interpreting mass spectra since branched chain compounds differing by one or two carbons in backbone or chain length may elute from the column at nearly the same time. To address this confusing situation, relative gas chromatography (GC) retention times are presented for typical mono-, di-, tri-, and tetramethylalkanes comprising most of the commonly appearing series of homologous methyl-branched alkanes up to 53 carbons that are found in insect cuticular hydrocarbons. Typical insect-derived methylalkanes with backbones of 33 carbons were characterized by Kovats indices (KI); monomethyl alkanes elute between KI 3328 and 3374, dimethylalkanes elute between KI 3340 and 3410, trimethylalkanes elute between KI 3378 and 3437, and tetramethylalkanes elute between KI 3409 and 3459, depending upon the positions of substituents. A protocol is described for identification of methyl-branched hydrocarbons eluted from nonpolar polysiloxane DB-1 capillary GC columns. In this protocol, retention indices (KI values) are assigned to peaks, then the patterns in GC peaks that probably contain homologs are marked to assist subsequent GC-mass spectrometric (GC-MS) interpretation. Use of the KI allows assignment of likely structures and the elimination of others, with demonstrative consistency, as there are no known exceptions. Interpretation of electron ionization mass spectra can then proceed within narrowed structural possibilities without the necessity of chemical ionization GC-MS analysis. Also included are specific examples of insect hydrocarbons that were assembled from 30 years of the literature, and these are intended to help with confirmation of confusing or contradictory structures.

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Key Words—Hydrocarbons, alkanes, methyl-branched hydrocarbons, cuticular hydrocarbons, insects, GC-MS data, retention indices.

INTRODUCTION

Gas chromatography (GC) elution times may overlap for methyl-branched alkanes that differ by one or even two carbons in chain length, causing difficulty in interpretation of their mass spectra. Interpretation of the mass spectra of methylalkanes in many insect systems is time consuming because few synthetic standards are available for comparison of retention times, particularly for insect-produced cuticular hydrocarbons having chain lengths (carbon backbones) of 21–53 carbons. Existing mass spectral databases are very difficult to use for this purpose, because they contain few methyl-branched alkanes and computerized matching is poor, even for normal alkanes, particularly when mixtures of isomers are present in the same peak. Fortunately, the various classes of methyl-branched hydrocarbon compounds may be separated on nonpolar capillary columns by GC. Each class shows consistent elution patterns with increasing chain length and positions of methyl branching. Normal alkanes can be plotted on a nomogram as boiling point versus log of retention time (Hupe, 1965). Kovats retention indices (KI) (Kovats, 1965) are convenient for assigning relative retention times and for determining the numbers of carbons in the backbones, although this is usually done isothermally. Linear temperature programming is typically used for practical reasons, and true KIs are not obtained. However, KI values may be estimated or interpolated from consecutive *n*-alkane standards injected together on the same column with the same temperature program (Takeda, 1991).

Kissin and Feulmer (1986) described KI for the difference between the elution time of a standard *n*-alkane and a methyl-branched isomer with the same molecular weight, and systematized some examples [$KI = KI_{n\text{-alkane Cn}} - KI_{\text{isoalkane Cn}}$ (for OV-1)]. In this system the molecular weight must be known, which can be difficult to determine in hydrocarbons in general, particularly when multiple compounds coelute. Relative retention times are well documented for monomethyl-branched hydrocarbons (Kissin and Feulmer, 1986) and for some polymethylalkanes (Kissin et al., 1986) that included calculated retention times for "terminal" polyisoprenoid alkanes of less than 20 carbons. However, confusion of di-, tri-, and tetramethyl-branched hydrocarbons having different backbones and overlapping elution times remains common. Examples of specific isomers are often difficult to find in the literature for comparison of retention times, and often chromatograms are published without a useful scale or without retention indices.

We previously described insect hydrocarbons that display consistent

schemes of methyl branches with increasing chain length, often with visible differences of methyl-branching patterns between closely related species (Carlson et al., 1993). Plentiful examples can be found of mass spectra, particularly of biologically active compounds (Nelson and Blomquist, 1994). Early papers on insect methylalkanes of 20–40 carbons showed consistent increases of 0.25–0.3 carbon equivalents for each additional methyl branch when the carbon backbone was held constant (Nelson and Sukkestad, 1970; Nelson et al., 1972), with internal methylene bridges of three carbons being most common. Useful examples are found in the alkanes of the tsetse fly *Glossina morsitans morsitans* that have series of internally substituted di-, tri-, and tetramethylalkanes of 25–39 carbons in which the major components had three methylene interruptions only (Nelson and Carlson, 1986), but were later shown to include dimethylalkanes having longer interruptions (Sutton and Carlson, 1997a). Tsetse flies of other species, *G. pallidipes* and *G. tachinoides*, showed dimethylalkanes with larger interruptions of 7, 9, and 11 methylenes, and a later study of *G. palpalis* describes "terminal" trimethylalkanes (Nelson et al., 1988). Lange et al. (1989) primarily described methylalkanes and only a few dimethylalkanes.

We describe herein representative retention times on nonpolar columns for several rather complete series of di-, tri-, and tetramethylalkanes found in tsetse flies (Carlson et al., 1993; Sutton and Carlson, 1997a) and extended data for internal monomethyl alkanes to 60 carbons found in grasshoppers (Sutton et al., 1996). We include data from the literature covering most of the homologous methylalkanes from insects, where detailed retention data were included. Several novel structures from *Blattella* cockroaches (Brenner et al., 1993) are included, as are selected structures from beetles that were verified with synthetic compounds, parasitoid wasps (Bernier et al., 1998; Geden et al., 1998), mosquitoes (Carlson et al., 1997), and horseflies (Sutton and Carlson, 1997b). Work by others includes that on termites (Haverty et al., 1988), pine engraver beetles (Page et al., 1997), Colorado potato beetles (Szafranek et al., 1994), and screwworm flies (Pomonis, 1989; Pomonis et al., 1989). We describe retention times from capillary GC that were confirmed by electron ionization (EI) gas chromatography–mass spectrometry (GC-MS) with nonpolar capillary columns and chemical ionization (CI) GC-MS.

We also describe a protocol for identification of methyl-branched hydrocarbons by assigning KI (retention indices), then identifying patterns in clusters of GC peaks with the charted retention indices of hydrocarbons on a representative scale. This is the first extensive presentation of several series of di-, tri-, and tetramethyl hydrocarbons in which relative retention times were determined by capillary column GC-MS and were shown to correlate with positions of methyl substituents and with changes in lengths of interruptions between methyl branches.

METHODS AND MATERIALS

Insects. Laboratory-reared and wild insects were used as fresh or dried intact specimens for extraction of hydrocarbons. Crude hexane or ether extracts of males and females of different ages were used. Museum-deposited voucher specimens were also used as a source of hydrocarbons. Hydrocarbons were obtained from many species of tsetse flies (Nelson and Carlson, 1986; Nelson et al., 1988; Carlson et al., 1993), *Blattella* cockroaches (Carlson and Brenner, 1988), horseflies (Sutton and Carlson, 1997b), grasshoppers (Sutton et al., 1996), and parasitoid wasps (Bernier et al., 1998; Geden et al., 1998).

Chemical Samples. Cuticular hydrocarbons were usually extracted from individual specimens by rinsing with hexane (ca. 1 ml) or by soaking the insect in at least three changes of hexane over 1 hr. Pinned specimens were submerged from 1 hr to overnight in a vial containing 3 ml of hexane, removed by grasping the pin with clean forceps, and rinsed once. Pooled extracts were eluted with hexane from a 4-mm × 20-mm or 20-mm × 450-mm column of silica gel. The hydrocarbon-containing fractions that eluted with hexane were concentrated under a stream of nitrogen and subjected to argentation thin-layer chromatography or column chromatography on 5% silver nitrate impregnated silica gel to separate the alkane fraction from the smaller amounts of alkenes (Carlson and Langley, 1986). Alternatively, larger samples of total hydrocarbons were reconstituted in hexane, aliquots were eluted with hexane from small columns (4 mm ID × 40 mm) containing silver nitrate-impregnated silica gel to obtain alkanes, and alkenes and alkadienes were eluted with 1% and 5% ether in hexane, respectively.

Several synthetic samples were available from previous efforts, including tsetse fly sex pheromone compounds mentioned above, 33-, 35-, and 37-carbon backbone di- and trimethylalkanes (Carlson et al., 1978), and a series of synthetic 2,*X*-dimethylalkanes (Pomonis et al., 1989).

GC and GC-MS. Gas chromatography (GC) of the hydrocarbons was performed on a Tracor model 540 instrument fitted with a nonpolar capillary column (30 m × 0.32 mm ID, 0.25 μm film of DB-1) in the on-column mode (OCI-3, SGE, Dallas, Texas) with hydrogen carrier gas. The oven temperature was programmed from 60° to 325°C at 6°/min. The instrument was equipped with a flame ionization detector and a Nelson Analytical model 9000 PC data system. The detector was operated at 360°C with hydrogen carrier gas at 1.2 ml/min. Later analyses were performed on a Hewlett-Packard model 6890 instrument fitted with a nonpolar capillary column (25 m × 0.20 mm ID, 0.25 μm film of DB-1) in the on-column mode with hydrogen carrier gas. The oven temperature was programmed from 60° (hold 2 min) to 220°C at 20°/min, then 220°C to 310°C at 3°/min (hold 30 min). The flame ionization detector was held at 340°C, and a Perkin-Elmer Turbochrom model 4.1 PC-based system was used

to record data. All samples were diluted as necessary, and each was coinjected with consecutive *n*-alkane standards of 22–40 carbons for determination of relative retention indices. Based on KI, an *n*-alkane of 33 carbons was assigned KI 3300 and an internally monomethyl-branched alkane with 34 total carbons was assigned KI 3328 (33.28 carbons equivalent chain length or 0.28 carbons added to a 33-carbon alkane). If the column was overloaded, the KI value was 3335. Mass spectra were recorded on a Hewlett-Packard model 5988 quadrupole instrument interfaced to a HP 5890 Series II GC. The 30-m DB-1 column described above was used with an on-column injector and helium carrier gas at a linear flow rate of 35 cm/min, with a hold at 60°C for 2 min, then temperature programmed from 60° to 220°C at 20°/min, and finally programmed from 220 to 310°C at 3°/min (hold for 20 min).

We have not attempted correlate isothermal GC-calculated KIs and temperature-programmed KIs. About 80% of the data presented are from recent analyses in our laboratory, with the remaining data obtained from the literature or from unpublished data. Minor variation is possible with changes in temperature programs, column length, film thickness, possibly with injection technique, and certainly with overloading.

RESULTS

The interpretation of mass spectra of methylalkanes is difficult, particularly when a large number of isomers are present, as in many insects. Interpretation is facilitated by the recognition of patterns and can be improved by using a protocol that involves recognition of methyl branching patterns from known structures. For the discussion presented in this paper, "isomers" all have the same molecular weight but different branching patterns, whereas "homologs" have the same methyl branching pattern, but the backbones have different lengths.

The peak sizes of standards and unknowns should be as close as possible to get good KI values. Overloading the column's stationary phase should be avoided to minimize the shifting of peak centers. It may be more useful to use the front of the peak than the center, especially if methylalkanes are smeared together. The KIs provide useful information, allowing elimination of some structures, while strongly suggesting others. Polymethylalkanes with even-numbered methylene bridges are biosynthetically unlikely (de Renobles et al., 1991).

Protocol for Identification of Insect Methylalkanes

1. Assume that the most prominent peaks represent unbranched or normal *n*-alkanes with odd numbers of carbons in their chains (21–31 carbons, but rarely above 33), with smaller amounts of even-numbered homologs. These *n*-alkanes are often found in a bell-shaped distribution around a central peak,

sometimes with a separate minor distribution. Locate and mark these by comparing their retention times to standard *n*-alkanes run under the same conditions, or coinject an *n*-alkane standard (C_{20}–C_{40}) with both even- and odd-numbered backbones. Make a scale to mark the KI values for all peaks within the sample. This establishes the likely backbone length of each hydrocarbon, after which assigned KI values from GC analysis contribute significantly to interpretation of branching patterns. The protocol given in the following illustrative examples is based on straight-chain and methyl-branched hydrocarbons having 33 or 34 carbons in the backbone; 3300 refers to *n*-tritriacontane (KI 3300), and 3400 refers to *n*-tetratriacontane (KI 3400). Figure 1 shows that compounds eluting up to 160 KI units later than an *n*-alkane can have the same carbon chain length. However, there may be a bimodal distribution of peaks (C_{23-29}, C_{33-41}) if the largest peaks are mono- or dimethylbranched of more than 33 carbons.

2. Identify the odd-numbered backbone compounds first because of the relative simplicity of their mass spectra. These are generally the larger peaks, except in the 2-methylalkane series in which large peaks containing even-numbered backbone homologs may be present.

3. Locate and mark as methylalkanes the peaks ranging between KI 3328 and 3333. These are probably internally branched monomethyls (IMMs) with methyl branches near the center of the chains (typically at carbons 15, 13, and 11). The majority of methyl branches occur on odd-numbered carbons in odd-numbered backbone monomethylalkanes (15-methyl to 3-methyl, etc.), as they do in di-, tri-, and tetramethylalkanes. On the back shoulder of these peaks are 9-methyl isomers at KI 3336, then 7-methyl isomers at KI 3340, and 5-methyl isomers at KI 3350. The common 2-methyl isomers elute at KI 3362-65 just after 4-methylalkanes at KI 3358, although the latter are rare in insects. The last eluting monomethyl isomer at KI 3372-74 is the 3-methyl isomer.

4. Alkanes with even-numbered backbones will have very nearly the same last two digit KI values as do odd-numbered backbone homologs with methyl-branches in about the same location. Since one carbon may be added to either end of the carbon chain, the resulting isomers will have methyl branches residing on both even-numbered and odd-numbered carbons, while retaining the same methylene interruption. The mass spectra therefore become more difficult to interpret, particularly for those with multiple branches, so these should be addressed after finishing all odd-numbered backbone compounds. For simplicity, Figure 2 exhibits KIs for di-, tri-, and tetramethylalkanes having even-numbered backbones only.

5. Locate and interpret structures of peaks near KI 3355–3360. These are usually "terminal" dimethylalkanes (IDMs) with a short bridge having intervals (I) between methyl branch points of 3 methylenes (I = 3). Thus addition of one isoprene unit [containing three methylenes (I = 3) and a methyl-branched carbon] typically adds 0.25 carbons to monomethylalkane KI values, while longer

methylene bridges (I = 7, 9, 11, 13) add 0.33 carbons, and the compounds elute later, near 3363. Dimethylalkanes with even-numbered methylene bridges are rare, if not biosynthetically impossible, in internally branched alkanes, and intervals of five methylenes (I = 5) are rare. Common near KI 3410 are "terminal" 3,*X*-dimethyl branched alkanes (I = 3) with the first branch on carbon 3, in which addition of one isoprene unit to a starting 3-methyl alkane has typically added 0.35 carbons. For larger intervals between methylene branches (I = 9, 11, 13), the compounds elute earlier, near KI 3403, an addition of 0.28 carbons. Less common are 2,*X*-dimethyls that elute on either side of an *n*-alkane of the same chain length depending on the interruption. Thus, 2,*X*-dimethyl isomers elute near KI 3395, when the methylene bridge is longest (I = 9, 11, 13), but together near the *n*-alkane when I = 5 or 7, and elute later near KI 3405 when I = 3.

6. Locate and interpret structures of peaks near KI 3380. These may be trimethylalkanes having short methylene bridges (I = 3/3) between internal methyl branches. Mixed length bridges eluting slightly later (KI 3383) are encountered less often (I = 3/5, 3/7 and sometimes 5/3). It is necessary to first locate and eliminate fragments from 2- and 3-methyl alkanes from these spectra before attempting interpretation of the rest of the fragments. Thus, addition of a third methyl branch with one methylene bridge (I = 3) typically adds 0.25 carbons to the KI value of a dimethylalkane. Peaks eluting near KI 3416–3420 may be "terminal" trimethyl-branched even-numbered backbone alkanes (I = 3/3) with the first branch on the 4-carbon, typically a 4,8,12-trimethyl. Peaks eluting near 3437 may be I = 3/3 homologs that have one more or one less carbon in the chain, with the first branch on the 3-carbon, and typically have 3,7,11-trimethyl branching: These homologs may coelute with IMMs of the next chain length, making interpretation tricky.

7. Locate peaks that elute at KI 3405–3410, or about 0.25–0.30 carbons after internal trimethyl isomers and just after the next (34-carbon) *n*-alkane. Peaks at 3405 may be internal tetramethylalkanes with the first methyl at 13- or 11- and I = 3/3/3 or some small interval. A tetramethyl hydrocarbon with the first branch at 9- and I = 3/3/3 elutes near KI 3418, and with a first branch at 7- it elutes near KI 3430. With a first (terminal) methyl branch at 3-, it elutes near KI 3459 (I = 3/3/3). We have examples of tetramethylalkanes with I = 3/3/3, and none with longer methylene interruptions.

8. The principles and procedures outlined above can be applied to all other sets of alkanes with a single backbone chain length.

Specific Examples in Support of the Protocol and the Figures

Figure 1 illustrates elution patterns measured or estimated for typical methylalkanes of 33-carbon backbones and is meant to be general for compounds with odd-numbered backbones. It shows typical methyl-substituted tritriacon-

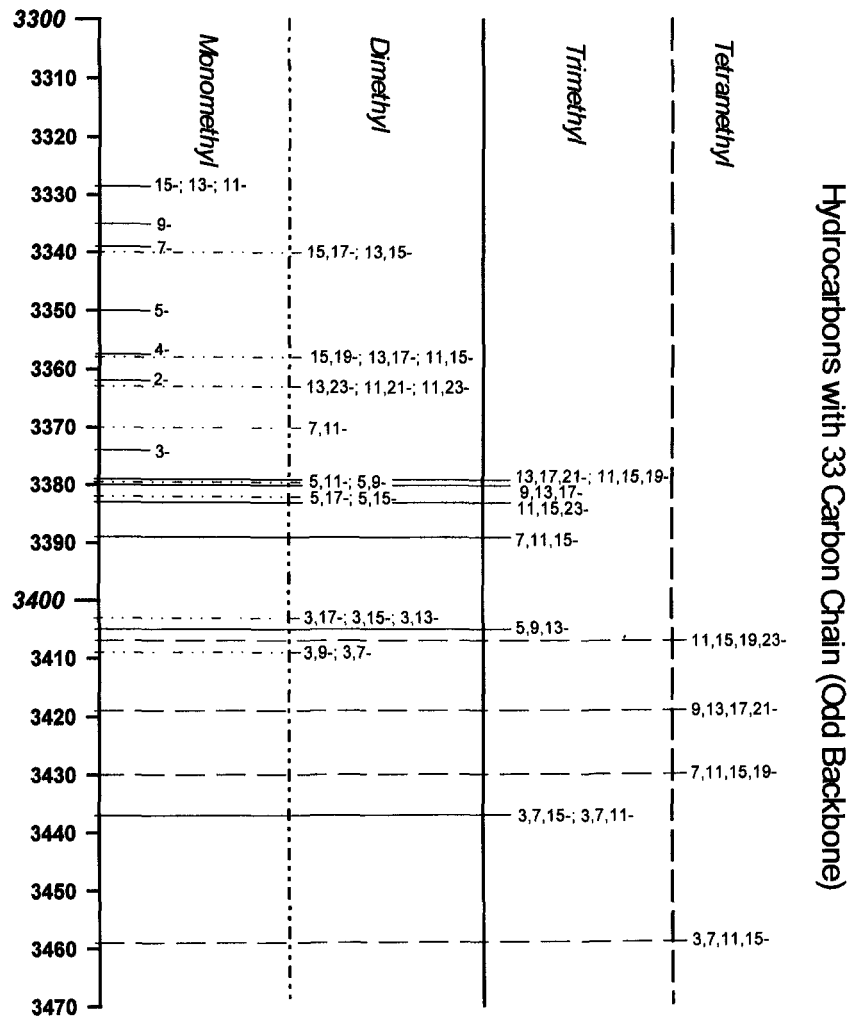

FIG. 1. Measured or estimate elution patterns of mono-, di-, tri-, and tetramethylalkanes with an odd-numbered backbone length of 33 carbons in insect hydrocarbons. KI values are shown in the legend and may be applied generally from C_{23} to C_{40} insect-derived alkanes with caveats as noted in the text.

tanes (alkanes with backbones of 33 carbons) with observed or estimated KI values, in which all monomethyl tritriacontanes elute between KI 3328 and 3374 (total 34 carbons), dimethyl tritriacontanes elute between KI 3340 and 3410 (total 35 carbons), trimethyl tritriacontanes elute between KI 3377 and 3437 (total 36 carbons), and tetramethyl tritriacontanes elute between KI 3407 and

3459 (total 37 carbons). This illustration is valid for methyl-branched alkanes of 25–40 carbons, with the caveats noted below for shorter or longer chain lengths, as illustrated specifically in Figures 3–5 below.

Figure 2 illustrates elution patterns measured or estimated for typical methylalkanes with 34 carbons in the backbone and is meant to be general for com-

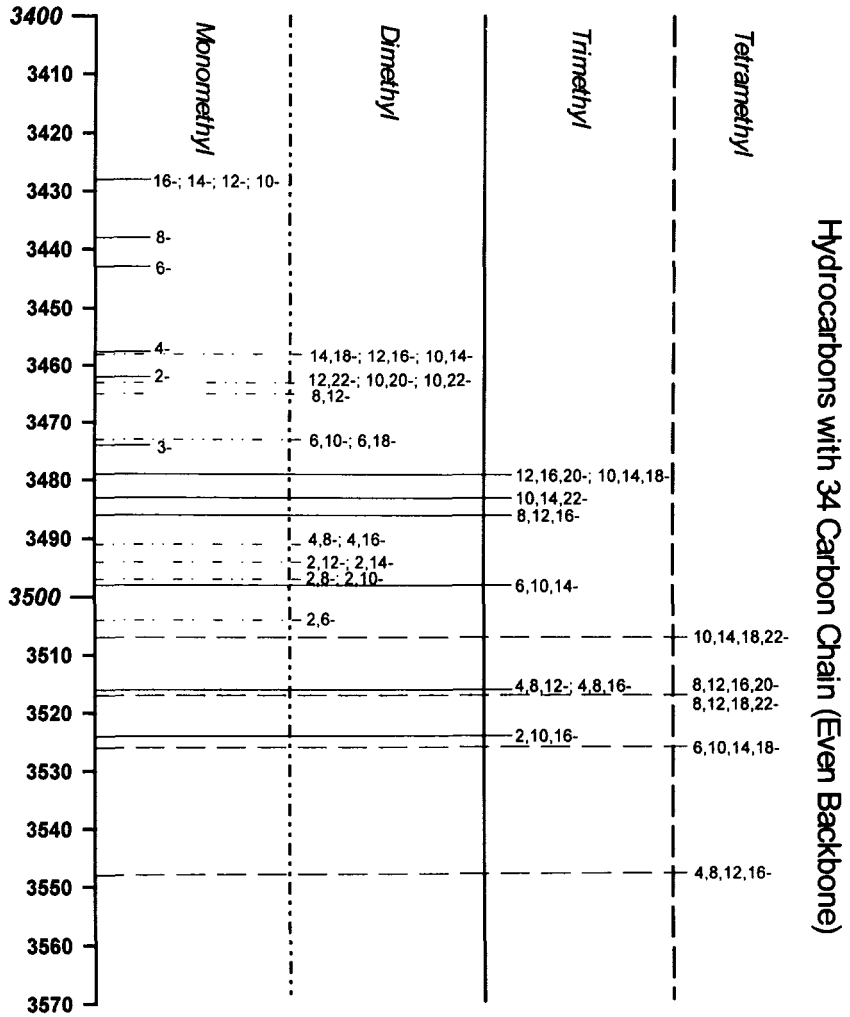

FIG. 2. Measured or estimated elution patterns of mono-, di-, tri-, and tetramethylalkanes with an even-numbered backbone length of 34 carbons in insect hydrocarbons. KI values are shown in the legend and may be applied generally from C_{23} to C_{40} insect-derived alkanes with caveats as noted in the text.

pounds with even-numbered backbones. Methyl branches at odd-numbered positions may be seen on alkanes with even-numbered backbones, but a number of compounds do not appear in Figure 2, as we did not observe them in odd-numbered backboned methylalkanes (i.e., 5- and 7-IMM, and 5,*X*-, and 7,*X*-IDM).

Methylalkanes. We concur with the assignments of Kissin and Feulmer (1986), who showed that relative retention times of methylalkanes increase smoothly when the chain length is held constant and the methyl branch is moved from the center carbon toward the 2-position at the end of the chain. 3-Methylalkanes elute last. We found close agreement with Kissin and Feulmer (1986) for plotted relative retention times of IMMs that successively eluted earlier after 30 carbons with increasing chain length, as in KI 3725 (Figure 3). The present work extends the data for IMMs to 53 carbons, with the heaviest homologs eluting at KI 5319.

Dimethylalkanes. We observed that the plotted relative retention times of IDMs decline smoothly with increasing chain length (KI 2765 to KI 3950) (Figure 4). KI values for Figure 1 were estimated from Figure 4 if an actual value was not available. Relative retention times of internal dimethylalkanes increase smoothly by about 0.14 carbons as the methylene bridge length increases (I = 7, 9, or 11) because the second methyl branch in dimethylalkanes can be considered to be moved past the center of the chain toward the other end.

The slight declines or increased shifts in retention indices observed with increasing chain length for the dimethylalkanes are real and are discussed here as they appear at the lower part of Figure 4, with the IDMs eluting earliest. The IDMs with lower interruptions (I = 3) included 9,*X*-, 11,*X*-, 13,*X*-, and 15,*X*-isomers and these showed the largest range of shifts with increasing chain length. However, peaks also containing the internally branched series with higher interruptions are split (I = 9, 11), because the latter isomers eluted considerably later than those with I = 3, typically by 8 KI units at 35, 36, and 37 carbons. This was seen in hydrocarbons from *Melanoplus* grasshoppers (Sutton et al., 1996) and tsetse flies (Sutton and Carlson, 1997a).

The 8,*X*-, 7,*X*-, and 6,*X*-series ranges were relatively narrow, with downward trends of 4 or 5 KI units observed with increasing chain length. There is a potential anomaly for 7,15-C_{27}, that appears to elute 3 KI units too early.

The values for 5,*X*-dimethyls were split, and the lower methylene-interrupted 5,*X*-series (I = 3) was shifted by 8 KI units to lower values over the range of 14 carbons, with a potential anomaly at 5,9-dimethyl-C_{35} that eluted 4 KI units too late. The 5,*X*-series with larger interruptions (I = 11) was nearly flat at KI 2192–2791, adding about 91 KI units to each backbone value. An anomaly was 5,13-Me_2C_{23} (I = 7), found at KI 2377, rather lower than expected by 7 KI units.

Relative retention times of "terminal" dimethylalkane isomers decrease

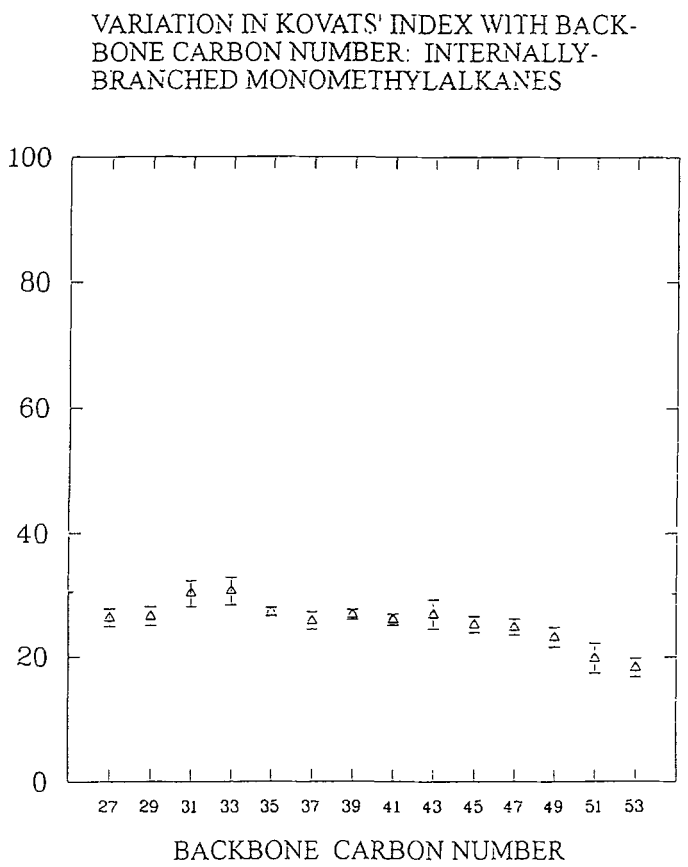

FIG. 3. Variation in KI with backbone carbon number: internal methylalkanes from *Melanoplus sanguinipes* coinjected with alkane standard up to 60 carbons (25 m × 0.20 mm ID, SBP-1, Supelco) (means of 10 analyses).

smoothly with increasing methylene bridge length because the second methyl branch can be considered to be moving toward the center of the chain. The overriding principle is that with a methyl branch moving toward the end of a chain, the isomer elutes later, and the effect of the terminal methyl is most important. With a methyl branch moving toward the center of a chain, the isomer elutes earlier. The 4,*X*-series appears to decline slightly over the range of compounds observed, from KI 2694 to 3489, changing by about 5 KI units over the range of C_{25}–C_{35}.

The 2,*X*-series was obtained from literature values, except for a synthetic 2,*X*-C_{27} series including 2,6- (KI 3005), 2,8-, 2,10- (KI 3002), 2,12-, and 2,14-

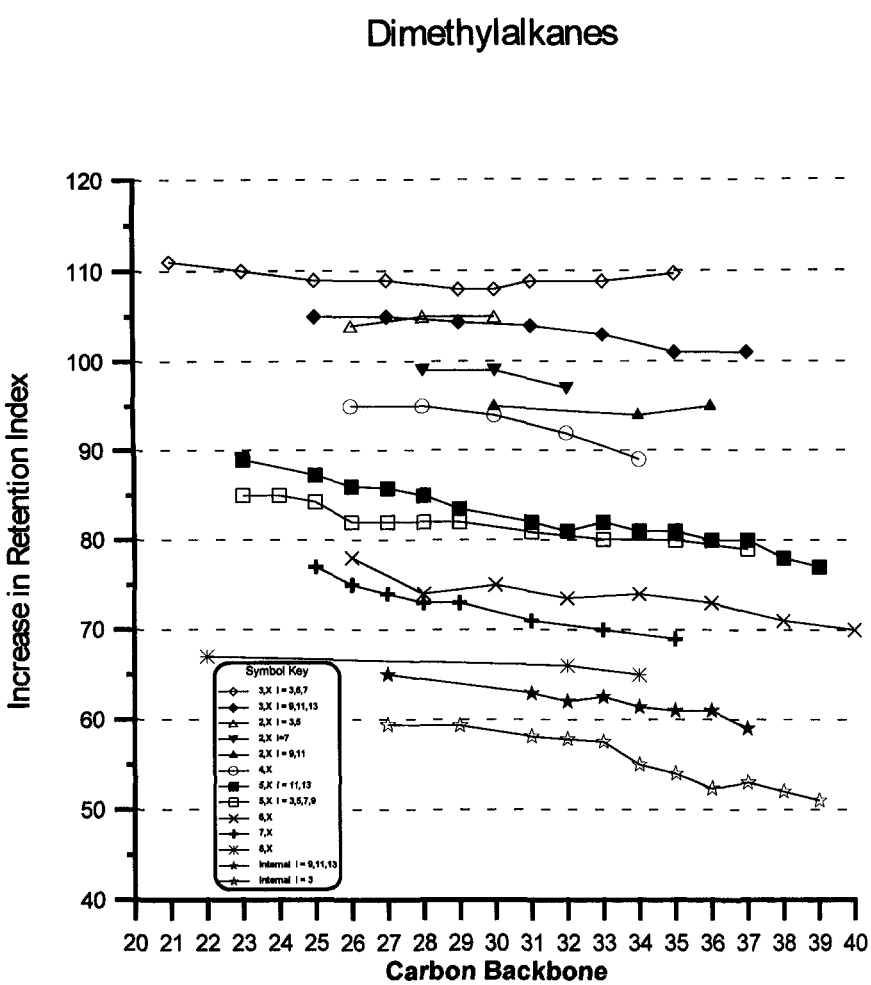

FIG. 4. Variation in KI with backbone carbon number compared to *n*-alkane standards: dimethylalkanes. Example-experimental values for internal dimethyls (I = 3/3) declined incrementally from KI 2759 (13,17-Me_2C_{27}) to KI 3952 (15,19-Me_2C_{39}).

dimethylnonacosanes (KI 2994). These results show that the lower methylene-interrupted 2,*X*-series (I = 3) did not shift to lower values over the range examined, but the 2,*X*-series with larger interruptions (I = 7, 9, 11) showed a shift downward.

The lower methylene-interrupted 3,*X*-series (I = 3, 5, 7) eluted after all other dimethyls and tended not to shift to lower values over the range examined,

declining from KI 2609 to KI 3007, then increasing slightly to KI 3609. The overall shifts in KI values were 107 to 110 KI units from C_{25} to C_{35}; this KI value is added to the carbon backbone for each homolog. Thus the measured retention value for 3,7-dimethyl C_{33} is 3409 (see Figure 1). In contrast, the 3, *X*-series with larger interruptions (I = 11) declined over the series from C_{25} to C_{37} by 8 KI units, starting at 2609 and ending at 3801. Thus, the measured retention value for 3,15-dimethyl-C_{33} is KI 3403.

Trimethylalkanes. The slight declines or declining shifts in retention indices observed with increasing chain length for the trimethylalkanes are discussed as they appear from the top of Figure 5, with the 3,*X*,*Y*-trimethylalkanes eluting

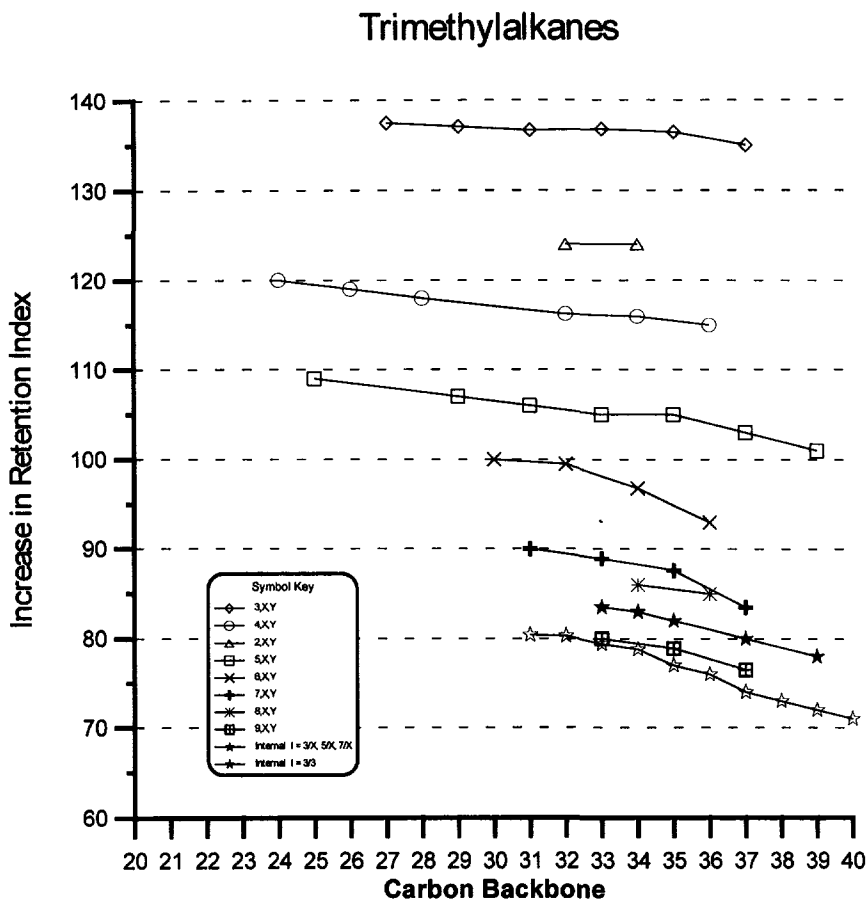

FIG. 5. Variation in KI with backbone carbon number compared to *n*-alkane standards: trimethylalkanes.

latest, and the internal trimethylalkanes eluting earliest. In each series, most of the trimethylalkanes contained two 3-methylene bridges (I = 3), but this typically varied when the corresponding dimethylalkanes had larger interruptions, as the third methyl branch was often added internally into a I = 9 or 11 methylene bridge.

Tetramethylalkanes. Internal tetramethylalkanes (I = 3/3/3) eluted near KI 3395, or 0.21 carbons after internal trimethylalkanes marked as 3374. External tetramethyl compounds such as 3,7,11,15-series eluted last at 3459, about KI 160 units later than the corresponding alkane, or 0.22 carbon equivalents after the corresponding external trimethylkanes. (Figure 6).

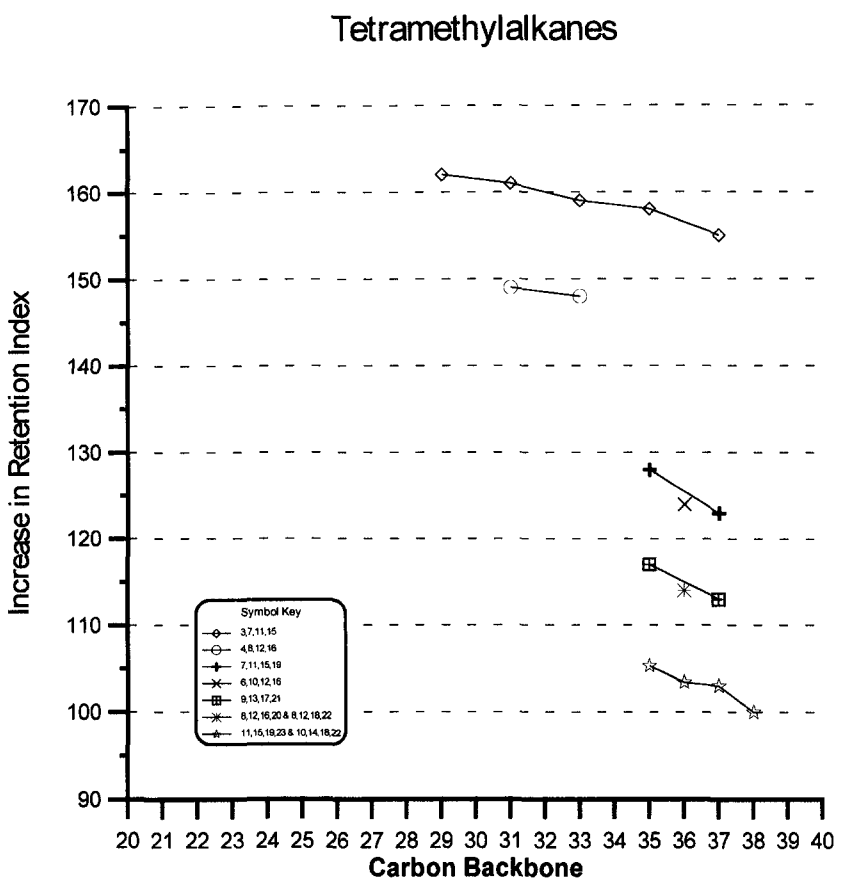

FIG. 6. Variation in KI with backbone carbon number compared to *n*-alkane standards: tetramethylalkanes.

DISCUSSION

We believe that the figures shown here will assist those attempting to decipher insect polymethylalkane structures, for which there are a restricted number of overlying patterns (Blomquist et al., 1987). These structures could be considered to rise from the apparent addition of isoprene units, although this is not meant to apply in the context of biosynthesis. The most common IDMs observed have 3-methylene interruptions, with 7-, 9-, and 11- bridges less common, while trimethylalkanes often have two 3-methylene interruptions, and all tetramethylalkanes had only 3-methylene interruptions. Most data in the literature do not include KI values or have few calculated KI values or carbon equivalents in which exact measurements were reported. In some cases, particularly GC-MS runs in which identifications were critical, sample overloading caused late elution of one or two peaks, and these late KI values were not included here. In these cases, we inspected GC-MS runs that first had been run very dilute, to get more accurate data for the larger peaks. It must be pointed out that recent KI values obtained from long narrow-bore (0.10–0.15 mm) capillary columns of 5% phenyl siloxanes such as DB-5 are substantially lower for polymethylalkanes than values described here. Observations that tri- and tetramethylalkanes elute more rapidly on these columns has not been previously reported, to our knowledge, and will be reported elsewhere.

The discussion of hydrocarbon identification by Lockey (1988) is illustrative with many examples given. However, the present effort refutes Lockey's statement that retention indices are "an unreliable indicator of carbon number owing to the effect which methyl branches have on retention times." Use of high-resolution columns and avoidance of overloading the column allow more precise, consistent, and indicative KI values to be assigned. Lockey (1988) stated that "An *n*-alkane of a hydrocarbon mixture frequently elute[s] with terminally-branched dimethylalkanes," with his examples (Figure 7, p. 625) showing 2,*X*-dimethyl nonacosanes eluting (at about KI 2895) just ahead of *n*-C_{29}, and 3,*X*-dimethyl triacontanes eluting (at about KI 3006) just after *n*-C_{30}. These statements are consistent with the present findings.

Lange et al. (1989) describe a strategy for identification of insect hydrocarbons that involves silica gel and argentation chromatography followed by GC and GC-MS with CI-GC-MS. Their mass spectral interpretation followed Nelson and Sukkestad (1970) and Nelson et al. (1972), as does ours, although we also referred to Nelson (1978) and Blomquist et al. (1987).

Methylalkanes. Lange et al. (1989) presented one table that showed carbon equivalent retention times to two decimals and contained IMMs, but only a few dimethylalkanes with mass spectra were presented. The KIs of IMM from *Reticulitermes* termites showed a decrease with increasing chain length from C_{25} (KI 2533) to C_{31} (KI 3131) with temperature-programmed values (L. Nelson,

unpublished data). Kissin and Feulmer (1986) showed similar trends for monomethylalkanes of 36 carbons or less, the upper limit of their published data.

Dimethylalkanes. The earliest-eluting dimethylalkane, 13,15-Me_2C_{29}, is a rare variant that has monomethylene spacing (I = 1). It occurs in the imported fire ant *Solenopsis invicta* and elutes near KI 2940. It occurs also in several termites including *Coptotermes formosanus* (Haverty et al., 1990), but has slightly longer retention times (KI 2947). This was ascribed to the presence of two diastereomers with very similar mass spectra. The early-eluting enantiomer appears consistently in termites, in which it elutes just after internally branched monomethylalkanes. Other GC-MS data presented for dimethylalkanes with 1-, 2-, and 4-methylene bridge carbons from *Hypoponera eduardi* ants (Lange et al., 1989) appeared dubious, but since no retention indices were presented, any suggestion of alternative structures is not possible. Although CI-GC-MS was performed in that study, we believe that even-numbered bridges in dimethylalkane structures are rare in insects, if they exist at all. However, synthetic enantiomers of 9,11-Me_2C_{29} eluted separately, just after internal methylalkanes (Pomonis et al., 1980).

The earliest eluting dimethyl isomers usually found are the symmetrical IDMs (I = 3) that elute near KI 3355, 3555, 3755, and 3955 in *G. morsitans* (Nelson and Carlson, 1986) (Figure 4). The 15,19- and 15,21-dimethyl alkanes elute at KI 3355 with I = 3 and 5 in *G. austeni*, although the latter are rare. Isomeric 9-methylene interrupted (I = 9) alkanes were present in *G. pallidipes* (Nelson and Carlson, 1986), and both natural and synthetic 13,23- and 15,25-dimethylheptatriacontane eluted at KI 3763. These compounds were incorrectly described as isomers of 13,17-dimethylheptatriacontane in another report on the same insect, although the mass spectra were consistent with the 13,23- and 15,25-isomers, as were the retention times (McDowell et al., 1981). Isomeric IDMs in *G. tachinoides* contained 11,19- and 13,21-dimethylheptatriacontane having longer methylene bridges (I = 11) that eluted at KI 3763 (Nelson and Carlson, 1986) together with synthetic 11,19-, 13,23-, and 15,25-dimethylheptatriacontane (Carlson, unpublished data). Long series of isomeric I = 9 and I = 11 IDMs were present in *Melanoplus* (Sutton et al., 1996). The 7,*X*-dimethyls also appeared to elute with this group. Several 5,*X*-(KI 3382) and 4,*X*-dimethyls (KI 3392) were found in *G. brevipalpis* (Nelson et al., 1988), and 5,*X*-dimethyls appeared in termites (Haverty et al., 1988).

Much more common are 3,7-dimethylnonacosane and its 3,*X*-isomers, such as those in *Blattella* cockroaches that coelute at KI 3010. The 3,*X*-isomers with 27 (KI 2810) and 31 (KI 3210) carbon backbones eluted at the same relative position, although we did not detect 33 carbon backbone homologs (Carlson and Brenner, 1988) (Figure 4).

Szafranek et al. (1994) described the retention times of terminal di- and trimethyl-branched alkanes from two species of Coleoptera and the mass spectral

interpretation by utilizing daughter ion mass spectra. Daughter ions derived from the molecular ion were usually observed as a large fragment from cleavage internal to the longest alkyl chain, but smaller fragments were not diagnostic. They observed several 2,*X*-dimethylalkanes that elute close to *n*-alkanes and their trimethyl homologs. Curiously, the major components had even-carbon-numbered backbones. Szafranek et al. (1994) showed KIs quite consistent on OV-I columns for 2,10-dimethylalkanes as well as those with higher interruptions that eluted slightly earlier (KI 2899, 3096, 3297, 3497) and 2,6-dimethylalkanes (KI 2704, 2905, 3105, 3305). KIs for their 2,10,18-trimethyl alkanes (I = 7/7) were internally consistent (KI 3324 and 3524) eluting about 10 KI units before internal monomethylalkanes with one more carbon backbone. Pomonis et al. (1989) described 2,6-dimethylalkane isomers that are not often seen except in screwworm flies, and obtained GC-MS data for synthetic isomers of 2,14- and 2,12-dimethylheptacosane (KI 2795), whereas 2,10- and 2,8-eluted just after the *n*-alkane (KI 2802), and the 2,6-dimethyl isomer (KI 2806) coeluted with a 3,*X*-isomer (Pomonis, 1989). We obtained essentially the same results with these synthetic isomers. Thus, it may still be useful to separate unbranched from branched alkanes with molecular sieving, especially if there is an excess of *n*-alkane. Although better separation can be obtained on a higher-resolution column, these are the only two common classes of insect-produced dimethylalkanes that could be confused with *n*-alkanes (Figure 2). Numerous other examples from different insect groups could be cited. Many are listed in Nelson (1993) and Nelson and Blomquist (1994).

Trimethylalkanes. The earliest-eluting trimethylalkane is the symmetrical internal 15,19,23-trimethylheptatriacontane at KI 3775, with homologs having 33- to 39-carbon backbones. All of these contain internally branched 3-methylene bridges (I = 3) and are present in *G. morsitans centralis* and *G.m. submorsitans* female tsetse flies (Figure 7). The series may include 13,*X*,*Y*- and 11,*X*,*Y*-isomers, with partially separated 9,*X*,*Y*-, followed by clearly separated 7,*X*,*Y*-isomers.

The next eluting isomer is the 5,9,13-trimethylnonacosane (KI 3005) in *G. tachinoides* males (Nelson and Carlson, 1986). A commonly found terminal trimethyl variation with an odd-numbered backbone is 4,8,12-trimethylheptacosane (KI 2820) and its 3,7,11-C_{29} homolog at KI 2920 in *G. tachinoides* males. A rare 2,6,10-trimethyl homolog was observed that eluted at KI 2825, just after these 4,8,12-isomers, which seems consistent with the elution patterns of the 4,8- and 4,10-isomers at KI 3392 eluting before the 2,*X*-dimethyl isomers. It seems to contradict Figure 1 of Kissin and Feulmer (1986), which has a 2-methyl alkane isomer eluting at KI 3363 but the 4-methyl isomer eluting at KI 3368, and a 3-methyl isomer at KI 3372. This was the only obvious contradiction that we found with Kissin and Feulmer (1986). A series of several other short 5,*X*,*Y*-trimethylalkanes were described from *Reticulitermes* termites (Hav-

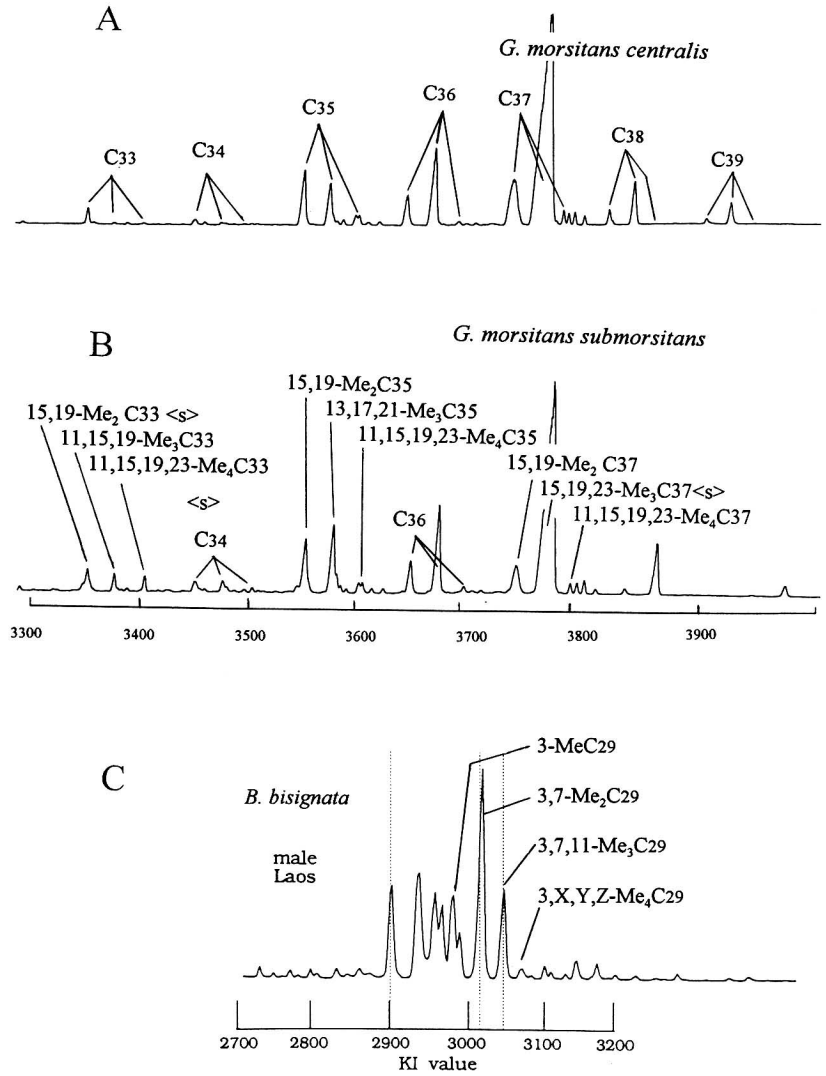

FIG. 7. Elution pattern of 15,19-dimethyl-, 15,19,23-trimethyl-, and 15,19,23,27-tetramethylalkanes of 33- to 39-carbon backbones from tsetse fly females. Add 0.20–0.25 carbons for each additional methyl branch. ⟨s⟩: symmetrically substituted. (A) *G. m. morsitans*. (B) *G. m. submorsitans*. (C) Elution pattern of 3-methyl-, 3,7-dimethyl-, 3,7,11-trimethyl- and 3,*X*,*Y*,*Z*-tetramethyl C_{29} from male *Blattella bisignata* cockroaches.

erty et al., 1996; Page et al., 1997; L. Nelson, unpublished data). The latest-eluting trimethylalkanes include "terminal" 3,7,11-trimethylhexacosane (KI 2740) and its 28-carbon backbone homolog (KI 2940) in *G. p. palpalis* males and several other species of tsetse flies in this group (Nelson et al., 1988).

Tetramethylalkanes. Internal tetramethylalkanes (I = 3/3/3) elute near KI 3390, or 0.16 carbons after internal trimethylalkanes marked as 3374. The specific example is 11,15,19,23-tetramethyl-C_{37}, found at KI 3790 in *G. m. morsitans* females (Nelson and Carlson, 1986), the first report of a homologous series of di-, tri-, and tetramethylalkanes in the same insect. An unusual terminal tetramethyl homolog eluted just after 2-methyltriacontane at KI 3065 in *Blattella* cockroaches. Reexamination of the spectra of male and female *B. asahinai* clearly shows the same homologous series that was not interpreted in the original paper (Carlson and Brenner, 1988). Inspection of the small, broad KI 3065 peak in *B. bisignata* and *B. asahinai* by EI- and CI-MS confirmed the presence of 2-methyltriacontane [*m/z* 393 (primary ion), 435 (M − 1)] that eluted just ahead of a tetramethyl homolog [*m/z* 463 (M − 1)] of the KI 3010 peak (3,7-dimethyl-C_{29}) and KI 3040 peak (3,7,11-trimethyl-C_{29}) (Figures 6 and 7C). The EI spectra contained fragment ions (*m/z* 127, 197, 224/225, 267, 295, and 365) consistent with 3,7,11,15-tetramethylnonacosane. This tetramethylalkane is the latest-eluting C_{29}-backbone compound known in insects and completes an homologous series of di-, tri-, and tetramethylalkanes that were also observed in the closely related *B. asahinai* (Brenner et al., 1993). *Glossina morsitans morsitans* females showed a nearly complete series of I = 3 internally branched, di-, tri-, and tetramethylalkanes (11,*X*-, 11,*X*,*Y*-, and 11,*X*,*Y*,*Z*-), and the monomethylalkanes were present at very low levels (Nelson and Carlson, 1986). There were externally branched series in *Muscidifurax* (Bernier et al., 1988) and *G. brevipalpis* (3-, 3,*X*-, 3,*X*,*Y*-, and 3,*X*,*Y*,*Z*-; 5,*X*-, 5,*X*,*Y*-, and 5,*X*,*Y*,*Z*-) (Nelson et al., 1988). These were also present as a complete series in *Melanoplus* (Sutton et al., 1996).

The compounds plotted here comprise most of the known insect hydrocarbon structures published or available to us that were accompanied by reference to reliable standards. The plots are mostly based on recent capillary GC-MS runs with standards. We are confident in the descriptive data and have not attempted to develop a formula to calculate retention times. If overloaded conditions are avoided, we suggest that the experimental values will fall within a few KI units of what is reported here.

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