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SYNTHETIC METHYL- AND DIMETHYLALKANES
Kovats Indices, [^{13}C]NMR and Mass Spectra of Some Methylpentacosanes and 2,X-Dimethylheptacosanes

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Abstract—All possible isomeric mono-methylpentacosanes as well as 2,6-, 2,8-, 2,10-, 2,12-, and 2,14-dimethylheptacosane were synthesized. The [^{13}C]NMR shifts and the Kovats indices were determined, and the relationship to separation of isomeric mixtures of insect cuticular waxes are discussed. When several homologous series of hydrocarbon isomeric mixtures are to be separated, it is not practical to attempt complete gas-chromatographic separation of all possible isomers. The mass spectra of the 2,X-dimethylheptacosanes are presented and discussed.

Key Words—Insect hydrocarbon, methylpentacosane, 2,X-dimethylalkane(s), [^{13}C]NMR, mass spectra, Kovats index.

INTRODUCTION

Insect hydrocarbons and their role in the growth, development, and behavior of insects have been widely studied and reviewed (Howard and Blomquist, 1982; Lockey, 1985, 1988; Blomquist and Dillwith, 1985; Blomquist et al., 1987). Characterization of hydrocarbon structure has been accomplished by mass spectrometry with some ambiguity because of the lack of standard compounds for comparison. Several papers have described the synthesis and mass spectra of some dimethylheptacosanes (Pomonis et al., 1980), methylpentatriacontanes, methylheptatriacontanes, a dimethylpentatriacontane, and a dimethylheptatriacontane (Pomonis et al., 1978) for the purpose of corroborating the structure of

these compounds, which had first been isolated from the tobacco hornworm (Nelson and Sukkestad, 1970; Nelson et al., 1972). The structures were assigned solely on the interpretation of the mass spectra of the natural products.

Many hydrocarbon mixtures that are isolated from insects are blends of not only homologs but also of isomers. Separation of the component compounds from these mixtures is usually accomplished by gas chromatography (GC). Structural characterization is performed by determining their Kovats indices (KI) (Kovats, 1965) and combining GC with mass spectrometry (GC-MS). Although homologs may easily be separated by GC, separation of isomers is not so readily accomplished. Capillary GC provides the best resolution of the mixtures. However, because the natural product mixtures contain compounds that range from 23 carbons ($C_{23}H_{48}$) to greater than 40 carbons ($C_{40}H_{82}$), the GC conditions require temperature programming over a wide range to obtain a separation. Theoretical as well as technical considerations do not allow complete GC separations, even by the best capillary chromatography, of all isomers in a series, e.g., not all isomeric methylalkanes can be separated from each other. The reasons for these limitations will be shown and discussed.

Recent biosynthetic studies using ^{13}C-labeled precursors showed incorporation of the label into insect hydrocarbons (Blomquist et al., 1980; Dwyer et al., 1981; Dillwith et al., 1982). Analysis of the isolated hydrocarbons by ^{13}C nuclear magnetic resonance spectrometry ([^{13}C]NMR) enabled investigators to identify the exact location of the incorporated label by nondestructive analytical means. The above authors used [^{13}C]NMR data obtained from our synthetic compounds to assign structure to their biosynthetic hydrocarbons.

All possible 12 monomethylpentacosanes ($C_{26}H_{54}$) were synthesized. The GC Kovats index and [^{13}C]NMR for each isomer was determined and will be discussed. The spectra obtained from a tandem mass spectrometer (MS-MS) of each of the monomethylpentacosanes were reported elsewhere (Cerny et al., 1986). In addition to the methylpentacosanes, five 2,X-dimethylheptacosanes were synthesized, viz., 2,6-, 2,8-, 2,10-, 2,12-, and 2,14-dimethylheptacosane. This class of dimethylalkanes was recently reported for the first time and was isolated from the screwworm (Pomonis, 1989). This report verifies the structure of these natural products by synthesis, chromatography, and spectrometry of some analogs.

METHODS AND MATERIALS

Gas Chromatography. The purity of the synthetic intermediates and final products was evaluated by GC. Analyses were performed with a Varian model 3700 flame ionization instrument using a 12- or a 25-m × 0.20-mm-ID fused silica capillary column [Hewlett-Packard (H-P) cross-linked methyl silicone 19091102, 0.33μm film]. A Varian multipurpose type injector in the split mode

with a glass frit insert was used for all capillary chromatography. Sample introduction was by injection using a 100:1 split ratio. The GC was programmed from 150 to 320°C at a variable rate (usually at 4 or 2°/min) with a 2-min initial isothermal and an 8-min final isothermal hold. Helium was the carrier gas at a flow rate of 0.77 ml/min (20 cm/sec) with a head pressure of 12.5 psig. Chromatographic data was reported by a Hewlett-Packard model 3390A integrator interfaced to the GC. The KI (Kovats, 1965) of each methylpentacosane or artificial mixtures of the methylpentacosanes were determined by isothermal gas chromatography at 210°C and *n*-$C_{25}H_{52}$ and *n*-$C_{26}H_{54}$ were used as the reference points. The KI of the dimethylheptacosanes were determined with *n*-$C_{27}H_{56}$, *n*-$C_{28}H_{58}$, and *n*-$C_{29}H_{60}$ as the reference points at an isothermal oven setting of 225°C.

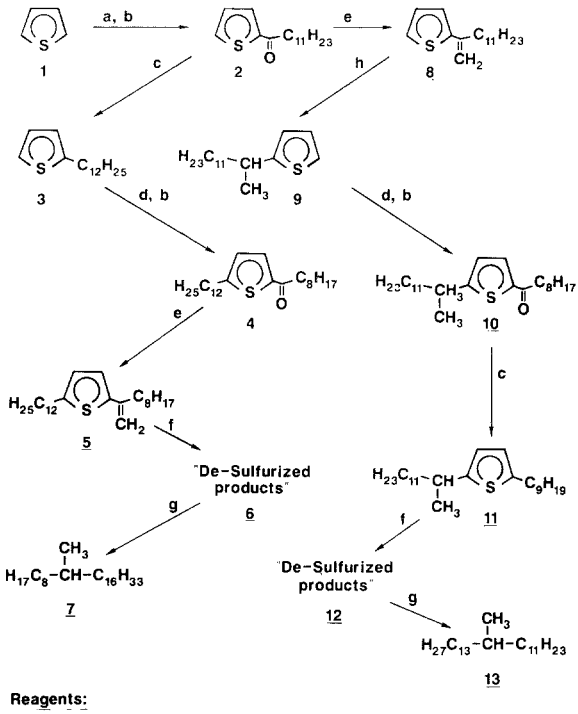
Gas Chromatography–Mass Spectrometry. These analyses were performed on a Hewlett-Packard model 5790 GC fitted with a 12.5-m methylsilicone fused silica capillary column with the exit inserted directly into the ionizing chamber of a Finnigan MAT model 1125 mass spectrometer. The transfer line from the gas chromatograph to the spectrometer was at 320°C. Ionizing voltage was 70 eV. The GC was programmed with a 2-min initial isothermal hold at 60°C, then heated to 150°C at 28°/min, then from 150 to 325°C at 3°/min using helium as carrier gas at 1 ml/min. An MS scan was made very 4.8 sec. Data acquisition and storage were made with a PDP 11/34 RSX-11 3.2 version computer dedicated to the spectrometer.

Synthesis. The procedures for synthesis of thiophene intermediates and methyl alkanes via Raney nickel desulfurization have been reported earlier (Pomonis et al., 1976a, b, 1978, 1980) and have not been modified significantly. Infrared and proton magnetic resonance spectra were in agreement with those previously reported (Pomonis et al., 1976a).

Carbon-13 Magnetic Resonance. The spectra were determined in $CDCl_3$ as 15% solutions with chemical shifts assigned relative to added tetramethylsilane (TMS) using a Jeol FX90Q magnetic resonance spectrometer at 22.5 MHz. Frequency assignments to carbons were calculated from the Lindeman and Adams (1971) formula shown in Levy and Nelson (1972).

RESULTS

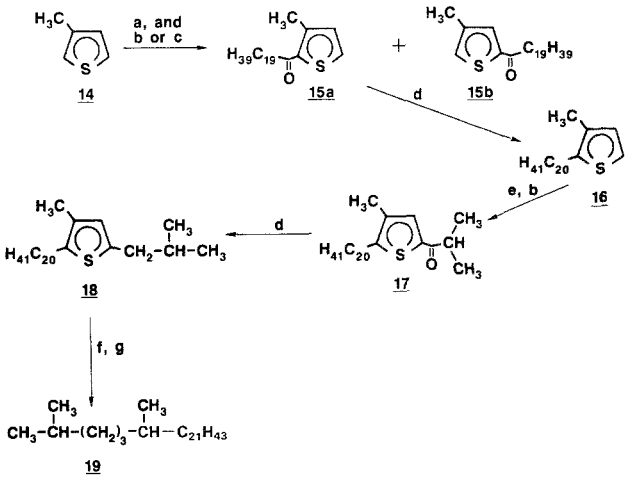
Synthesis. Synthesis of two of these methylpentacosanes is shown in Scheme 1. Friedel-Craft acylation of thiophene **(1)** with dodecanoyl chloride in benzene as solvent using stannic chloride as the Lewis acid gave 2-dodecanoylthiophene **(2)** in 82% yield. Modified Wolff-Kishner reduction of **2** gave the 2-dodecylthiophene **(3)** in 78% yield. Further acylation of **3** with nonanoyl chloride and stannic chloride gave **4** in near quantitative yield. Subsequent reaction of **4** with methyl Wittig reagent yielded the methylidine **5** (71% yield),



SCHEME 1.

which was desulfurized with Raney nickel and hydrogenated over palladium–carbon catalyst to give 9-methylpentacosane **(7)** in 61% yield from **5.** Compound **2** was also used to synthesize **13.** Methyl Wittig reagent, when reacted with **2,** gave **8** in 83% yield. To avoid desulfurization and/or poisoning of the heterogeneous catalyst during reduction of the methylidene double bond, the homogeneous catalyst Tris-triphenylchlororhodium (Osborn et al., 1966; Harmon et al., 1969) was used to synthesize **9** from **8** (61%). Friedel-Crafts acylation, Wolff-Kischner reduction, desulfurization, and catalytic reduction (Pd/C) of **9** yielded **13** (**10,** 99%; **11,** 65%; **13,** 89%).

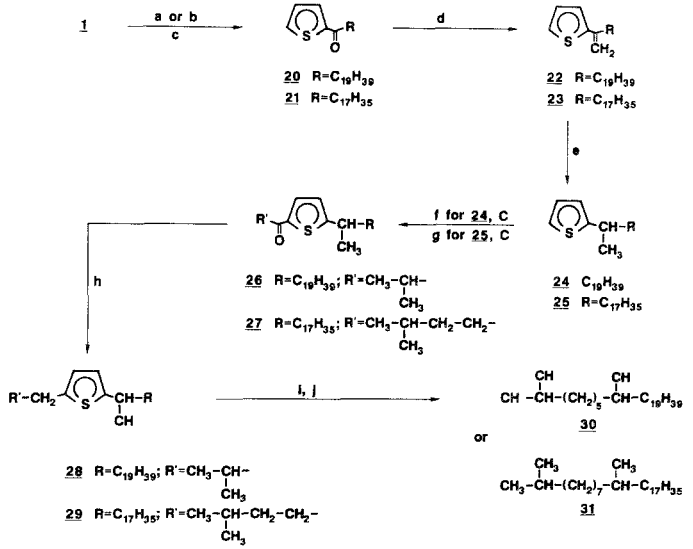
The 2,X-dimethylheptacosanes were synthesized as outlined in Schemes 2 and 3. Acylation of 3-methylthiophene **(14)** with eicosanoyl chloride gave a mixture of varying proportions of the two possible isomers: 2-acyl-3-methylthiophene **(15a)** and the 5-acyl-3-methylthiophene **(15b).** The ratio of 2-acyl to 5-acyl product was controlled by the type of Lewis acid used. Thus, when



Reagents:

(a) $C_{19}H_{39}COCl$; (b) $SnCl_4$/benzene; (C) $AlCl_3/CS_2$; (d) H_2NNH_2, Diethylene glycol, KOH; (e) $CH_3-CH(CH_3)-COCl$; (f) Raney Ni; (g) $Pd/C/H_2/CH_3CH_2OH$

SCHEME 2.



Reagents:

(a) $C_{19}H_{39}COCl$; (b) $C_{17}H_{35}COCl$; (c) $SnCl_4$, benzene; (d) BuLi, $Et_2O, \Phi_3PCH_3^+ Br^-$; (e) $[(\Phi)_3P]_3ClRh$, H_2, CH_3CH_2OH: benzene; (f) $CH_3CH(CH_3)COCl$; (g) $CH_3CHCH_2CH_2COCl$; (h) H_2NNH_2, diethylene glycol, KOH; (i) Raney-Ni/benzene; (j) Pd/C, H_2, CH_3CH_2OH

SCHEME 3.

aluminum chloride was used, greater than 98 : 1 yield of **15a : 15b** was obtained, while a ratio of 66 : 44 **(15a : 15b)** was obtained when stannic chloride was employed. The two isomers were easily separated by alumina column chromatography. Compound **15a** was reduced to **16** by the Wolff-Kischner reaction (73%) and then acylated with 3-methylpropanoyl chloride and stannic chloride to give **17** (90%). Reduction of **17** to **18** (80%) followed by desulfurization and hydrogenation provided the 2,6-dimethylheptacosane (**19,** 90%). Thiophene **(1)** was acylated with either eicosanoyl or octadecanoyl chloride and stannic chloride to provide **20** (99%) or **21** (Scheme 3). Compound **20** was purified by column chromatography through alumina. These acylthiophenes were reacted with methyl Wittig reagent to provide either **22** (62%) or **23,** and the methylidene double bond was subsequently reduced in a hydrogen atmosphere with homogeneous catalyst to prevent premature ring desulfurization to give **24** (92%) or **25.** Compounds **24** and **25** were acylated with 2-methylpropanoyl chloride and 4-methylpentanoyl chloride to give **26** (99%) and **27,** respectively, which were then reduced to give **28** (79%) and **29.** Raney nickel desulfurization of the dialkylthiophenes **28** and **29** followed by catalytic reduction with hydrogen over palladium on charcoal gave 2,8- and 2,10-dimethylheptacosane (**30** and

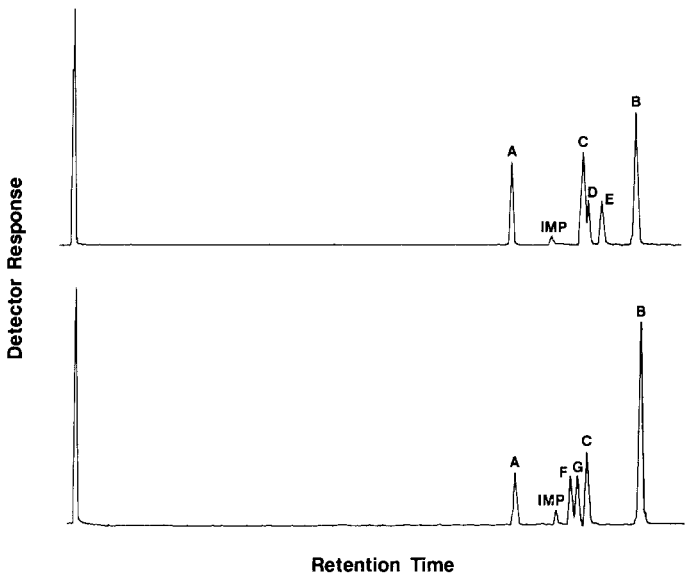


FIG. 1. Top: Capillary GC trace of A: n-$C_{25}H_{52}$ (KI 2500); B: n-$C_{26}H_{54}$ (KI 2600); C: 4-methylpentacosane (KI 2557); D: 2-methylpentacosane (KI 2562); E: 3-methylpentacosane (KI 2574). Isothermal at 210°C, 12.5-m methylsilicone fused silica column. Bottom: Capillary GC trace of A: n-$C_{25}H_{52}$; B: n-$C_{26}H_{54}$; C: 4-methylpentacosane; F: 6-methylpentacosane (KI 2544); G: 5-methylpentacosane (KI 2549). Isothermal at 210°C, 12.5-m methylsilicone fused silica column.

TABLE 1. KOVATS INDICES (KI) OF ALL POSSIBLE MONOMETHYLPENTACOSANES[a], SOME INTERNALLY BRANCHED DIMETHYLALKANES,[b,c] AND SOME 2,X-DIMETHYLHEPTACOSANES[b]

Compound	KI
3-Methylpentacosane	2574
2-Methylpentacosane	2562
4-Methylpentacosane	2557
5-Methylpentacosane	2549
6-Methylpentacosane	2544
7-Methylpentacosane	2541
8-Methylpentacosane	2538
9-Methylpentacosane	2536
10-Methylpentacosane	2534
11-Methylpentacosane	2533
12-Methylpentacosane	2532
13-Methylpentacosane	2532
9,11-Dimethylheptacosane	2773
8,14-Dimethylheptacosane	2771
9,14-Dimethylheptacosane	2768
9,13-Dimethylheptacosane	2765
2,6-Dimethylheptacosane	2808
2,8-Dimethylheptacosane	2802
2,10-Dimethylheptacosane	2802
2,12-Dimethylheptacosane	2794
2,14-Dimethylheptacosane	2794
3,11-Dimethylnonacosane	3006
10,14-Dimethyltriacontane	3061
13,17-Dimethylheptatriacontane	3752
15,19-Dimethylpentatriacontane	3551

[a] Isothermal 210°C, 12.5-m methylsilicone (MeSi) fused silica capillary column.
[b] Isothermal 225°C, 12.5-m MeSi fused silica capillary column.
[c] Pomonis et al. (1980).

31, 88%) respectively. The 2,12- and 2,14-dimethylheptacosanes were similarly prepared.

Gas Chromatography. Each of the methylpentacosanes were gas chromatographed using various parameters on capillary columns to determine the best conditions for optimum resolution of isomers. The Trennzahl number (separation number) for these compounds was determined also using various chromatographic conditions. Chromatograms of mixtures of groups of three methylalkanes are shown in Figure 1. The best separation for this series was achieved on a 12-m × 0.22-mm-ID column with 1.0 or 0.33 μm film thickness at 210°C isothermal using helium as carrier gas at 30 cm/sec. The KI values of all methylalkanes that were determined in this study are listed in Table 1.

TABLE 2. CARBON-13 CHEMICAL SHIFTS (PPM) OF ALL POSSIBLE MONOMETHYLPENTACOSANES[a]

	C_1[b]	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9
2-Methylpentacosane	22.62	31.98[c]	39.10	27.98	29.35	29.74	29.74	29.74	29.74
3-Methylpentacosane	11.40	29.35	34.42[c]	36.66	27.10	30.03	29.74	29.74	29.74
4-Methylpentacosane	14.43	20.18	39.49	32.57[c]	37.15	27.10	30.13	29.74	29.74
5-Methylpentacosane	14.14	23.11	29.35	36.86	32.76[c]	37.15	27.11	30.03	29.74
6-Methylpentacosane	14.14	22.82	32.86	26.91	37.25	32.74[c]	37.25	27.21	30.13
7-Methylpentacosane	14.13	22.72	31.98	29.74	27.11	37.15	32.76[c]	37.15	27.11
8-Methylpentacosane	14.14	22.82	32.08	29.55	30.13	27.21	37.25	32.86[c]	37.25
9-Methylpentacosane	14.14	22.82	32.06	29.45	29.83	30.13	27.21	37.25	32.86[c]
10-Methylpentacosane	14.15	22.73	31.99	29.46	29.75	29.75	30.04	27.12	37.16
11-Methylpentacosane	14.14	22.72	31.98	29.35	29.74	29.74	29.74	30.03	27.11
12-Methylpentacosane	14.06	22.74	31.91	29.38	29.77	29.77	29.77	29.77	30.06
13-Methylpentacosane	14.04	22.72	31.98	29.35	29.74	29.74	29.74	29.74	29.74

The KI values for the 2,X-dimethylheptacosanes show a trend toward decreasing values as the number of methylene groups between the methyl branches increases.

Carbon-13 Nuclear Magnetic Resonance Spectrometry ([^{13}C]NMR). The NMR spectra of all 12 methylpentacosanes as well as the five 2,X-dimethylheptacosanes were experimentally determined and the chemical shifts are listed in Tables 2 and 3, respectively. The tertiary carbon that bears the branched methyl group in the straight-chain portion has, as predicted by calculation (Levy and Nelson, 1972), a constant chemical shift for all possible isomers between 5- and 13-methylpentacosane. However, the tertiary carbons of 2-, 3-, or 4-methylpentacosane have chemical shifts that are significantly different from the more internally located tertiary carbons of the remaining isomers. This difference reflects the influence of the terminal methyl group at position 1. The branched methyl group, which is carbon-26 in all of the methylpentacosanes (Table 2), also has a fairly constant chemical shift in all possible isomers except for 2-methylpentacosane, in which the two ultimate methyl groups are equivalent. The chemical shift for carbon-26 (in 3-methylpentacosane also reflects the effect of the proximal carbons at positions 2 and 3.

Mass Spectrometry. The synthesis of the complete series of methylpentacosanes made compounds available for mass spectrometric fragmentation studies. These reactions were consistent with the well-known patterns of electron-impact-induced fragmentations for natural or synthetic methylalkanes (McCarthy et al. 1968; Pomonis et al., 1978, 1980). The spectra are not included in this paper.

TABLE 2. Continued

C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17-21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}^{d}
29.74	29.74	29.74	29.74	29.74	29.74	29.74	29.74	29.35	31.98	22.62	14.04	22.62
29.74	29.74	29.74	29.74	29.74	29.74	29.74	29.74	29.35	31.88	22.72	14.04	19.20
29.74	29.74	29.74	29.74	29.74	29.74	29.74	29.74	29.35	31.98	22.72	14.14	19.69
29.74	29.74	29.74	29.74	29.74	29.74	29.74	29.74	29.35	31.98	22.72	14.14	19.69
29.83	29.83	29.83	29.83	29.83	29.83	29.83	29.83	29.44	32.08	22.82	14.14	19.79
30.03	29.74	29.74	29.74	29.74	29.74	29.74	29.74	29.35	31.98	22.72	14.13	19.70
27.21	30.13	29.84	29.84	29.84	29.84	29.84	29.84	29.55	32.08	22.82	14.14	19.79
37.25	27.21	30.13	29.83	29.83	29.83	29.83	29.83	29.45	32.06	22.82	14.14	19.79
32.77[c]	37.16	27.12	30.04	29.75	29.75	29.75	29.75	29.46	31.99	22.73	14.15	19.70
37.15	32.76[c]	37.15	27.11	30.03	29.74	29.74	29.74	29.35	31.98	22.72	14.14	19.70
27.13	37.08	32.79[c]	37.08	27.13	30.06	29.77	29.77	29.38	31.91	22.74	14.06	19.72
30.03	27.11	37.15	32.76[c]	37.15	27.11	30.03	29.74	29.35	31.98	22.72	14.04	19.70

[a] 15% solutions in $CDCl_3$.
[b] Carbon number of pentacosane backbone.
[c] Denotes the carbon carrying the methyl substituent.
[d] Carbon number of the methyl substituent.

The mass spectra of the 2,X-dimethylheptacosanes (Figures 2 and 3) reveal that a pair of peaks that are characteristic of fragmentation reactions typical of the more frequently encountered dimethylalkane compounds are missing. The spectra appear to be those of methylalkanes whose gas chromatographic KI values are about 40 units lower than those expected, i.e., very near the KI value of the *n*-alkane of one methylene unit less than the molecular ion mass of the experimental value.

DISCUSSION

Chromatography. In a companion paper (Pomonis, 1989) we described the isolation and characterization of a large number of hydrocarbons from the screwworm fly. We also attempted to quantify the individual hydrocarbons with some success. However, it is not always possible to separate an individual hydrocarbon component from all of its companion isomers even with capillary GC. Quantification of some individual hydrocarbons may therefore become difficult or impossible, causing ambiguity to be introduced; that is, only the mass of the mixture of hydrocarbons is represented by the chromatographic peak and measured.

The separation of two isomers on a GC column is a function of the col-

TABLE 3. CARBON-13 CHEMICAL SHIFTS (PPM) OF SOME SELECTED 2,X-DIMETHYLHEPTACOSANES[a]

	1	2[b]	3	4	5	6	7	8	9
2,6-dimethylheptacosane	23.01	28.27	39.69	25.15	37.63	33.06[c]	37.44	27.40	30.32
2,8-dimethylheptacosane	22.72	27.99	39.10	27.11	30.33	27.50	37.15	32.76[c]	37.15
2,10-dimethylheptacosane	22.62	27.98	39.10	27.11	30.03	29.74	30.03	27.11	37.15
	1	2	3	4	5	6	7-8	9	10
2,12-dimethylheptacosane	22.72	28.08	39.20	27.20	30.03	29.83	29.83	30.03	27.49
	1	2	3	4	5	6	7-10	11	12
2,14-dimethylheptacosane	22.62	27.98	39.10	27.10	30.05	29.93	29.93	30.05	27.40

umnn's separation number (n_{sep}) also known as the Trennzahl number, TZ (Ettre, 1977; Grob, 1977; Jennings, 1980). The TZ number is a measure of the possible number of resolved peaks between peaks of two members of a homologous series differing by one CH_2 unit. The TZ is inversely related to temperature, i.e., the higher separation numbers occur at lower temperatures. Thus, at lower temperatures, the peaks are separated by greater distances, but there is a point beyond which peak shape and resolution suffer. This implies that for efficient separation of moderate to higher molecular weight hydrocarbons at lower temperatures and large retention coefficients, inordinately long analysis times are required.

Our column, under optimized conditions, gave a TZ = 34 for separation of n-$C_{25}H_{52}$ and n-$C_{26}H_{54}$ according to the equation shown (Jennings, 1980):

$$TZ = [t_{R(n+1)} - t_{R(n)}/w_{0.5n} + w_{0.5n+1}] - 1. \quad (1)$$

It is possible to calculate the separation number necessary to separate two compounds whose KIs are known and which fall between the two n-paraffins (Jennings, 1980). The TZ is related to KI by the equation:

$$TZ = [100/(KI_2 - KI_1)] - 1 \quad (2)$$

thus for 4-methylpentacosane (KI 2557) and 2-methylpentacosane (KI 2562) at 210°C isothermal on a 12.5-m capillary column, the relationship becomes:

$$TZ = [100/(2562 - 2557)] - 1 = 19$$

Thus, the efficiency of the column has to be 19 or greater to resolve the two isomers. An example of this separation is shown in Figure 1 and includes a

TABLE 3. Continued

10	11	12	13	14-22	23	24	25	26	27	28[d]	29[e]
30.03	30.03	30.03	30.03	30.03	30.03	29.74	32.28	23.01	14.43	23.01	19.89
27.50	30.03	29.74	29.74	29.74	29.74	29.35	31.98	22.72	14.14	22.72	19.70
32.76[c]	37.15	27.11	27.50	29.74	29.74	29.44	31.98	22.62	14.14	22.62	19.70
11	12	13	14	15	16-23	24	25	26	27	28[d]	29[e]
37.25	32.86[c]	37.25	27.49	30.03	29.83	29.74	32.08	22.72	14.14	22.72	19.80
13	14	15	16	17	18-23	24	25	26	27	28[d]	29[e]
37.05	32.76[c]	37.05	27.40	30.05	29.93	29.74	31.59	22.62	14.04	22.62	19.69

[a] 15% solutions in $CDCl_3$.
[b] Carbon bearing 1st methyl substituent (constant).
[c] Carbon bearing 2nd methyl substituent (variable).
[d] Carbon number of methylsubstituent on carbon 2.
[e] Carbon number of methyl substituent on variable carbon number.

third isomer, 3-methylpentacosane. Other factors that improve column efficiency are (1) increasing the linear flow velocity with use of hydrogen in preference to helium as carrier gas since hydrogen has a flatter van Deemter curve and (2) increasing the length of the column since resolution is a square root function of the column length (Jennings, 1980). However, increasing the column length to 50 m (4 × 12.5) only doubles the resolution ($R_s = 4^{1/2} = 2$). It also increases the analysis time and, under isothermal conditions, causes peak broadening. From equation 2 and from the KI values (Table 1) for various methylpentacosanes, it is seen that in order to separate some isomers a TZ = 99 (10-methyl- vs. 11-methylpentacosane) would be required. However, 10-methylpentacosane may more easily be separated from 4-methylpentacosane (TZ = 3.4).

From the foregoing argument, it is seen that the analyst who is dealing with a mixture of homologous hydrocarbons composed of several isomeric series such as those isolated from the insect surface lipids faces a formidable task if it is desired to separate the mixture into its individual component parts.

Magnetic Resonance Spectrometry. Isotope-labeled precursors such as malonic, 2-methylmalonic, propionic, and succinic as well as tetracosanoic acids have been used in studies to determine the metabolic origin of the methyl branches in insect hydrocarbons (Blomquist et al., 1980; Dwyer et al., 1981; Dillwith et al., 1982; Pomonis and Hakk, 1987). When radiolabel is biochemically incorporated in a molecule, the position of that label in the product is

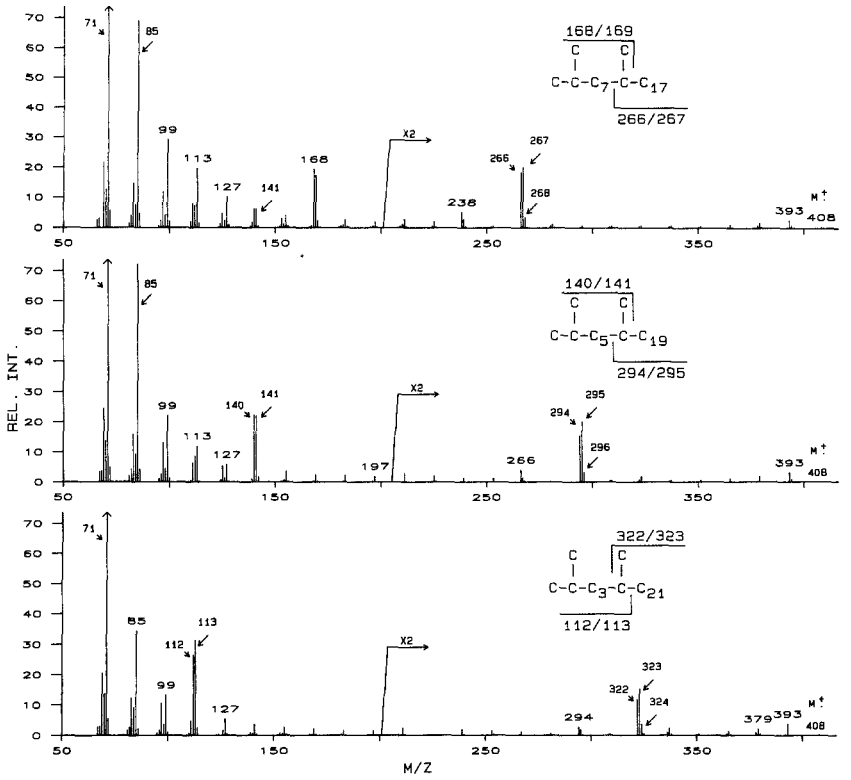


FIG. 2. EI mass spectra of synthetic compounds. Top: 2,10-dimethyl heptacosane (KI 2802). Middle: 2,8-dimethylheptacosane (KI 2802). Bottom: 2,6-dimethylheptacosane (KI 2808).

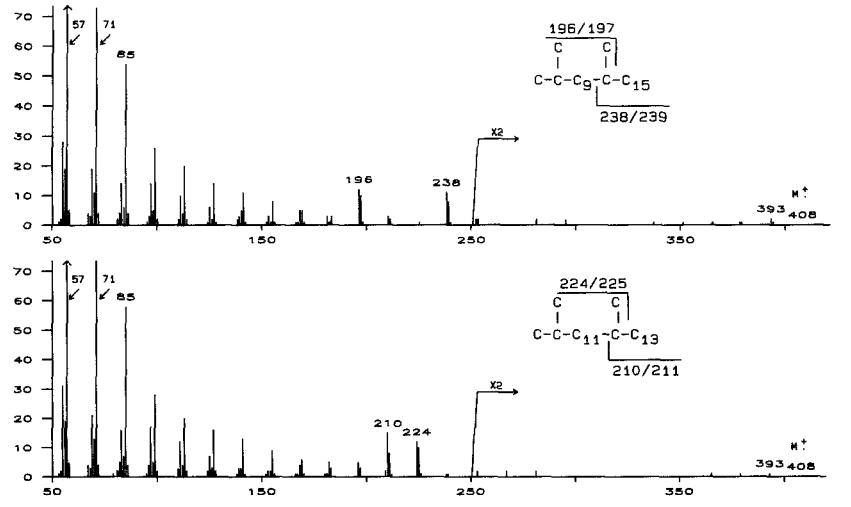


FIG. 3. EI mass spectra of synthetic compounds. Top: 2,12-dimethylheptacosane (KI 2794). Bottom: 2,14-dimethylheptacosane (KI 2794).

often determined by tedious and meticulous oxidative degradation. Alkanes are resistant to oxidative reactions, and the products of the oxidation are those of randomized cleavages leading to ambiguity as to the position of the label. However, ^{13}C-labeling allows the investigator to use nondestructive [^{13}C]NMR spectrometry to precisely observe the position of the incorporated label in the metabolic product. For example, [^{13}C]NMR analysis of the isolated hydrocarbon fractions from housefly surface lipids showed that the 2-methyl group in 2-[^{13}C]methylmalonic acid was the origin of the methyl branch in internally branched methylalkanes by isotope enrichment (Dillwith et al., 1982). The synthesis of all 12 methylpentacosanes permitted a systematic assignment of ^{13}C chemical shift values for each carbon in the molecules, with the experimental values (Table 2) being in very good agreement with the calculated values computed from an equation taken from Levy and Nelson (1972). A few of these experimentally determined chemical shift values were used in locating the position of the incorporated label (enrichment) in biosynthetic studies in the termite (Blomquist et al., 1980) and housefly (Dillwith et al., 1982).

Mass Spectra. In the companion paper (Pomonis, 1989) are described the isolation and characterization of a number of alkanes from the screwworm fly adult. Of special interest is the report of the characterization of 2,X- and 3,X-dimethylalkanes. The 2,X-dimethylalkanes are a new catagory of insect alkanes and were reported for the first time. Characterization of this group of alkanes was difficult because (1) they were present in very low concentration; (2) they were easily masked, chromatographically, under the *n*-alkane peak; and (3) most confounding of all, when all *n*-alkanes were removed by molecular sieving, two chromatographic peaks gave the same mass spectra. The unknown compounds were represented by a peak that chromatographed with a nominal KI of 2900 but presented mass spectra nearly identical to methylnonacosanes ($C_{30}H_{62}$), which are included in a peak with a nominal KI 2935 (Pomonis, 1989).

After considering several possible structures, the 2,X-dimethylalkane alternative seemed to fit the data but did not adhere to one of the several useful empirical rules of fragmentation for dimethylalkanes under electron impact (McCarthy et al., 1968; Nelson et al., 1972; Pomonis et al., 1980). Missing were those characteristically prominent single masses due to cleavage yielding fragment ions that include both methyl groups (Pomonis et al., 1980) and those due to loss of C_3H_7 (M-43). To test the 2,X-dimethylalkane hypothesis, several 2,X-dimethylheptacosanes were synthesized and the MS that were determined are shown in Figures 2 and 3. The fragmentation reactions of the synthetic compounds supported our earlier interpretations and assignment of structures as the 2,X-dimethylalkanes.

We have synthesized a number of 2,X-dimethylheptacosanes and determined and reported their mass spectra, [^{13}C]NMR, as well as their chromatographic retention indices (KI). There data corroborate the structures of a number of previously unreported compounds isolated from the screwworm fly (Pomonis,

1988). We also reported the synthesis, some spectral properties, and KI of the possible monomethylpentacosanes. The relationship of these physical properties to those of a few other methyl alkanes were compared and discussed.

REFERENCES

BLOMQUIST, G.J., and DILLWITH, J.W. 1985. Cuticular lipids, pp. 117–154, *in* G.A. Kerkut and L.I. Gilbert (eds.). Comprehensive Physiology, Biochemistry and Pharmacology, Vol. 3. Pergamon Press, Oxford.

BLOMQUIST, G.J., CHU, A.J., NELSON, J.H., and POMONIS, J.G. 1980. Incorporation of [2,3-^{13}C]succinate into methyl-branched alkanes in a termite. *Arch. Biochem. Biophys.* 204:648–650.

BLOMQUIST, G.J., NELSON, D.R., and DE RENOBALES, M. 1987. Chemistry, biochemistry and physiology of insect cuticular lipids. *Arch. Insect Biochem. Physiol.* 6:227–265.

CERNY, R.L., POMONIS, J.G., FATLAND, C.F., and NELSON, D.R. 1986. The determination of aliphatic branch points by tandem mass spectrometry. Proceedings American Society of Mass Spectrometry 33rd Annual Congress, Cincinnati, OH. pp. 913–914.

DILLWITH, J.W., NELSON, J.H., POMONIS, J.G., NELSON, D.R., and BLOMQUIST, G.J. 1982. A ^{13}C NMR study of methyl-branched hydrocarbon biosynthesis in the housefly. *J. Biol. Chem.* 257:11305–11314.

DWYER, L.A., BLOMQUIST, G.J., NELSON, J.H., and POMONIS, J.G. 1981. A ^{13}C-NMR study of the biosynthesis of 3-methylpentacosane in the American cockroach. *Biochim. Biophys. Acta* 663:536–544.

ETTRE, L.S. 1977. Basic Relationships of Gas Chromatography. Perkin-Elmer Corp. Norwalk, Connecticut. iv + 65 pp.

GROB, R.L. 1977. Modern Practice of Gas Chromatography. John Wiley & Sons, New York xvi + 654 pp.

HARMON, R.E., PARSONS, J.L., COOKE, D.W., GUPTA, S.K., and SCHOOLENBERG, J. 1969. Homogeneous catalytic hydrogenation of unsaturated organic compounds. *J. Org. Chem.* 34:3684–3685.

HOWARD, R.W., and BLOMQUIST, G.J. 1982. Chemical ecology and biochemistry of insect hydrocarbons. *Annu. Rev. Entomol.* 27:149–172.

JENNINGS, W. 1980. Gas Chromatography with Glass Capillary Columns. Academic Press. New York. x + 320 pp.

KOVATS, E. 1965. Gas chromatographic characterization of organic substances and the retention index system. *Adv. Chromatogr.* 1:229–247.

LEVY, G.C., and NELSON, G.L. 1972. Carbon-13 Nuclear Magnetic Resonance for Organic Chemists. Wiley-Interscience, New York. xiii + 222 pp.

LINDEMAN, L.P., and ADAMS, J.Q. 1971. Carbon-13 nuclear magnetic resonance spectrometry chemical shifts for the paraffins through C_9. *Anal. Chem.* 43:1245–1252.

LOCKEY, K.H. 1985. Minireview. Insect cuticular lipids. *Comp. Biochem. Physiol.* 81B:263–273.

LOCKEY, K.H. 1988. Review. Lipids of the insect cuticle: origin, composition and function. *Comp. Biochem. Physiol.* 89B:595–645.

MCCARTHY, E.D., HAN, J., and CALVIN, M. 1968. Hydrogen atom transfer in mass spectrometric fragmentation patterns of saturated aliphatic hydrocarbons. *Anal. Chem.* 10:1475–1480.

NELSON, D.R., and SUKKESTAD, D.R. 1970. Normal and branched aliphatic hydrocarbons from the tobacco hornworm. *Biochemistry* 9:4601–4611.

NELSON, D.R., SUKKESTAD, D.R., and ZAYLSKIE, R.G. 1972. Mass spectra of methyl-branched hydrocarbons from eggs of the tobacco hornworm. *J. Lipid Res.* 13:413–421.

OSBORN, J.A., JARDINE, F.H., YOUNG, J.F., and WILKINSON, G. 1966. The preparation and properties of tris(triphenylphosphine)halegenorhodium(I) and some reactions thereof including catalytic homogeneous hydrogenation of olefins and acetylenes and their derivatives. *J. Chem. Soc.* (A) 1966:1711–1732.

POMONIS, J.G. 1989. Cuticular hydrocarbons of the screwworm, *Cochliomyia hominivorax* (Diptera: Calliphoridae). Isolation, identification and quantification as a function of age, sex and irradiation. *J. Chem. Ecol.* 1989.

POMONIS, J.G., and HAKK, H. 1987. Synthesis of methyl[2-^{13}C-2,2-d_2]- and [3-^{13}C-3,3-d_2]tetradecanoate. *J. Labelled Comp. Pharm.* 24:1077–1085.

POMONIS, J.G., FATLAND, C.F., and TAYLOR, F.R. 1976a. Mono-, di-, and trisubstituted acyl and alkyl thiophenes I. Synthesis, ultraviolet and proton magnetic resonance spectra. *J. Chem. Eng. Data* 21:233–236.

POMONIS, J.G., FATLAND, C.F., and ZAYLSKIE, R.G. 1976b. Mono-, di-, and trisubstituted acyl- and alkylthiophenes. 2. Mass spectrometry. *J Chem. Eng. Data* 21:380–385.

POMONIS, J.G., FATLAND C.F., NELSON, D.R., and ZAYLSKIE, R.G. 1978. Insect hydrocarbons. Corroboration of structure by synthesis and mass spectrometry of mono- and dimethylalkanes. *J. Chem. Ecol.* 4:27–39.

POMONIS, J.G., NELSON, D.R., and FATLAND, C.F. 1980. Insect hydrocarbons 2. Mass spectra of dimethylalkanes and the effect of the number of methylene units between methyl groups on fragmentation. *J. Chem. Ecol.* 6:965–972.