Cuticular Hydrocarbons of the Colorado Beetle
Leptinotarsa decemlineata say

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The cuticular hydrocarbons of the Colorado beetle Leptinotarsa decemlineata say consist of 2-methylalkanes, internally branched monomethylalkanes, dimethyl-, trimethyl- and tetramethyl-alkanes. The hydrocarbons differ from those of most of the other insects so far investigated. The major components were found to be the dimethyl branched alkanes, 2,6- and/or 7,22-dimethyl-octacosane and the tetramethylalkane, 3,10,16,23-tetramethyltritriacontane. The hydrocarbons identified are members of homologous series which differ by two methylene groups. The identification of hydrocarbons was achieved by mass spectrometry and gas chromatographic determination of Kovats retention indices on two high efficiency capillary columns coated with Dexsil 300 and OV-1.

1. Introduction

Hydrocarbons constitute a major component of insect surface waxes [1]. The surface lipid layers of insects have important physiological functions e.g.

1. restricting water movement through the cuticle to prevent desiccation of the insects,
2. protecting the insects against being infected by microorganisms and
3. may play an important role in chemical communication. The interest in insect hydrocarbons increased significantly when differences were observed in them with ageing and between sexes [2, 3]. Thus, at least some of the hydrocarbons have semiochemical functions [4, 5]. The semiochemical functions include sex attractants and aphrodisiacs, species and cast recognition cues, territory marking, recruitment and alarm pheromones, defense secretions and kairomones [6].

Many defense secretions of insects contain paraffinic and olefinic terpenes [7].

Complex mixtures of normal and methyl branched alkanes have been reported in many insects. Among them, mono-, di- and trimethylalkanes with terminal (position 2 or 3) and internal branching have been reported [8, 9].

Unlike many thoroughly investigated insects, little has been done in studying the common potato vermin Colorado beetle. In this work we present the results of the study of Colorado beetle surface lipids by gas chromatography and gas chromatography-mass spectrometry.

2. Results

The epicuticular hydrocarbons amount to 1 mg/insect which adds up to 13% of total lipid extract. But it seems that methylene chloride might be penetrating inside the insect and extract triglycerides, too. Thus, the figures for lipids might not be representative for cuticular layers alone. According to L. L. Jackson [10] the ratio of epicuticular hydrocarbons to internal hydrocarbons was 28:1.

Chromatographic separations of hydrocarbons (Fig. 1) indicate the presence of more than 30 hydrocarbons in the range of 2700 to 4000 units of Kovats retention indices. The mass fingerprinting by field desorption mass spectrometry (Fig. 2) reveals hydrocarbons with carbon numbers ranging from C_{28} to C_{30}. The major components consist of 29 (11%), 30 (25%), 31 (10.5%), 32 (12.5%) and 37 (12.5%) carbon numbers. As no n-alkanes were found by coinjection of the standards, the mixture seems to be composed solely of branched hydrocarbons.

The structural assignment of the individual hydrocarbons was made on the basis of mass spectrometry (GC-MS), Kovats retention indices and their differences on liquid phases Dexsil 300 and OV-1. The four homologous series of branched chain alkanes

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found in Colorado beetle surface layer were distinguished by the following features:

- For homologous series of 2-methylalkanes, Kovats retention indices are decreased by 40 units relatively to their straight chain counterparts. Their retention indices are little dependent on temperature.
- The order of elution of monobranched hydrocarbons is 5-, 4-, 2- and 3-methyl isomers [11].
- The internally branched dimethylalkanes have

7-Methylalkanes elute 70 units earlier than \( n \)-alkanes of the same molecular weight. It appears, that beyond 7-methyl substitution of the chain, the retention index differences of isomeric methylhydrocarbons are getting lower, which makes the separation extremely difficult. The differences of retention indices of all monomethyl alkanes on Dexsil 300 and OV-1 are 4 units.

- The internally branched dimethylalkanes have
retention indices of about 100 units lower than \( n \)-alkanes of the same total number of carbon atoms on a Dexsil 300 column.

- In turn the internally branched trimethylalkanes have Kovats indices 150 units lower than \( n \)-alkanes. The differences between retention indices on two liquid phases amount to \( ca. \) 11 units. This can distinguish monomethylalkanes from trimethylalkanes when those incidentally interfere.

- \( Ca. \) 200 units shift corresponds to tetramethylalkanes with 14 units difference between both phases. This is consistent with the literature data.

Table 1. Kovats retention indices of cuticular hydrocarbons of Colorado beetle on Dexsil 300 and OV-1 liquid phases.

<table>
<thead>
<tr>
<th>Peak number</th>
<th>Retention index on Dexsil 300</th>
<th>Retention index on OV-1</th>
<th>Relative intensity</th>
<th>Structure</th>
<th>Total carbon number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>2692.5</td>
<td>2700.0</td>
<td>0.63</td>
<td>11,14-dimethylhexacosane</td>
<td>C_{28}</td>
</tr>
<tr>
<td>2.</td>
<td>2700.0</td>
<td>2704.2</td>
<td>1.45</td>
<td>7,20- and/or 2,6-dimethylhexacosane</td>
<td>C_{28}</td>
</tr>
<tr>
<td>3.</td>
<td>2832.1</td>
<td>2836.1</td>
<td>0.73</td>
<td>10- and 11-methylloctacosane</td>
<td>C_{29}</td>
</tr>
<tr>
<td>4.</td>
<td>2860.2</td>
<td>2864.3</td>
<td>5.48</td>
<td>2-methyloctacosane</td>
<td>C_{29}</td>
</tr>
<tr>
<td>5.</td>
<td>2892.2</td>
<td>2899.2</td>
<td>6.90</td>
<td>11,18- and/or 2,18-dimethylloctacosane</td>
<td>C_{30}</td>
</tr>
<tr>
<td>6.</td>
<td>2900.0</td>
<td>2905.3</td>
<td>20.20</td>
<td>7,22- and/or 2,6-dimethylloctacosane</td>
<td>C_{30}</td>
</tr>
<tr>
<td>7.</td>
<td>2918.0</td>
<td>-</td>
<td>2.60</td>
<td>2,12,18-trimethylloctacosane</td>
<td>C_{31}</td>
</tr>
<tr>
<td>8.</td>
<td>2920.5</td>
<td>2929.3</td>
<td>4.40</td>
<td>7,10,18- and 7,12,18-trimethylloctacosane</td>
<td>C_{31}</td>
</tr>
<tr>
<td>9.</td>
<td>2931.3</td>
<td>2934.5</td>
<td>3.16</td>
<td>11-methylnonacosane</td>
<td>C_{30}</td>
</tr>
<tr>
<td>10.</td>
<td>2959.5</td>
<td>2966.9</td>
<td>3.26</td>
<td>2,19- and 2,12-dimethylnonacosane</td>
<td>C_{31}</td>
</tr>
<tr>
<td>11.</td>
<td>3031.1</td>
<td>3033.9</td>
<td>0.94</td>
<td>12-methyltriacontane</td>
<td>C_{31}</td>
</tr>
<tr>
<td>12.</td>
<td>3062.2</td>
<td>3065.1</td>
<td>2.30</td>
<td>2-methyltriacontane</td>
<td>C_{31}</td>
</tr>
<tr>
<td>13.</td>
<td>3091.1</td>
<td>3096.5</td>
<td>3.80</td>
<td>11,18- and/or 2,18-dimethyltriacontane</td>
<td>C_{32}</td>
</tr>
<tr>
<td>14.</td>
<td>3100.0</td>
<td>3105.2</td>
<td>5.50</td>
<td>7,24- and/or 2,6-dimethyltriacontane</td>
<td>C_{32}</td>
</tr>
<tr>
<td>15.</td>
<td>3131.1</td>
<td>3133.0</td>
<td>1.00</td>
<td>11- and 13-methylhentriacontane</td>
<td>C_{32}</td>
</tr>
<tr>
<td>16.</td>
<td>3227.4</td>
<td>3233.7</td>
<td>0.53</td>
<td>12-methyldotriacontane</td>
<td>C_{33}</td>
</tr>
<tr>
<td>17.</td>
<td>3290.6</td>
<td>3297.2</td>
<td>2.80</td>
<td>11,22- and/or 2,22-dimethyldotriacontane; 13,20- and/or 2,20-dimethyldotriacontane</td>
<td>C_{34}</td>
</tr>
<tr>
<td>18.</td>
<td>3310.6</td>
<td>3324.0</td>
<td>5.30</td>
<td>3,10,16,21-tetramethylhentriacontane</td>
<td>C_{34}</td>
</tr>
<tr>
<td>19.</td>
<td>3326.9</td>
<td>3331.7</td>
<td>1.30</td>
<td>11-, 13-, 15- and 17-methyltritriacontane</td>
<td>C_{34}</td>
</tr>
<tr>
<td>20.</td>
<td>3350.1</td>
<td>3361.1</td>
<td>3.80</td>
<td>2,10,16- and/or 2,12,16-trimethyltriacontane</td>
<td>C_{35}</td>
</tr>
<tr>
<td>21.</td>
<td>3449.3</td>
<td>-</td>
<td>2.20</td>
<td>10,16,24-trimethyltriacontane</td>
<td>C_{36}</td>
</tr>
<tr>
<td>22.</td>
<td>3510.3</td>
<td>3524.2</td>
<td>10.10</td>
<td>3,10,16,23-tetramethyltritriacontane</td>
<td>C_{37}</td>
</tr>
<tr>
<td>23.</td>
<td>3550.1</td>
<td>3560.2</td>
<td>6.60</td>
<td>2,10,18- and/or 2,12,18-trimethyltetriacontane</td>
<td>C_{37}</td>
</tr>
</tbody>
</table>
for tetramethyl branched alkanes, pristane and phytane, on an OV-1 capillary column, where \( \Delta I \) was found to be ca. 190.

The Kovats retention indices of hydrocarbons on both liquid phases, Dexsil 300 and OV-1, are included in Table I.

Group recognized hydrocarbons by gas chromatography were later positively identified by mass spectrometry. Upon electron impact branched hydrocarbons fragment by cleavage on either side of the methyl branch to give two major ions with an odd mass number \( (C_nH_{2n-1}) \) and two ions with an even mass number \( (C_nH_{2n}) \) [13, 14]. The ratio of the intensity of odd and even ions formed is controlled by the chain length and additional branching [15].

However, often some branching positions may be indistinguishable. The close similarity of the mass spectra of 2-methyloctane and 2,6-dimethylheptane is a good example [13]. Furthermore, a serious difficulty was encountered in distinguishing between asymmetrical and symmetrical substitution (Scheme 1). In this case the mass spectra may exhibit identical pairs of complementary ions only, e.g. 337 + 113, corresponding in nominal mass to \( M+28 \) and signals at \( M-15^\circ \) and \( M-43^\circ \), which reveal that 2-methyl substitution is negligible. In these cases both structures were proposed in our discussion (Fig. 3–6).

Fig. 3. GC/MS-EI (70 eV) mass spectrum of 11,8- and/or 2,18-dimethyloctacosane from cuticular wax of Colorado beetle.
Fig. 4. GC/MS-EI (70 eV) mass spectrum of 7,22- and/or 2,6-dimethyloctacosane from cuticular wax of Colorado beetle.

Fig. 5. GC/MS-EI (70 eV) mass spectrum of 3,10,16,23-tetramethyltritriacontane from cuticular wax of Colorado beetle.
Due to the difficulties in separation, some GC peaks were found to be the mixtures of closely related compounds. In such cases the retention indices were used to predict the tentative structure and molecular weight. Later the positive identifications by mass spectrometry were performed. Some discrepancy may be encountered about the identity of GC peak 22. The mass spectrum may be ascribed to a mixture of dimethylalkanes (M. W. 506) and tetramethylalkanes (M. W. 520). Kovats retention index alone on a Dexsil 300 column does not distinguish them because it may correspond to a shift of 100 or 200 units. However, we believe that this is a tetramethylalkane because of 14 units difference for Dexsil 300 and OV-1 as well as the intensive ion of m/z 520 in the FD mass spectrum of the hydrocarbon mixture.

In this way the major individual hydrocarbons were identified. They are listed in Table I.

3. Discussion

Two serious problems are encountered in the study of a cuticular hydrocarbon mixture, namely 1. achievement of a sufficient separation to be sure that a GC peak corresponds to a single component and 2. accomplishing an unambiguous structure identification. These studies should be supported by synthesis to confirm both positive identification and biological activity. The first two problems of Colorado beetle cuticular hydrocarbons are the subjects of the present study. During the study a capillary column with Dexsil 300 was used, which was earlier found to be the most efficient liquid phase for separation of isoalkane mixtures [11]. We believe this is true for the multibranched hydrocarbons as well.

The structure identification methods used in this study represent the state of the art for such complicated mixtures on a microgram scale.
The cuticular hydrocarbons of Colorado beetle considerably differ from those of most other insects so far investigated. The mixture consists of mono-, di-, tri- and tetramethylalkanes ranging from C_{28} to C_{46} without a trace of n-alkanes, which are the major constituents of plant cuticular hydrocarbon mixtures including potatoes [16]. These have been found as the common constituents of insect cuticular lipids where the odd chain length predominates. According to M. von Ardenne et al. [17] larvae of potato beetle contain hydrocarbons of surprisingly high molecular weight, particularly tetrapentacontane (C_{54}H_{110}), pentapentacontane (C_{55}H_{112}) and heptapentacontane (C_{57}H_{116}). Although the results need to be confirmed by modern methods, a conclusion can be drawn. Both larvae and adult insects do not show a structural relationship between food and native hydrocarbons, which suggests an insect biosynthetic process providing these compounds.


The most abundant components of the cuticular lipids of Colorado beetle are branched hydrocarbons of 29 (2-methyloctacosane), 30 (11,18- and/or 2,18-, 7,22- and/or 2,6-dimethyloctacosane), 32 (7,24- and/or 2,6-dimethyltriacontane) and 37 carbon atoms (3,10,16,21-tetramethyltritriacontane).

Terminally branched monomethylalkanes include two 2-methylalkanes. Similarly to literature data [8] their chains consist of an even number of carbon atoms. The additional methyl group adds up to an odd carbon number. Both 2-methylalkanes are the members of homologous series separated by two methylene groups.

Internally branched monomethylalkanes represent minor components of the hydrocarbon mixture. They have been found to be odd (C_{29}, C_{31}, C_{33}) and even (C_{30}, C_{32}, C_{34}) carbon number hydrocarbons, with even and odd carbon numbers of the main chain. The positions of branching were at even (10 or 12) and odd (11, 13, 15, 17) carbon numbers, respectively.

Positively identified dimethyl- and trimethylalkanes of the epicuticles of the potato beetle have been found to be considerably different from those ever since found in an insect. The main feature of dimethylalkanes already found in insects is the separation by 1, 3, 5, 7, 9, 11 or 13 methylene groups of the branching position but the number of carbon atoms in the chain is odd. Many dimethylalkanes show isoprenoid spacing, in which the first methyl branch is positioned at an odd carbon number while the second methyl branch occurs three methylene units further along the chain. The distance in methyl branching is consistent with the incorporation of methylmalonyl-CoA in place of a malonyl-CoA in the biosynthetic process [5]. In contrast those of Colorado beetle possess a chain with an even number of carbon atoms showing the first branch at odd or even carbon numbers and the second one at an even carbon atom, which causes the even or odd number of carbon atoms in between. This is difficult to be explained by incorporation of 3 carbon atom units by methylmalonyl-CoA in the biosynthetic process.

Some dimethylalkanes proposed in the Colorado beetle lipids reveal symmetrical positions of methyl branches e.g. 11,18-dimethyloctacosane and 7,22-dimethyloctacosane. These consist of a homologous series of C_{30} and C_{32} hydrocarbons. The structural assignment is tentative and a positive identification will be obtained from a comparison with synthetic compounds. The symmetrical compounds might be biosynthesized via head-to-head condensation.

As we mentioned before, certain dimethylalkanes could only be identified by their retention indices. For instance, the EI mass spectrum of the most abundant GC peak 6 reveals only one pair of complementary ions, 112/113 and 336/337. This might suggest 7-methylnonacosane. But the Kovats retention index of I = 2900 precisely indicates the dimethylalkane structure.

Although trimethylalkanes have not often been identified in insect lipids, we found two mixtures of homologous trimethylalkanes of 35 and 37 carbon atoms.

According to our knowledge, tetramethylalkanes have not been found so far in cuticular insect lipids. The structural proof is presented in the results section. The tetramethylalkanes identified have 35 and 37 carbon atoms and their main carbon chains are odd, too.

Many publications showed the possibility to use the composition of the hydrocarbon fraction for chemotaxonomy of insects. The highly branched long chain alkanes (Table I) seem to be a characteristic feature for this insect species.

4. Materials and Methods

Adult Colorado beetles (males and females) were collected from wild populations in northeast-
ern Poland in August 1978. Cuticular lipids were extracted by immersing the insects into methylene chloride. The total yield of lipid fraction was 1.5 g per 250 insects. Hydrocarbons were separated from the remaining lipids by eluting the extract with cyclohexane through a neutral Al₂O₃ column (75×2.5 cm). In this way 240 mg of a hydrocarbon mixture was obtained which constitutes 13% of the total lipid fraction. Hydrocarbons were separated by gas chromatography (GC) on a 40 m capillary column with Dexsil 300 [11] (0.25 mm i.d., liquid phase film thickness 0.1 μm) or on a 40 m OV-1 capillary column. The separations were carried out with a Varian Aerograph Model 1400 gas chromatograph, which had been modified for operation with a glass capillary column. The GC oven was programmed from 200 °C at 2 °C/min with a linear velocity of carrier gas (helium) of 16 cm/s (200 °C). Kovats retention indices (I) on Dexsil 300 and OV-1 columns were determined at 240 °C and 270 °C. The retention indices were measured within an error of ±1.0 unit for k’ greater than 5.

Electron impact mass spectra were recorded on a Varian MAT 311 A mass spectrometer at an ionization energy of 70 eV. The sample was introduced by GC-MS using a Dexsil 300 capillary column. The column was coupled to the mass spectrometer by an open split interface [18].

Field desorption fingerprinting of the sample was performed with a Varian MAT 711 mass spectrometer equipped with a combined FD-FI-EI source.

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