Cuticular hydrocarbons from the bed bug *Cimex lectularius* L.

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1. Subject and source

The common bed bug *Cimex lectularius* L. (Hemiptera: Cimicidae) is an obligate, blood-sucking insect that attacks humans; other members of the Cimicidae feed primarily upon bats and birds (Usinger, 1966). The chemical ecology of bed bugs is receiving interest with the idea that bed bug semiochemicals could be incorporated into detection and control strategies for this blood-sucking pest (Haynes et al., 2010; Weeks et al., 2010).

The bed bugs used in this study were originally collected in 1973 from an infestation at the U. S. Army barracks in Ft. Dix NJ by Harold J. Harlan. The bugs were identified as *C. lectularius* L. first by Dr. Harlan and subsequently by Prof. Woodbridge A. Foster (Ohio State) and by the late Dr. Richard C. Froeschner, principal Heteropterist, Smithsonian Institution, Washington, DC. Since their initial collection they have been maintained by feeding on human blood. In 2008, a sub-colony of these bed bugs (“Harlan strain”) was established at Beltsville MD and has been fed weekly on human red blood cells (RBC) fortified with plasma, using an artificial feeding system (Feldlaufer et al., 2010).

2. Previous work

The chemical composition of alarm pheromones extracted from nymphal (immature) and adult *C. lectularius* has been well-documented as a mixture of *C*6 and *C*8 aldehydes (Collins, 1968; Feldlaufer et al., 2010; Levinson et al., 1974a; Schildknect, 1964; ). However, no reports on the chemical composition of bed bug cuticular hydrocarbons can be found in the literature, though cuticular extracts have been shown to play a role in gregariousness and arrestment of bugs (Domingue et al., 2010). Other unidentified compounds may also act by contact as aggregation or arrestment semiochemicals (Siljander et al., 2007; Olson et al., 2009).

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3. Present study

A colony of the Harlan strain of *C. lectularius* was kept at 27°C and 40±5% RH. Ten adult bed bugs (5 males and 5 females) were placed in a conical vial to which 200 μL of analytical grade pentane was added. After gentle agitation the pentane was transferred using a glass capillary tube to a 1 mL total recovery vial. A 2 μL aliquot (equal to 0.1 bed bug equivalent) was analyzed by a GC–MS (7890A gas chromatograph/5975C mass spectrometer; Agilent Technologies, Santa Clara CA) equipped with an RTx 5 MS column (30 m × 250 μm i.d.; 0.25 μm film thickness; Restek, Bellefonte PA). The temperature was programmed for 50°C for 2 min, increased to 280°C at 10°C/min, and then held at 280°C for 15 min. All mass spectra were conducted by electron impact. Identities of *n*-alkanes were accomplished by comparing retention times and mass spectra of unknowns to commercial standards. Methylalkanes were identified by known fragmentation patterns resulting in diagnostic ions (Blomquist et al., 1976; Jackson and Blomquist, 1976).

Seventeen hydrocarbons accounting for nearly 99% of the compounds eluting between 24 and 38 min were identified in the pentane extract of *C. lectularius* (Fig. 1, Table 1). The sample contained mostly *n*-alkanes (76.7%) of 24–34 carbons, with odd-numbered components predominating. The two most abundant compounds were *n*-nonacosane (*n*C29) and *n*-hentriacontane (*n*C31), which together accounted for more than 55% of the total hydrocarbons identified. Monomethyl-branched hydrocarbons represented the remainder of the sample with 2-methyltriacontane (2MeC30) accounting for >10% of the total hydrocarbons. The 2-methylalkanes were identified by their characteristic fragmentation patterns with strong M-15 and M-43 ions (Blomquist et al., 1976; Jackson and Blomquist, 1976).

Groups of adult male and female bugs were subsequently examined. No qualitative differences were detected in the hydrocarbon profiles of the two sexes, or in the exuviae (cast skins) of last-instar nymphs destined to become either adult males or females. Exuviae for these latter analyses were collected by rearing last-stage nymphs individually. When the nymph molted to either an adult male or an adult female, we knew the sex of the cast skin.

### Table 1
Cuticular hydrocarbons identified from *C. lectularius.*

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Compound</th>
<th>CLE</th>
<th>Rt</th>
<th>Abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>n</em>-Tetracosane (<em>n</em>C24)</td>
<td>24.0</td>
<td>23.980</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td><em>n</em>-Pentacosane (<em>n</em>C25)</td>
<td>25.0</td>
<td>24.772</td>
<td>0.20</td>
</tr>
<tr>
<td>3</td>
<td><em>n</em>-Hexacosane (<em>n</em>C26)</td>
<td>26.0</td>
<td>25.562</td>
<td>0.28</td>
</tr>
<tr>
<td>4</td>
<td>2-Methylhexacosane (2MeC26)</td>
<td>26.6</td>
<td>26.097</td>
<td>0.13</td>
</tr>
<tr>
<td>5</td>
<td><em>n</em>-Heptacosane (<em>n</em>C27)</td>
<td>27.0</td>
<td>26.441</td>
<td>6.30</td>
</tr>
<tr>
<td>6</td>
<td><em>n</em>-Octacosane (<em>n</em>C28)</td>
<td>28.0</td>
<td>27.449</td>
<td>2.75</td>
</tr>
<tr>
<td>7</td>
<td>2-Methyloctacosane (2MeC28)</td>
<td>28.6</td>
<td>28.170</td>
<td>2.02</td>
</tr>
<tr>
<td>8</td>
<td><em>n</em>-Nonacosane (<em>n</em>C29)</td>
<td>29.0</td>
<td>28.659</td>
<td>31.70</td>
</tr>
<tr>
<td>9</td>
<td><em>n</em>-Hentriacontane (<em>n</em>C30)</td>
<td>30.0</td>
<td>30.067</td>
<td>4.91</td>
</tr>
<tr>
<td>10</td>
<td>2-Methyltriacontane (2MeC30)</td>
<td>30.6</td>
<td>31.120</td>
<td>10.21</td>
</tr>
<tr>
<td>11</td>
<td><em>n</em>-Hentriacontane (<em>n</em>C31)</td>
<td>31.0</td>
<td>31.828</td>
<td>24.13</td>
</tr>
<tr>
<td>12</td>
<td>11, 13, 15-Methylhentriacontane</td>
<td>31.3</td>
<td>32.403</td>
<td>0.29</td>
</tr>
<tr>
<td>13</td>
<td>3-Methyl hentriacontane (3MeC31)</td>
<td>31.7</td>
<td>33.351</td>
<td>0.54</td>
</tr>
<tr>
<td>14</td>
<td><em>n</em>-Doptriacontane (<em>n</em>C32)</td>
<td>32.0</td>
<td>33.931</td>
<td>3.13</td>
</tr>
<tr>
<td>15</td>
<td>2-Methyldotriacontane (2MeC32)</td>
<td>32.6</td>
<td>35.516</td>
<td>8.09</td>
</tr>
<tr>
<td>16</td>
<td><em>n</em>-Doptriacontane (<em>n</em>C33)</td>
<td>33.0</td>
<td>36.553</td>
<td>3.19</td>
</tr>
<tr>
<td>17</td>
<td>13, 15, 17-Methyltriacontane.</td>
<td>33.3</td>
<td>37.413</td>
<td>0.98</td>
</tr>
</tbody>
</table>

CLE = chain length equivalents; Rt = retention time.
4. Chemotaxonomic and ecological significance

Since an initial study (Levinson et al., 1974b) on bed bug olfaction and behavior in response to their aldehyde alarm pheromones, there has been renewed interest in this area, involving both C. lectularius (Harraca et al., 2010; Ryne, 2009) and its tropical cousin Cimex hemipterus (Liedtke et al., 2011; Prakash et al., 1996). The contributions of bed bug cuticular hydrocarbons to the behaviors of attraction, aggregation, and arrestment, however, are not as well understood, although cuticular extracts have been shown to be bioactive (Domingue et al., 2010; Weeks et al., 2011). In addition, canines trained on live bed bugs and used to subsequently detect field infestations, will also alert to a pentane extract of live adults (Pfeister et al., 2008). However, the study of Pfeister et al. (2008) study was not designed to distinguish between alarm pheromones and cuticular hydrocarbons, both of which would be present in the solvent extract. Our first report of cuticular hydrocarbons from the bed bug should contribute to research aimed at elucidating the role of these compounds in bed bug behavior. Ultimately, such information can be used in designing more-effective traps and lures for C. lectularius as well as act as a basis for comparison with other Cimex sp. that attack humans such as the tropical bed bug C. hemipterus.

References