

## 4-Oxo-Aldehydes From the Dorsal Abdominal Glands of the Bed Bug (Hemiptera: Cimicidae)

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**ABSTRACT** Analyses of the dorsal abdominal glands of fourth- and fifth-instar nymphs of the bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae), indicated the predominant constituents were (*E*)-2-hexenal and (*E*)-2-octenal, with lesser amounts of 4-oxo-(*E*)-2-hexenal and 4-oxo-(*E*)-2-octenal. The latter two compounds have not been reported previously as occurring in bed bugs. There were no differences in the chemical composition of the dorsal abdominal glands excised from exuviae left behind by either male or female adults, nor from glands excised from fourth-instar exuviae. Because the two oxo-aldehydes made up at least 16% of the gland contents, further study of the functional role of these chemicals seem advisable.

**KEY WORDS** bed bugs, *Cimex lectularius*, semiochemicals, dorsal abdominal glands

The common bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae), is an obligate, blood-sucking insect that has been associated with humans for thousands of years (Usinger 1966). Despite feeding only on blood and requiring a bloodmeal to molt and reproduce, there remains no evidence that *C. lectularius* or the tropical bed bug *Cimex hemipterus* (F.) act as biological vectors of disease pathogens, although mechanical transmission of certain viruses remains a possibility (Jupp et al. 1983, Jupp and Lyons 1987, Webb et al. 1989, Blow et al. 2001, Silverman et al. 2001). Nonetheless, apart from disease transmission bed bugs are annoying, difficult to control, and cause allergic reactions ranging from little if any irritation, to both immediate and delayed immune reactions of varying degree, to relatively severe allergic hypersensitivity (Leverkus et al. 2006, Reinhardt et al. 2009). There has been a huge resurgence of bed bug populations worldwide, and explanations for this resurgence include, but are not limited to, increased international travel, changes in pest control practices for other urban pests, and insecticide resistance (Boase 2004, 2008, Romero et al. 2007, Harlan et al. 2008).

The chemical ecology of bed bugs has received renewed attention, owing in large part to the potential for using pheromones and semiochemicals in control strategies. Early research with bed bug scents and odors demonstrated that the bed bug produced or released predominantly two C<sub>6</sub> and C<sub>8</sub> aldehydes,

(*E*)-2-hexenal and (*E*)-2-octenal (Schildknecht et al. 1964). Scent glands and whole body extracts of adult *C. lectularius* were shown to produce greater amounts of (*E*)-2-hexenal than (*E*)-2-octenal, whereas nymphs produced greater amounts of (*E*)-2-octenal (Collins 1968, Levinson et al. 1974a). Referred to as both “alerting” (=alarm pheromone) and “assembling” (=aggregation pheromone) scents, both aldehydes were shown to be bioactive in filter paper bioassays (Levinson and Bar Ilan 1971), and in studies involving bed bug antennae (Levinson et al. 1974b). More recently, Siljander and colleagues (2007) have presented evidence for an age-specific contact pheromone in bed bugs, and an airborne aggregation pheromone consisting of 10 components, including (*E*)-2-hexenal and (*E*)-2-octenal as the major constituents, has also been identified by this group (Siljander et al. 2008a). The addition of (*E*)-2-hexenal and (*E*)-2-octenal to desiccant dusts used for bed bug control has been shown to enhance dust effectiveness (Benoit et al. 2009).

Because the contents of the dorsal abdominal glands (DAGs) are retained in the exuviae of other true bugs (Aldrich et al. 1991, Borges and Aldrich 1992), we analyzed by gas chromatography-mass spectrometry (GC-MS) the DAGs from *C. lectularius* exuviae to determine their chemical composition and to determine whether there are sex-specific differences in gland content. In this article, in addition to (*E*)-2-hexenal and (*E*)-2-octenal, we report the identification of two oxygenated aldehydes, namely, 4-oxo-(*E*)-2-hexenal and 4-oxo-(*E*)-2-octenal from these glands.

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## Materials and Methods

**Insects.** A colony of *C. lectularius* was established from bugs obtained from Harold Harlan (Crownsville, MD). The colony was kept at  $27 \pm 2^\circ\text{C}$  and  $40 \pm 5\%$  RH and fed weekly through a Parafilm membrane on packed red blood cells and plasma (1.25:1; vol:vol; Takano-Lee et al. 2003) or on red blood cells, alone (Walter Reed Army Medical Center, Washington, DC). During feeding, the bloodmeal was maintained at  $39^\circ\text{C}$  in a jacketed beaker (Kontes Glass Co., Vineland, NJ) attached to a recirculating water bath.

**Collection of Exuviae and DAGs.** After blood feeding, fifth-instar nymphs (Unger 1966) were placed individually in 8-ml screw-cap vials containing a small strip of filter paper, which provided a substrate for the bug to rest. After molting, the sex of each adult was determined so exuviae left behind from males and females could be collected. The three DAGs were carefully excised as a group from each exuvia with microscissors under a dissecting microscope. Any DAGs that were punctured or otherwise ruptured were discarded. Excised DAGs ( $n = 180$ , from 60 males;  $n = 180$ , from 60 females) from the same sex were combined, placed in Total Recovery minivials (Waters, Milford, MA), and refrigerated (in the dark) at  $-5^\circ\text{C}$  until analysis. DAGs were also collected from fourth-instar nymphs ( $n = 60$ , from 20 individuals) without attempting to determine the sex of the nymphs.

**Analyses.** Immediately before analyses, spectral grade pentane (Sigma-Aldrich, St. Louis, MO) was added to the vials ( $50 \mu\text{l}$  each for males and females;  $25 \mu\text{l}$  for fourth instar) containing the DAGs and the DAGs were gently crushed using a hand-made glass pestle fashioned from a Pasteur pipette. A  $1\text{-}\mu\text{l}$  aliquot was analyzed by a GC-MS (7890A gas chromatograph/5975C mass spectrometer; Agilent Technologies, Santa Clara, CA) equipped with an RTx 5 MS column ( $30 \text{ m} \times 250 \mu\text{m}$  i.d.;  $0.25\text{-}\mu\text{m}$  film thickness; Restek, Bellefonte, PA). The temperature was programmed for  $50^\circ\text{C}$  for 2 min, increased to  $280^\circ\text{C}$  at  $10^\circ\text{C}/\text{min}$ , and then held at  $280^\circ\text{C}$  for 15 min. All mass spectra were conducted by electron impact. Identifications of gland constituents were accomplished by comparing retention times and mass spectra of unknowns to authentic standards.

**Chemicals.** (*E*)-2-Hexenal and (*E*)-2-octenal were obtained from Bedoukian Research (Danbury, CT). 4-Oxo-(*E*)-2-hexenal and 4-oxo-(*E*)-2-octenal were prepared by the method of Moreira and Millar (2005), and their mass spectra were compared with published spectra (Moreira and Millar 2005, Kawai et al. 2006).

**Statistics.** Because pooled samples were analyzed and variance is a function of the number of individuals ( $n$ ), we calculated variance by using the standard formula for proportions coming from a binomial distribution [ $p(p-1)/n$ ]. However, because our data were not binomial, we calculated that this measure of uncertainty, had the data been normally distributed, corresponds to a coefficient of variation of  $\approx 28\%$ .

**Table 1.** Percentage  $\pm$  SE of major constituents from DAGs of *C. lectularius*

Compound	Fifth instar		Fourth instar
	Males	Females	Mixed
( <i>E</i> )-2-Hexenal	$19.9 \pm 5.1$	$21.0 \pm 5.2$	$22.0 \pm 9.2$
( <i>E</i> )-2-Octenal	$61.6 \pm 6.2$	$58.6 \pm 6.3$	$57.7 \pm 11.2$
4-Oxo-( <i>E</i> )-2-hexenal	$10.9 \pm 4.0$	$11.9 \pm 4.2$	$11.8 \pm 7.2$
4-Oxo-( <i>E</i> )-2 octenal	$4.6 \pm 2.7$	$4.9 \pm 2.7$	$6.4 \pm 5.5$

Exuviae left behind by males or females were collected by rearing fifth instars individually and determining the sex of the resulting adult (180 DAGs for males and females, 60 individuals each). No determination of sex was made with fourth instars (60 DAGs, 20 individuals). DAGs were extracted in pentane and analyzed by GC-MS. Identifications were based on a comparison of retention times and mass spectra with authentic standards.

## Results

The DAGs of fourth and fifth instar bed bug exuviae contained the same compounds, predominantly  $\text{C}_6$  and  $\text{C}_8$  aldehydes (Table 1). The most abundant compound in all samples examined was (*E*)-2-octenal, which was  $\approx 3$  times more abundant than (*E*)-2-hexenal, the next most abundant compound. 4-oxo-(*E*)-2-hexenal and 4-oxo-(*E*)-2-octenal made up  $\approx 11$  and  $5\%$  of the total, respectively, in all samples. There were no significant differences between the chemical compositions of fourth- and fifth-instar DAGs or between DAGs excised from the exuviae of bugs that were male or female adults. Lesser amounts ( $<0.5\%$ ) of 2,4-octadienal and nonanal were also detected in our samples, although no attempts were made to assign a particular geometric isomer to these compounds.

## Discussion

Nymphs of true bugs (Heteroptera) produce defensive chemicals in their abdominal scent glands that include aldehydes of six to ten carbons (Aldrich 1988, Millar 2005, and references therein), and our results with bed bugs are consistent with these findings. However, although 4-oxo-aldehydes have been identified previously from several primarily phytophagous true bug families (Aldrich et al. 1991, Borges and Aldrich 1992, Farine et al. 1992, Pavis et al. 1994, Fucarino et al. 2004), these compounds have never been reported from *C. lectularius*. In this regard, chemicals produced by bed bugs more closely resemble those produced by phytophagous bugs than they resemble the semiochemicals produced by other hematophagous Heteroptera such as triatomine bugs (Cruz-López et al. 2001, Guerenstein and Guerin 2004).

The resurgence of bed bugs worldwide has led to the development of traps and lures to reduce or limit bed bugs. Some traps incorporate chemicals found in human emanations (Anderson et al. 2009), whereas others are based in part on the bug's semiochemistry. Siljander et al. (2008a) found 10 compounds, including (*E*)-2-hexenal and (*E*)-2-octenal, to be essential components of an airborne aggregation pheromone for bed bugs and are included in a patent application

(Siljander et al. 2008b). Other research has demonstrated that the addition of blends of (*E*)-2-hexenal and (*E*)-2-octenal enhances the efficacy of desiccant dusts used to control bed bugs (Benoit et al. 2009). Given that the 4-oxo-aldehydes we identified in this study constitute >16% of DAG compounds, it may be germane to determine their functional roles as semiochemicals. This may lead to their consideration in the design of new trapping or control strategies.

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