

Compounds in Metathoracic Glands of Adults and Dorsal Abdominal Glands of Nymphs of the Stink Bugs, *Chlorochroa uhleri*, *C. sayi*, and *C. ligata* (Hemiptera: Pentatomidae)

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Hsiao-Yung Ho and Jocelyn G. Millar (2001) Compounds in metathoracic glands of adults and dorsal abdominal glands of nymphs of the stink bugs *Chlorochroa uhleri*, *C. sayi*, and *C. ligata* (Hemiptera: Pentatomidae). *Zoological Studies* 40(3): 193-198. The contents of metathoracic glands of adults and dorsal abdominal glands of nymphs of 3 *Chlorochroa* species, (*C. sayi*, *C. uhleri*, and *C. ligata*) were analyzed. Compounds were identified by gas chromatography (GC) and coupled GC-mass spectrometry, matching retention times and mass spectra with those of authentic samples. Tridecane was the major component in the defensive glands of both adults and nymphs. Other compounds identified include (*E*)-2-hexenal, (*E*)-4-oxo-2-hexenal, (*E*)-2-octenal, (*E*)-2-octenyl acetate, (*E*)-4-oxo-2-octenal, (*E*)-2-decenal, undecane, dodecane, tetradecane, and pentadecane. <http://www.sinica.edu.tw/zool/zoolstud/40.3/193.pdf>

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Stink bugs, as their name suggests, are characterized by the production of large quantities of strong-smelling and irritating defensive chemicals, which are released when the bugs are disturbed or molested (Aldrich 1988). Odorous compounds are produced by both adults and immatures (Staddon 1979, Aldrich 1988, Pavis et al. 1994), and numerous reports attest to their efficacy as effective defenses against predation (Staddon 1979, Aldrich 1988, Krall et al. 1999). They also may have a role as alarm pheromones (Kou et al. 1989), as has been demonstrated for similar types of compounds produced by bug species in other families (Gunawardena and Bandumathie 1993, Leal et al. 1994). Stink bug defensive compounds have received considerable study, in part because they constitute such an obvious defense, and because they are produced in simple mixtures in comparatively large quantities, making them easy to analyze and identify. Furthermore, the types of compounds constituting the defensive chemical blends appear to be similar for the

several dozen species for which data are available, typically consisting of alkane hydrocarbons and saturated and unsaturated aldehydes and esters (Aldrich 1988). In adults, the defensive compounds are produced in large, well-defined, and usually colored metathoracic glands, which are not present in the immature stages (Aldrich 1988). Instead, the nymphal defensive compounds are produced in dorsal abdominal glands (Staddon 1979).

Sex pheromones have been identified from only a few phytophagous stink bug species to date (McBrien and Millar 1999). In all cases reported, components from metathoracic glands have not been part of the pheromone blend, unlike the case with pheromone blends of bugs from other families (e.g., *Campyloma verbasci*, Smith et al. 1991). However, because stink bugs release defensive compounds readily when disturbed, it is difficult if not impossible to collect extracts of sex pheromones uncontaminated by these components. Thus, the objectives of this study were: 1. to characterize the

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defensive compounds produced in the metathoracic glands (MTGs) of adult bugs and the dorsal abdominal glands (DAGs) of immature bugs; and 2. to compare the defensive gland contents of adults and nymphs between the 3 *Chlorochroa* species.

MATERIALS AND METHODS

Analysis of metathoracic gland contents of adult bugs

Metathoracic gland contents were analyzed from 5 adult bugs of each sex. To prevent the premature discharge of gland contents, mature bugs were anesthetized with CO₂ and then killed by freezing. The legs and wings of freshly killed bugs were clipped off with scissors, and the carcass was pinned through the head, dorsal side up. The outer edges of the abdomen were cut open with a pair of fine iris scissors. Then the dorsal cuticle was flipped open, exposing the contents of the abdomen. The tissues inside the body cavity were carefully removed to expose the paired orange-colored metathoracic glands. The gland contents were sampled by piercing the gland with a drawn-out glass microcapillary tube. Then the tube was broken in 20 µl of pentane in a conical glass vial insert (200 µl) inside a screw-capped vial to release the extracted gland contents. Extracts were concentrated by evaporation under a gentle stream of nitrogen, as required, and analyzed by splitless gas chromatography on a DB-17 column (30 m x 0.25 mm, J&W Scientific, Folsom, CA), with a temperature program of 50 °C for 1 min, then 10 °C/min to 250 °C. Injector and detector temperatures were 250 and 280 °C, respectively, with helium carrier gas. Extracts were also analyzed by splitless coupled gas chromatography-mass spectrometry (GC-MS) with a Hewlett-Packard 5890 GC fitted with a DB5-MS column (20 m x 0.2 mm i. d.) and interfaced to an HP 5970B mass selective detector (electron impact ionization, 70 eV). The GC was programmed at 40 °C/1 min, then 10 °C/min to 250 °C, with injector and transfer line temperatures of 250 and 280 °C, respectively. Compounds were tentatively identified by GC/MS, and identifications were confirmed by comparison of the retention time(s) and mass spectra of the unknowns with those of authentic samples (see below). The relative amount of each compound was determined from the area under the GC peaks.

Analysis of dorsal abdominal gland contents of nymphs

Dorsal abdominal gland contents were analyzed from 5 last instar nymphs of each species. To prevent the discharge of gland contents, nymphs were anesthetized with CO₂ and killed by freezing. Then the legs were clipped off, and the bug was pinned through the head, ventral side up. The outer edge of the abdomen was cut open, and the ventral cuticle was flipped open exposing the contents of the abdomen. After carefully removing the tissue inside the body cavity, 3 pairs of dorsal abdominal glands were exposed. The 1st pair was very small. The 2nd and 3rd pairs appeared like orange lobes attached to the cuticle. The contents of the latter 2 sets of glands were collected in a drawn-out glass microcapillary tube, and diluted with pentane for analysis. These extracts were then analyzed as described above for adult metathoracic glands.

Chemical standards

(*E*)-2-hexenal, (*E*)-2-octenal, (*E*)-2-hexenyl acetate, (*E*)-2-octenyl acetate, undecane, dodecane, tridecane, tetradecane, and pentadecane were purchased from Aldrich Chemical Company (Milwaukee WI). (*E*)-4-oxo-2-hexenal was synthesized as described below, and (*E*)-4-oxo-2-octenal was a gift from Prof. Francisco de Assis Marques, Federal Univ. of Parana, Curitiba, Brazil.

Synthesis of 4-hydroxy-1-[(tetrahydropyranyl)oxy]-2-hexyne

Tetrahydropyranyl ether-protected 2-propyn-1-ol (3.5 g, 25 mmol) and a few milligrams of triphenylmethane indicator in 50 ml freshly distilled dry tetrahydrofuran (THF) under Ar were cooled to about -25 °C in a dry ice-acetone bath. Butyllithium in hexane (1.6 M, 17.2 ml, 27.5 mmol) was added dropwise and the solution turned orange. The solution was allowed to warm to 0 °C over ~15 min, then was cooled again to less than -15 °C, and propanal (1.74 g, 30 mmol) in 10 ml THF was added dropwise. The reaction was stirred until complete as determined by GC (~30 min), then quenched with water and extracted with hexane (3 x 50 ml). The combined hexane extracts were washed with brine, dried over anhydrous Na₂SO₄, concentrated, and pumped briefly under vacuum with warming (0.5 mmHg, 50 °C) to remove traces of solvent and unreacted propanal. The crude product (4.6 g, 93%) was > 95% pure as determined by GC and was used without further purification.

Synthesis of (E)-2-hexen-1,4-diol

LiAlH₄ (0.92 g, 24 mmol) was added in portions to 25 ml of THF in a 250-ml 3-neck flask under Ar,

chilled to about 0 °C in an icebath with stirring. The crude alcohol from above (1.98 g, 10 mmol) was added dropwise by syringe (This caused extensive foaming!). Once the addition was complete and the foaming had subsided, the mixture was heated with gentle reflux for 6 h under Ar. The cooled mixture was then quenched by dropwise sequential addition of water (1 ml), 6 M aqueous NaOH (0.75 ml), and water (3 ml) (This also caused extensive foaming!). Petroleum ether (25 ml) was then added, and the mixture was stirred a further 15 min to allow the aluminum salts to granulate. The mixture was then filtered, and the filtrate was dried over Na₂SO₄ and concentrated. The crude product was taken up in methanol (50 ml), *p*-toluenesulphonic acid monohydrate (150 mg) was added, and the mixture was stirred at room temperature for 4 h. A saturated NaHCO₃ solution (2 ml) was then added, and the mixture was stirred for 15 min. The solvent was removed under reduced pressure at 35 °C, and the crude product was taken up in Et₂O (70 ml). The ether solution was dried over Na₂SO₄, filtered, and concentrated in a vacuum, and the residue was purified by silica gel flash chromatography (hexane/EtOAc 2: 1, then EtOAc). After Kugelrohr distillation (bp ~118-122 °C, 6 mmHg), 1.74 g (60%) of (*E*)-2-hexen-1,4-diol was obtained. ¹H NMR (CDCl₃, 300 MHz): δ 5.88 (dt, J = 15.5, 4.9 Hz, 1 H), 5.76 (br dd, J = 16.0, 6.0 Hz, 1 H), 4.19 (d, J = 5.1 Hz, 2 H), 4.10 (overlapped dt, J ~ 6.5, 6.1 Hz, 1 H), 1.90 (br s, 2 H), 1.51-1.70 (m, 2 H), 0.95 (t, J = 7.4 Hz, 3 H). ¹³C NMR (CDCl₃, 75.48 MHz): δ 134.1, 129.9, 73.6, 62.9, 30.0, 9.7. EIMS: m/z: 87 (18), 85 (13), 69 (70), 57 (87), 55

(18), 45 (18), 43 (59), 41 (100).

Synthesis of (*E*)-4-oxo-2-hexenal

Pyridinium chlorochromate (388 mg, 1.8 mmol) and neutral activated aluminum oxide (388 mg) were ground together to a fine powder in a mortar and pestle and transferred to a round-bottomed flask containing 5 ml CH₂Cl₂. A solution of (*E*)-2-hexen-1,4-diol (32 mg, 0.28 mmol) in CH₂Cl₂ was added dropwise. The mixture was stirred for 1 h at room temperature, then concentrated under reduced pressure until < 1 ml of liquid remained. The residue was applied to a 5-cm column of silica gel, and the product was eluted with hexane/Et₂O (1: 1) affording 23 mg (77%) of (*E*)-4-oxo-2-hexenal. ¹H NMR (CDCl₃, 300 MHz): δ 9.79 (d, J = 7.0 Hz, 1 H), 6.91 (d, 16.2 Hz, 1 H), 6.79 (dd, J = 16.26, 6.9 Hz, 1 H), 2.75 (q, J = 7.2 Hz, 2H), 1.17 (t, 7.2 Hz, 3 H). ¹³C NMR (CDCl₃, 75.48 MHz): δ 200.3, 193.4, 144.7, 137.3, 34.5, 7.5. EIMS m/z: 112 (M⁺, 15), 84 (15), 83 (100), 57 (22), 55 (98).

RESULTS

A typical gas chromatogram of the metathoracic gland contents of an adult male *C. ligata* is shown in figure 1. The volatile gland constituents consisted primarily of saturated hydrocarbons, short-chain unsaturated aldehydes, and an unsaturated ester (Tables 1 and 2). Tridecane was the most abundant component, with lesser and approximately equal amounts of (*E*)-2-hexenal, (*E*)-4-oxo-2-hexenal, and

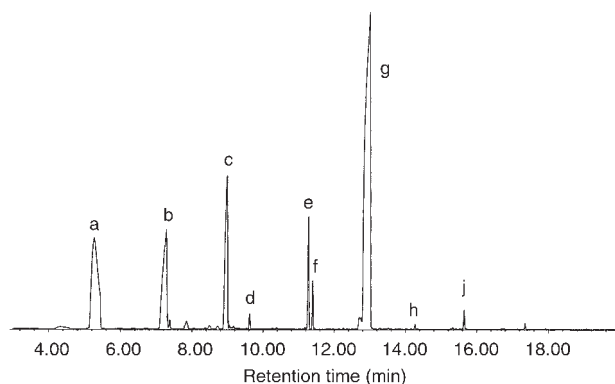


Fig. 1. Gas chromatogram of metathoracic gland contents from an adult male *Chlorochroa ligata*. GC conditions: initial temp. 40 °C (1 min), 10 °C/min – 250 °C, then 250 °C (10 min); injector: 250 °C, detector: 280 °C. Column: DB-5MS. Compounds: a: (*E*)-2-hexenal; b: (*E*)-4-oxo-2-hexenal; c: (*E*)-2-octenal; d: undecane; e: dodecane; f: (*E*)-2-octenyl acetate; g: tridecane; h: tetradecane; j: pentadecane.

Table 1. Percentages of compounds found in metathoracic glands of male *Chlorochroa* bugs (mean ± SD) relative to the most abundant compound, tridecane ($n = 5$)

Compound	Species		
	<i>C. ligata</i>	<i>C. sayi</i>	<i>C. uhleri</i>
(<i>E</i>)-2-hexenal	20.6 ± 7.1	21.1 ± 3.8	10.8 ± 9.1
(<i>E</i>)-4-oxo-2-hexenal	12.9 ± 6.2	23.7 ± 14	22.5 ± 19.7
(<i>E</i>)-2-octenal	9.5 ± 4.4	22.7 ± 14	37.3 ± 29.6
Undecane	0.27 ± 0.08	0	0.12 ± 0.04
Dodecane	2.3 ± 0.4	2.3 ± 0.2	1.8 ± 0.1
(<i>E</i>)-2-octenyl acetate ^a	5.8 ± 6.8	17.9 ± 9.7	1.5
Tridecane	100	100	100
Tetradecane	0.08 ± 0.03	0	0.15 ± 0.03
Pentadecane	0.2 ± 0.04	0	0.35 ± 0.09

^a(*E*)-2-octenyl acetate was found in only one of the 5 *C. uhleri* male adults sampled, but (*E*)-2-octenyl acetate was found in every metathoracic gland extract of the 5 *C. ligata* and *C. sayi* male adults sampled.

(*E*)-2-octenal. (*E*)-2-octenyl acetate was the only ester component detected. Analogous extracts from adult males of the other 2 species were qualitatively very similar (Table 1), with tridecane being the most abundant compound in all cases, but with the amounts of the minor components varying between species.

Extracts from adult female bugs were qualitatively and quantitatively similar to those from the corresponding males, with a couple of minor exceptions. First, a significant quantity of (*E*)-2-hexenyl acetate (26% of the tridecane peak) was found in the extract from a single female *C. sayi*. Second, (*E*)-2-octenyl acetate was only detected in one of 5 *C. uhleri* males sampled.

A typical gas chromatogram of a dorsal abdominal gland (DAG) extract from a *C. ligata* nymph is shown in figure 2. DAG contents from nymphs of the 3 *Chlorochroa* spp. were similar (Table 3), with the exception that (*E*)-2-hexenal was detected only in *C. sayi*. Furthermore, the extracts were qualitatively similar to those from MTGs of adults, except that the minor and trace components were sometimes not detected. However, the relative proportions of DAG contents differed from those of the adult MTGs, being dominated by aldehyde components, and (*E*)-4-oxo-2-octenal and (*E*)-2-decenal were found in DAG extracts but not in MTG extracts of any of the 3 species.

DISCUSSION

The contents of metathoracic glands of adults of

Table 2. Percentages of compounds found in metathoracic glands of female *Chlorochroa* bugs (mean \pm SD) relative to the most abundant compound, tridecane ($n = 5$)

Compound	Species		
	<i>C. ligata</i>	<i>C. sayi</i>	<i>C. uhleri</i>
(<i>E</i>)-2-hexenal	13.9 \pm 6.7	22.7 \pm 11.8	15.3 \pm 6.1
(<i>E</i>)-4-oxo-2-hexenal	11.1 \pm 6.6	28.8 \pm 21.2	31.4 \pm 25
(<i>E</i>)-2-hexenyl acetate ^a	0	26	0
(<i>E</i>)-2-octenal	6.7 \pm 3.6	37.7 \pm 22.6	31.1 \pm 13
Undecane	0.17 \pm 0.07	0	0.21 \pm 0.04
Dodecane	2.2 \pm 0.3	2.2 \pm 0.3	2.4 \pm 1.5
(<i>E</i>)-2-octenyl acetate	3.8 \pm 6.1	20.5 \pm 32.4	0
Tridecane	100	100	100
Tetradecane	0.08 \pm 0.02	0	0.11 \pm 0.03
Pentadecane	0.3 \pm 0.07	0	0.42 \pm 0.14

^a(*E*)-2-hexenyl acetate was only found in only one of the 5 *C. sayi* female adults sampled, and no (*E*)-2-hexenyl acetate was found in the metathoracic gland extract of the 5 *C. ligata* and *C. uhleri* female adults sampled.

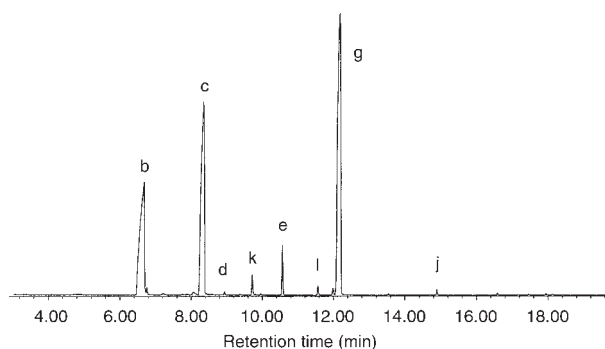


Fig. 2. Gas chromatogram of dorsal abdominal gland contents from a *Chlorochroa ligata* nymph. GC conditions: initial temp. 40 °C (1 min), 10 °C/min to 250 °C, then 250 °C (10 min); injector: 250 °C, detector: 280 °C. Column: DB-5MS. Compounds: b: (*E*)-4-oxo-2-hexenal; c: (*E*)-2-octenal; d: undecane; e: dodecane; g: tridecane; j: pentadecane; k: (*E*)-4-oxo-2-octenal; l: (*E*)-2-decenal.

both sexes of the 3 *Chlorochroa* spp. were similar and typical of what has been reported for several other pentatomid species (Aldrich 1988 1995). Thus, these blends of hydrocarbons with aldehydes and esters appear to be highly conserved, being shared both within and across genera, and even between bug families (Aldrich 1995). The aldehydes and esters are strongly scented and are strong irritants, providing both an easily detected warning signal and a strong defense. The function of the hydrocarbons is less clear, but they may serve as solvents and as controlled-release substrates for the more volatile aldehydes (Remold 1962, Gunawardena and Herath 1991). In addition to biosynthetic parsimony, the similarity in the defensive chemical blends shared by numerous species may provide another benefit of serving as a generic warning signal and strong deterrent to attack. That is, a predator that has been exposed to the blend once is

Table 3. Percentages of compounds found in dorsal abdominal glands of male *Chlorochroa* nymphs relative to tridecane (mean \pm SD, $n = 5$)

Compound	Species		
	<i>C. ligata</i>	<i>C. sayi</i>	<i>C. uhleri</i>
(<i>E</i>)-2-hexenal	—	1.9 \pm 1.1	—
(<i>E</i>)-4-oxo-2-hexenal	81 \pm 35	121 \pm 69	136 \pm 46
(<i>E</i>)-2-octenal	84 \pm 21	85 \pm 28	96 \pm 27
Undecane	0.5 \pm 0.4	0.8 \pm 0.4	0.3 \pm 0
(<i>E</i>)-4-oxo-2-octenal	1.7 \pm 1.0	2.3 \pm 1.1	2.0 \pm 1.2
Dodecane	2.1 \pm 0.7	2.1 \pm 0.9	2.3 \pm 0.9
(<i>E</i>)-2-decenal	0.7 \pm 0.3	0.7 \pm 0.7	0.4 \pm 0.1
Tridecane	100	100	100
Pentadecane	0.2 \pm 0.1	0.3 \pm 0.1	0.3 \pm 0.1

unlikely to attack another individual producing a similar scent, even though that individual may look entirely different and be of an entirely different species (Janaiah 1993).

All evidence suggests that the metathoracic gland components form no part of the sex pheromone chemistry of these species. The sex pheromones are exclusively male-produced, and the components distinctly differ in structure from the defensive compounds, being terpenoids rather than the products of acetogenin biosynthetic pathways (Ho 2000). Furthermore, unlike MTG contents, sex pheromone blends are generally species specific, even though individual components of a pheromone blend may be shared by more than 1 species (McBrien and Millar 1999). The hypothesis that MTG contents are primarily for defense and have no role as sex pheromones is further supported by the similarity between MTG and DAG contents of nymphs, which, being sexually immature, have no need of sex pheromones.

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椿象 *Chlorochroa uhleri*, *C. sayi* 與 *C. ligata* (Hemiptera: Pentatomidae)
成蟲與幼蟲臭腺之化學成分

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本研究利用氣相層析質譜儀與氣相層析儀鑑定三種椿象, *Chlorochroa uhleri*, *C. sayi* 與 *C. ligata* 之成蟲與幼蟲臭腺中所含的化學成分, 主要成分是十三烷, 其他包括烯醛類, 乙酸辛烯酯等。

關鍵詞：椿象成蟲, 幼蟲, 臭腺成分, 十三烷, 短鏈烯醛類。

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