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Article

Identification of Defensive Compounds in Metathoracic Glands of Adults of the Stink Bug *Dichelops melacanthus* (Hemiptera: Pentatomidae)

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Os conteúdos das glândulas metatorácicas de insetos adultos do percevejo *Dichelops melacanthus* (Hemiptera: Pentatomidae) foram analisados. Os compostos de defesa foram identificados combinando as técnicas de cromatografia gasosa (CG), cromatografia gasosa acoplada à espectrometria de massas (CG-EM) e por comparação de seus índices de retenção com os de compostos padrões. Tridecano foi o componente majoritário, seguido de quantidades menores e aproximadamente iguais de (*E*)-4-oxo-2-hexenal e (*E*)-2-octenal. Também foram identificados (*E*)-2-hexenal, decano, acetato de (*E*)-2-hexenila, undecano, (*E*)-4-oxo-2-octenal, dodecano, acetato de (*E*)-2-octenila, 1-trideceno, tetradecano e pentadecano.

The contents of metathoracic glands of adults of the stink bug *Dichelops melacanthus* (Hemiptera: Pentatomidae) were analyzed. Compounds were identified by gas chromatography (GC), coupled GC-mass spectrometry and matching retention indices and mass spectra with those of authentic samples. Tridecane was the major component followed by lesser and approximately equal amounts of (*E*)-4-oxo-2-hexenal and (*E*)-2-octenal. Other compounds identified include (*E*)-2-hexenal, decane, (*E*)-2-hexenyl acetate, undecane, (*E*)-4-oxo-2-octenal, dodecane, (*E*)-2-octenyl acetate, 1-tridecene, tetradecane and pentadecane.

Keywords: *Dichelops melacanthus*, defensive compounds, short-chain unsaturated aldehydes, unsaturated acetates, tridecane

Introduction

Insects have evolved a multitude of chemical and behavioral defenses against other offensive organisms. Stink bugs, as their name suggests, produce large quantities of strong-smelling and irritating defensive chemicals, which are released when the bugs are disturbed or molested.[1] In general, heteropterans produce allomones in dorsal abdominal scent glands as immatures, and in metathoracic scent glands as adults.[1-3] Numerous reports attest to the efficacy of the secretions of the metathoracic and abdominal scent glands as effective defenses against predation.[1,2,4] They also may have a role as alarm pheromones,[5] as has been demonstrated for similar types of compounds produced by bug species in other families.[6,7] Stink bug defensive compounds have received considerable study, and it has been shown that the types of compounds constituting the defensive chemical blends typically consist of alkane hydrocarbons and saturated and unsaturated aldehydes and esters. In adults, the defensive compounds are produced in large, well-defined, and usually colored metathoracic glands, which are not present in the immature stages.

Sex pheromones have been identified from only a few phytophagous stink bug species to date.[8,9] In all cases reported, components from metathoracic glands have not been part of the pheromone blend. However, because stink bugs readily release defensive compounds when disturbed, it is difficult if not impossible to collect extracts of sex pheromones uncontaminated by these components.

The main objective of this study was to characterize the defensive compounds produced in the metathoracic glands of adults of the stink bug *Dichelops melacanthus*. These components elicited dispersive behavior of conspecific individuals of this species (both larvae and adults)[10] in "puff" assays in which the content of the

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gland was applied on the bug using a glass pipette.[7] In Brazil, this bug has for several years been considered a secondary pest of soybean (*Glycine max* L.) Merril, pods, and has recently become a key pest of corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.).[11] The no-tillage system and late-season corn, after they were introduced, provided good conditions for the survival and rapid proliferation of this insect. When corn or wheat germinates, the insects suck on the stems of the plants, causing severe damage and significantly reducing yields.[12]

Experimental

Insects

Dichelops melacanthus was collected at the School Farm of the State University of Londrina, in Londrina County, northern Paraná State (latitude 23 °S and longitude 51 °W). Insects were provided with soybean seeds (*Glycine max*) and water at 22 ± 2 °C, 70 ± 10% relative humidity and a 14:10 h light-dark cycle.

Analysis of metathoracic gland contents of adult bugs

Metathoracic gland contents were analyzed from 5 adult bugs. To prevent the premature discharge of gland contents, mature bugs were anesthetized with CO_2 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 0.2 mL of hexane in a glass vial (2.5 mL) inside a screw-capped vial to release the extracted gland contents. Extracts were concentrated by evaporation under a gentle stream of argon, as required, and analyzed (1 μL) by splitless gas chromatography on a VF-5ms column (30 m × 0.25 mm × 0.25 μm, Varian, Lake Forest, CA), with a temperature program of 50 °C for 1 min, then 5 °C min^{-1} to 180 °C and hold for 15 min. Injector and detector temperatures were 250 and 300 °C, respectively, with helium carrier gas (1.0 mL min^{-1}). Extracts were also analyzed (1 μL) by splitless coupled gas chromatography mass spectrometry (GC-MS) with a Varian 3800 GC fitted with a CP-Sil 8 CB Low Bleed/MS column (30 m × 0.25 mm × 0.25 μm, Varian, Lake Forest, CA) and interfaced to an Varian Saturn 2000 MS/MS mass selective detector (ion trap, electron impact ionization (70 eV)). The GC was programmed at 50 °C for 3 min, then 5 °C min^{-1} to 280 °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 (EI and CI) of the unknowns with those of authentic samples or by comparative analysis of Kovats indeces. In order to determine the Kovats index of the compounds, a mixture of *n*-alkanes (C_8-C_{20}) was injected followed by natural extract in the GC-MS equipment programed at 50-240 °C at 3 °C min^{-1}. Injector and detector temperatures were 250 °C and 300 °C, respectively. The relative amount of each compound was determined from the area under the GC peaks.

Chemical standards

(*E*)-2-hexenal, (*E*)-2-octenal, (*E*)-2-hexenyl acetate, 1-tridecene, 1-dodecene, decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane, heptadecane, octadecane and eicosane were purchased from Aldrich Chemical Company (Milwaukee, Wisconsin, USA). (*E*)-2-Octenyl acetate was a gift from Dr. Jocelyn G. Millar, University of California, Riverside, USA. (*E*)-4-Oxo-2-hexenal and (*E*)-4-oxo-2-octenal were synthesized as described below.

*(E)-2-Hexen-1,4-diol (**2a**)*

A solution of *n*-BuLi in hexanes (22.9 mL, 27.5 mmol) was added dropwise to a stirred solution of THP-protected 2-propyn-1-ol, **1** (3.50 g, 25 mmol) in anhydrous THF (50 mL) at –30 °C. The solution was warmed to 0 °C, hold at 0 °C for 15 min, then recooled to –30 °C, followed by the addition of propionaldehyde (1.74 g, 30 mmol) in THF (10 mL). After stirring 1 h between –20 and –30 °C and 2 h at room temperature, the mixture was poured into water and the product was extracted with Et_2O. The ether extract was washed with brine, dried over Na_2SO_4 and concentrated.

The crude product in anhydrous THF (50 mL) was refluxed with $LiAlH_4$ (1.90 g, 50 mmol) under Ar for 3 h. Water (2 mL), 6 mol L^{-1} NaOH (1.5 mL), and a second portion of water (6 mL) were added sequentially dropwise at 0 °C. The solution was decanted from the granular solids, diluted with Et_2O, washed with brine, dried over Na_2SO_4, and concentrated.

The crude product was taken up in methanol (50 mL), *p*-toluenesulfonic acid monohydrate (150 mg) was added, and the mixture was stirred 4 h at room temperature. Saturated $NaHCO_3$ solution (2 mL) was then added and the mixture was stirred for 15 min. The MeOH was removed under reduced pressure, and the residue was extracted with ether. The ether extract was dried over Na_2SO_4, filtered, concentrated and purified by silica gel flash chromatography (hexane-EtOAc 2:1, then EtOAc). The oil obtained was distilled (bp 118-122 °C at 6 mm Hg) giving 1.74 g (60% overall yield) of diol, **2a**. ^{1}H NMR (300 MHz, $CDCl_3$): 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); ^{13}C NMR (75 MHz, $CDCl_3$): δ 134.1, 129.9, 73.6, 62.9, 30.0, 9.7. MS (70 eV) *m*/*z* (relative intensity): 87 (18), 85 (13), 69 (70), 57 (87), 55 (18), 45 (18), 43 (59), 41 (100).

*(E)-4-Oxo-2-hexenal (**3a**)*

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 CH_2Cl_2. A solution of (*E*)-2-hexen-1,4-diol (32 mg, 0.28 mmol) in CH_2Cl_2 was added dropwise. The mixture was stirred for 1 h at room temperature and then concentrated under reduced pressure until < 1 mL of liquid remained. The residue was applied to a 5 cm column of silica gel (60-230 Mesh), and the product was eluted with hexane/Et_2O (1:1) affording 23 mg (77%) of **3a**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.79 (d, *J* 7.0 Hz, 1 H), 6.91 (d, *J* 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, *J* 7.2 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 200.3, 193.4, 144.7, 137.3, 34.5, 7.5. MS (70eV) *m/z* (relative intensity): 112 (M+, 15), 84 (15), 83 (100), 57 (22), 55 (98).

*(E)-2-Octen-1,4-diol (**2b**)*

The diol **2b** was prepared as described above for preparation of **2a**, replacing propionaldehyde with pentanal, giving 2.19 g (61% overall yield) of diol **2b**. The oil obtained was distilled (bp ~134-38 °C at 6 mm Hg). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.86 (dt, *J* 15.6, 5.0 Hz, 1 H), 5.76 (dd, *J* 15.6, 5.8 Hz, 1 H), 4.18 (d, *J* 5.0Hz, 2 H), 4.15 (overlapped dt, *J* 6.4, 6.3 Hz, 1 H), 1.69 (br s, 2 H), 1.50-1.62 (m, 2 H), 1.27-1.42 (m, 4 H), 0.92 (t, *J* 6.8 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 134.5, 129.6, 72.3, 62.7, 36.8, 27.6, 22.6, 14.0. MS (70 eV) *m*/*z* (relative intensity): 113 (8), 87 (35), 69 (73), 57 (64), 55 (30), 45 (20), 43 (65), 41 (100).

*(E)-4-Oxo-2-octenal (**3b**)*

Diol **2b** (40 mg) was oxidized as described above for **2a**, giving 30 mg (80%) of (*E*)-4-oxo-2-octenal, **3b**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.78 (d, *J* 7.7 Hz, 1 H), 6.89 (d, *J* 16.2 Hz, 1 H), 6.77 (dd, *J* 16.1, 7.7 Hz, 1 H), 2.70 (t, *J* 7.3 Hz, 2 H), 1.65 (quin, *J* 7.5 Hz, 2 H), 1.36 (sex, *J* 7.5 Hz, 2 H), 0.93 (t, *J* 7.3 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 200.3, 193.3, 145.3, 137.5, 40.9, 25.7, 22.2, 13.8. MS (70 eV) *m*/*z* (relative intensity): 125 (8), 111 (42), 98 (47), 84 (20), 83 (51), 70 (36), 57 (28), 55 (100), 41 (58).

Results and Discussion

A typical gas chromatogram of the metathoracic gland content of an adult male *D. melacanthus* is shown in Figure 1.

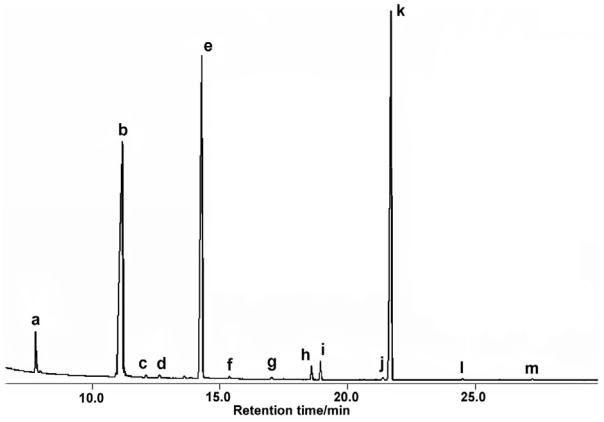


Figure 1. Gas chromatogram of metathoracic gland contents from *D. melacanthus*. Compounds: (*E*)-2-hexenal (**a**), (*E*)-4-oxo-2-hexenal (**b**), decane (**c**), (*E*)-2-hexenyl acetate (**d**), (*E*)-2-octenal (**e**), undecane (**f**), (*E*)-4-oxo-2-octenal (**g**), dodecane (**h**), (*E*)-2-octenyl acetate (**i**), 1-tridecene (**j**), tridecane (**k**), tetradecane (**l**), pentadecane (**m**).

The volatile gland constituents consisted primarily of saturated hydrocarbons, short-chain unsaturated aldehydes, and two unsaturated esters (Table 1).

Tridecane was the most abundant component, with lesser and approximately equal amounts of (*E*)-4-oxo-2-hexenal and (*E*)-2-octenal. All the compounds were unambiguously identified from their molecular ions and mass spectra and by comparison of retention indices and mass spectra with those of authentic standards. The stereochemistry and position of the double bonds in the unsaturated acetates was confirmed to be (*E*)-2 by comparison of the retention times with those of standards; the *E* and *Z* isomers, and the different positional isomers have retention times that are sufficiently different to allow unambiguous assignment of the double-bond positions and geometry.[13] The positions of the double bonds in (*E*)-2-hexenal and (*E*)-2-octenal were unambiguous because the conjugation of the carbonyl groups with the double bonds

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

Entry	Compound[a]	Mean ± SD	Range	Kovats index (found)	Kovats index (literature)[12]
1	(*E*)-2-Hexenal	2.58 ± 1.94	0.10 – 6.03	860	854
2	(*E*)-4-Oxo-2-hexenal	84.1 ± 9.63	65.5 – 92.4	976	
3	Decane	0.38 ± 0.24	0.12 – 0.67	1000	999
4	(*E*)-2-Hexenyl acetate	0.26 ± 0.09	0.09 – 0.33	1028	
5	(*E*)-2-Octenal	87.9 ± 10.3	71.8 – 99.6	1066	
6	Undecane	0.21 ± 0.04	0.17 – 0.30	1100	1099
7	(*E*)-4-Oxo-2-octenal	0.29 ± 0.16	0.15 – 0.59	1162	
8	Dodecane	2.16 ± 0.15	1.99 – 2.40	1200	1199
9	(*E*)-2-Octenyl acetate	1.16 ± 1.09	0.19 – 3.12	1223	
10	1-Tridecene[b]	0.54 ± 0.04	0.46 – 0.57	1292	1292
11	Tridecane	100		1302	1299
12	Tetradecane	0.40 ± 0.11	0.25 – 0.57	1401	1399
13	Pentadecane	0.32 ± 0.10	0.19 – 0.49	1500	1500

[a]Hexadecane, heptadecane, octadecane, eicosane and 1-dodecene were also detected in trace amounts in some extracts. [b]Tentative identification.

increased the retention times substantially beyond that of the saturated analogs. The geometry of the double bonds was confirmed to be *E* from the match of the retention times and mass spectra with those of authentic standards (2-octenal: *Z*: *m/z* 83 > *m/z* 70; *E*: *m/z* 83 < *m/z* 70 and 2-hexenal: *Z*: *m/z* 83 > *m/z* 69; *E*: *m/z* 83 < *m/z* 69).

To provide standards for conclusive identification of insect-produced compounds, short syntheses of (*E*)-4-oxo-2-hexenal and (*E*)-4-oxo-octenal were performed (Scheme 1).

Because of the instability of these substances, the syntheses were thought to prepare the corresponding diols **2a** and **2b**, more stable compounds, which could be readily oxidized to the oxo-aldehydes as needed. Attempted distillation of the 4-oxo-2-alkenals resulted in some isomerization, which for our purposes proved to be advantageous because it provided the *Z* isomers for GC retention-time determinations. The good match of the retention times of the natural products with those of the major synthetic compounds confirmed the stereochemistry of the double bonds of the natural products as *E*. The retention indices of the oxo-aldehydes, not found in the literature, were calculated, and can be employed in future studies to support the identification of the natural compounds.

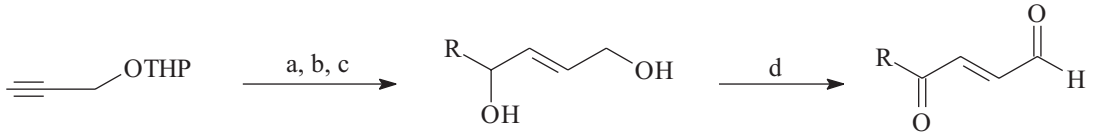
The identification of 1-tridecene remained tentative, based on comparison of the mass spectrum with database searching and retention index value, because the double bond position of this compound was not determined by microchemistry of the extract.

The identification of the structures of the alkanes found in the extracts was based on their mass spectra and good match with the retention indices of authentic standards.

The composition is also typical of what has been reported for several other pentatomid species.[1,15] The aldehydes and esters identified are strongly scented and strong irritants, providing both an easily detected warning signal and an effective 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.[16,17] However it has been reported for pentatomids that other *n*-alkanes, when combined with (*E*)-2-hexenal, were not as effective deterrents for other insects as *n*-tridecane, the major compound found in the extracts analyzed. Hence, *n*-tridecane appears to be the optimal *n*-alkane to work synergistically with the other scent compounds.[4]

Conclusions

The compounds extracted from the metathoracic scent gland of *D. melacanthus* were unambiguously identified from their molecular ions and mass spectra and from



1 **2a** R = Et; **2b** R = *n*-Bu **3a** = (*E*)-4-oxo-2-hexenal; **3b** = (*E*)-4-oxo-2-octenal

Scheme 1. Reaction conditions: (a) THF, *n*-BuLi; propanal or pentanal; (b) $LiAlH_4$, THF, reflux; (c) MeOH, PTSA (60% for **2a**, 61% for **2b**); (d) PCC, Al_2O_3, CH_2Cl_2 (77% for **3a**, 80% for **3b**).

comparison of their retention indices with those of authentic standards. The compounds have all been reported previously from other bug species.[1,15] Two of the compounds isolated were synthesized through a short route which allowed the preparation of more stable intermediates that could be converted into the natural compounds as needed.

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