Pheromone Blends of Predaceous Bugs (Heteroptera: Pentatomidae: Podisus spp.)

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Z. Naturforsch. 46c, 264–269 (1991); received October 9, 1990

Hemiptera, Stink Bug, Attractant, (E)-2-Hexenal, α-Terpineol

Male predaceous stink bugs (Pentatomidae: Asopinae) in the genus Podisus release long-range attractant pheromones from a pair of hypertrophied glands opening underneath the wings. Pheromone compositions are reported for four additional Podisus spp.: two Nearctic species (P. connexivus and an undetermined Podisus sp.), and two Neotropical species (P. placidus and P. mucronatus). Males of each species release (E)-2-hexenal, plus species-specific major components that include α-terpineol, linalool, 9-hydroxy-2-nonanone, and (E)-2-hexenyl tiglate. The pheromonal chemistry of the Neotropical species closely resembles that for the previously studied Nearctic species, P. maculiventris and P. fretus.

Introduction

Stink bugs in the New World genus Podisus (Pentatomidae: Asopinae) are obligatory predators, primarily of caterpillars and beetle larvae [1]. Adult Podisus males, and males in some other asopine genera, possess enormous dorsal abdominal glands (DAGs) opening between the third and fourth segments underneath the wings [2, 3]. Homologous sexually dimorphic DAGs have since been reported for two species of phytophagous pentatomoid bugs: the Australian spined citrus bug, Bipororulus bibax Breddin (Pentatomidae) [4]; and a West African shield bug, Sphaerocoris annulus (F.) (Scutelleridae), whose secretion consists of a mixture of C9 aliphatic aldehydes [5]. For two North American Podisus spp. the male-specific DAG secretions have been chemically elucidated, and shown to function as long-range attractant pheromones [6, 7]. Males of the spined soldier bug, P. maculiventris (Say), produce principally (E)-2-hexenal, benzyl alcohol and (E)-2-hexenyl tiglate. The male-specific DAG secretion also contains large amounts of small amounts of other monoterpenols [6]. In the sympatric species, P. fretus Olsen, the male-specific DAG secretion also contains large amounts of (E)-2-hexenal and benzyl alcohol, but S(+)-linalool (a minor constituent in the spined soldier bug DAG secretion) is the dominant monoterpenol of P. fretus males [7]. Besides attracting potential mates, calling Podisus males become vulnerable to a complex of parasitoids that exploit the pheromones as host-finding kairomones, and conspecific males and nymphs are attracted to calling males [8].

We report here on the male DAG chemical compositions of four additional Podisus spp.; two Nearctic species (P. mucronatus Uhler and P. placidus Uhler), and two Neotropical species (P. connexivus Bergroth and P. sp.).

Materials and Methods

Podisus placidus adults and eggs were collected during the last week in June, 1984, near Gay Head, Martha’s Vineyard Island, Massachusetts. Several generations of P. placidus were rearred in the laboratory on mealworm pupae, Tenebrio molitor L.
(Coleoptera: Tenebrionidae) (Rainbow Mealworms, Compton, CA). Four P. mucronatus adult males were collected near Tampa, Florida, on March 27, 1989. The Brazilian Podisus spp. were collected in a soybean field near Brasilia, on February 13, 1990. One generation of P. connexivus was reared in the laboratory on mealworm pupae; the analysis of P. sp. is based on a single field-collected male.

Extracts of the male DAGs were prepared as described in detail previously [6]. In short, the glands were dissected from CO2-anesthetized bugs submerged in tap water, fat body surrounding the excised glands was removed, and the glands were macerated in 50–200 μl of CH2Cl2 or heptane. Part of a male P. mucronatus DAG extract (30 μl) was esterified by addition of 10 μl of a diazomethane/ether solution, followed by acetic acid after 2 min. Airborne extracts of some P. placidus and P. connexivus males were prepared by confining single insects in a 150 ml glass column, drawing air by vacuum (40 ml/min) through ca. 30 mg of activated charcoal inside a Sweeny luer-lock filter holder (13 mm; Thomas Scientific, Philadelphia, PA), and extracting the filter with 200 μl of CH2Cl2. The contents of the two large nymphal DAGs are shed with each ecdysis making extraction of the exuviae a convenient method for isolation of the combined secretions [2, 3]. Therefore, P. connexivus exuviae were collected within a day of ecdysis and extracted with CH2Cl2 for analysis.

Samples were analyzed by gas chromatography (GC) on a bonded methyl silicone column (0.25 μm film, 14 m × 0.25 mm ID; DB-1™, J&W Scientific, Folsom, CA) using a Varian 3700 GC with helium as carrier (40 cm/sec), and a temperature program from 45 °C for 2 min to 230 °C at 15 °C/min. One extract from male P. connexivus DAGs was analyzed on a Cyclodex-B™ chiral column (0.25 μm film; 30 m × 0.25 mm ID; J&W Scientific) using a Hewlett-Packard 5890 GC with helium as carrier, isothermally at 110 °C. Reported compound percentages are based on mV output from the flame ionization detector using a Shimadzu C-R 3A recorder. Electron impact mass spectra (EI-MS) were obtained at 70 eV using a Finnigan 4510 GC-MS, equipped with a 30 m DB-1 column. Podisus placidus DAG extracts were also analyzed by chemical ionization mass spectrometry (CI-MS) using NH3 and ND3 as reagent gases.

Compounds identified by mass spectral data were cross-checked by GC coinjection of the natural product with an authentic standard under appropriate isothermal conditions. The following standards were obtained commercially: benzyl alcohol, benzaldehyde, linalool, terpinen-4-ol, tiglic acid, tiglyl aldehyde, n-tridecane, and 1-nonanol (Aldrich Chemical Co., Milwaukee, WI); (E)-2-hexenal, (E)-2-hexenyl, (E)-2-hexenoic acid, (E)-2-octenyl, (Z)-3-nonenol, and benzyl tiglate (Bedoukian Research Inc., Danbury, CN); trans-piperitol (PCR Research Chemicals Inc., Gainesville, FL); and α-terpineol (Hercules Inc., Wilmington, DE). (E)-2-Hexenyl tiglate, (E)-2-hexenyl, (E)-2-hexenoate, and (E)-2-hexenyl benzoate were synthesized as part of an earlier investigation [9], as were the enantiomers of α-terpineol [6]. (E)-4-Oxo-2-hexenal was synthesized according to Ward and VanDorp [10]. 9-Hydroxy-2-nonanone was synthesized from 6-bromo-1-hexanol (from 6-bromohexanoic acid and BH3, THF) and the sodium salt of ethyl acetoacetate in absolute ethanol by analogy with Vogel [11] (colorless liquid b.p. = 141–164 °C/9 mmHg; 95.3% pure by GC). A standard of 2-(4-hydroxyphenyl)ethanol was prepared by reduction of (4-hydroxyphenyl)acetic acid (Eastman Kodak, Rochester, NY) with LiAlH4 in tetrahydrofuran (pink crystals from water; m.p. = 89–91 °C).

Results

An airborne extract of a single 4-day-old P. connexivus male (Fig. 1A) and the extract of the DAGs from the same male (Fig. 1B) exhibited similar patterns by GC, except that the concentration of benzyl alcohol (2) is reduced in the aeration sample (2.4% versus 15% in the gland extract). α-Terpineol (5) is the major volatile of both the gland extract (72%) and the aeration extract (78%), plus (E)-2-hexenal (1) (3.2%, DAG extract; 6.0%, airborne extract), linalool (3) (2.5%; 3.2%), terpinen-4-ol (4) (1.5%; 2.1%), and trans-piperitol (6) (2.9%; 4.0%) (Fig. 1A and B). The enantiomers of α-terpineol were baseline separated on the Cyclodex-B column: S(−)-α-terpineol eluted at retention time (RT) = 21.5 min and R(+)-α-terpineol eluted at RT = 21.9 min. α-Terpineol from P. connexivus consisted of 98% of the R(+) and 2% of the S(−)-enantiomers. Coinjection of
synthetic $S(-)-\alpha$-terpineol with the insect-derived material increased the size of the peak at RT = 21.5 min without changing the peak shape (Fig. 1A, chart speed = 3 cm/min). The exuvial extract of *P. connexivus* is dominated by three compounds: 4-oxo-(E)-2-hexenal (7) (30%), linalool (3) (23%), and $n$-tridecane (8) (32%) (Fig. 1C). The DAG extract of the other Brazilian *Podisus* sp. contained (E)-2-hexenal (1) (36%), benzyl alcohol (2) (14%), and linalool (3) (45%) (Fig. 2).

Extracts of male *P. mucronatus* DAGs include a combination of hexenyl, tiglyl, and benzyl derivatives, but lack monoterpenols (Fig. 3). Based upon a pooled extract from 4 males, the principal components are (E)-2-hexenal (1) (38%), (E)-2-hexenol (10) (18%), benzyl alcohol (2) (13%), 266 J. R. Aldrich et al. • Pheromone Blends of Predaceous Bugs
Fig. 3. Gas chromatogram of the dorsal abdominal gland extract from four field-collected *Podisus mucronatus* males (9 = (E)-2-methyl-2-butenal (= tiglyl aldehyde), 1 = (E)-2-hexenal, 10 = (E)-2-hexenol, 11 = benzaldehyde, 12 = tiglic acid, 2 = benzyl alcohol, 13 = (E)-2-hexenoic acid, 14 = benzoic acid, 15 = (E)-2-hexenyl tiglate, 16 = (E)-2-hexenyl (E)-2-hexenoate, 17 = benzyl tiglate, and 18 = (E)-2-hexenyl benzoate).

and (E)-2-hexenyl tiglate (15) (15%). The percentages of (E)-2-hexenal and (E)-2-hexenol for these males analyzed separately by GC ranged from 40% aldehyde: 20% alcohol to 20% aldehyde: 36% alcohol. In addition, tiglic (12), (E)-2-hexenoic (13), and benzoic (14) acids were confirmed by GC-MS of their methyl-ester derivatives. Other identified minor components are tiglyl aldehyde (9), benzaldehyde (11), (E)-2-hexenyl (E)-2-hexenoate (16), benzyl tiglate (17), and (E)-2-hexenyl benzoate (18).

Gland and aeration extracts of *P. placidus* males also include both (E)-2-hexenal and (E)-2-hexenol in variable proportions, but the other identified components are heretofore unknown from *Podisus* or other asopines (Fig. 4). The EI-MS of the major component in the DAG extract (Fig. 4A, RT = 6.8 min) suggested 9-hydroxy-2-nonanone as a possible structure. NH₃ CI-MS of this component substantiated this possibility by indicating a molecular weight of 158 (base peak: m/z = 176; [M + NH₄]⁺), and ND₃ CI-MS confirmed the presence of one exchangeable proton in the molecule (base peak: m/z = 181; [M + ND₃ - H + D]⁺). The EI-MS and RT of synthetic 9-hydroxy-2-nonanone were identical to those for the natural product, verifying the assigned structure (23). Other aliphatic components (19–22) from male *P. placidus* DAG and aeration extracts were identified as for compound 23. The EI-MS of the minor component eluting just after compound 23 exhibited prominent ions at m/z (%) 77(20), 107(100), and 138(30, M⁺), suggesting an hydroxyphenyl ethanol structure for this component [12]. Synthetic 2-(4-hydroxyphenyl)ethanol coeluted with, and gave an identical EI-MS to, the insect-derived material. As for male *P. mucronatus* DAGs, the (E)-2-hexenal: (E)-2-hexenol ratio in *P. placidus* DAG extracts varied greatly, ranging from 13.5% of the aldehyde with no detectable alcohol to 4.4% aldehyde with 28.1% alcohol. A significant correlation between the age of a male (sampled from < 1-day-old to 29-days-old) and the (E)-2-hexenal: (E)-2-hexenol ratio was not detected (r = -0.0596, n = 11). Based upon a pooled sample of DAGs dissected from 90 lab-reared males (Fig. 4A), the *P. placidus* secretion consists of 5.7% (E)-2-hexenal (1), 20% (E)-2-hexenol (10), 0.30% (E)-2-octenal (19), 5.7% (E)-2-octenol (20), 3.1% (Z)-3-nonenal (21), 4.2% 1-nonanol (22), 57% 9-hydroxy-2-nonanone (23), and 0.4% 2-(4-hydroxyphenyl)ethanol (24). An airborne extract of
eight 17–29-day-old *P. placidus* males shows the same compounds as identified in the gland extracts (except for compound 24), but with concentrations increasing with decreasing molecular weights; 17% (E)-2-hexenal (1), 34% (E)-2-hexenol (10), 0.37% (E)-2-octenal (19), 6.4% (E)-2-octenol (20), 8.1% (Z)-3-nonenol (21), 5.6% 1-nonanol (22), and 21% 9-hydroxy-2-nonanone (23) (Fig. 4B).

**Discussion**

All the compounds identified from the DAG secretions of Brazilian *Podisus* males have been previously identified from North American *Podisus* spp. [2, 3]. In fact, the male DAG secretion of the *Podisus* sp. is quantitatively nearly identical to that for the North American species, *P. fretus* [7], and *P. connexivus* males produce a DAG secretion like that for males of the North American *P. maculiventris* except for a lower proportion of (E)-2-hexenal [6]. Furthermore, both *P. connexivus* and *P. maculiventris* [6] males produce predominantly *R*-(-)-a-terpineol in their DAGs. The exocrine blends from nymphs of *P. connexivus* and *P. maculiventris* are alike, as well [3]. While a specific determination was not achieved for the *Podisus* species chemically allied to *P. fretus*, morphologically this Brazilian asopine is clearly distinct from *P. fretus*. Thus, the first two South American *Podisus* spp. to be chemically characterized mirror the two North American species initially investigated. The ranges of the North American species do not overlap the ranges of the South American species [1].

The male DAG chemistry of *P. mucronatus* represents another interesting case of parallel semiochemical evolution among Heteroptera. (E)-2-Hexenyl tiglate and (E)-2-hexenyl (E)-2-hexenoate are the predominant volatiles produced in the male-specific ventral abdominal gland of the Central American bug, *Pachylis laticornis* (Coreidae) [9]. Benzyl tiglate is also a minor secre-
tory constituent of both the pentatomid and the corendid species.

The male DAG secretion of *P. placidus* resembles that of *P. mucronatus* in one respect: (E)-2-hexenol is an abundant secretory constituent in both species. Nevertheless, the remaining components identified from the *P. placidus* exocrine blend render the secretion highly distinctive and species-specific. One minor component from the DAG secretion of *P. placidus* provides yet another example of exocrinological parallelism in the Insecta; (Z)-3-nonenol is part of the male-produced pheromone of the Mexican fruit fly, *Anastrepha ludens* (Tephritidae) [13].

A pattern now appears to have emerged for *Podisus* attractant pheromones: (E)-2-hexenal is omnipresent, but idiosyncratic compounds are biosynthesized in sympatric species.

**Acknowledgements**

We thank Joseph E. Eger (DowElanco, Tampa, F.L.) for collecting and identifying *Podisus mucronatus*, and Thomas J. Henry (Systematic Entomology Laboratory, USDA-ARS) for identifications of the other North American *Podisus* species. Dr. Jocelia Grazia (Departamento de Zoologia, Instituto de Biociencias, U.F.R.G.S. – Porto Alegre – R.S.) kindly determined the South American species. Dr. Jocelyn Millar (Department of Entomology, University of California, Riverside) performed the chiral analysis, for which we are grateful. Susan Wilzer maintained the *P. placidus* colony and prepared the extract from 90 males. Mention of commercial products does not constitute an endorsement by the U.S. Department of Agriculture.