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Article

Alarm Pheromone System of Stink Bug *Piezodorus guildinii* (Heteroptera: Pentatomidae)

***Paulo H. G. Zarbin*[a*], *Miguel Borges*[b], *Alcindo A. dos Santos*[a], *Alfredo R. M. de Oliveira*[a], *Fábio Simonelli*[a] *and Francisco de A. Marques*[a]**

[a]*Universidade Federal do Paraná, Departamento de Química, CP 19081, 81531-990, Curitiba - PR, Brazil*

[b]*Embrapa, Cenargen, Área de Controle Biológico, CP 02372, 70849-970, Brasília - DF, Brazil*

Os compostos utilizados pelo percevejo da soja *Piezodorus guildinii* como feromônio de alarme foram caracterizados através da análise da composição química das secreções da glândula metatorácica dos insetos adultos. Além dos hidrocarbonetos característicos, (*E*)-2-hexenal e (*E*)-4-oxo-2-hexenal foram detectados como constituintes majoritários. Tais compostos já foram previamente descritos como feromônio de alarme em outras espécies de pentatomídeos.

The compounds utilized by the soybean stink bug *Piezodorus guildinii* as alarm pheromone were characterized by analysis of the chemical composition of the metathoracic scent gland secretions from adult bugs. In addition to characteristic hydrocarbons, (*E*)-2-hexenal and (*E*)-4-oxo-2-hexenal were detected as major constituents. These compounds were previously described as alarm pheromone in several other pentatomid species.

Keywords: *Piezodorus guildinii*, alarm pheromone,(E)-2-hexenal, (E)-4-oxo-2-hexenal

Introduction

Piezodorus guildinii is a member of the stinkbug complex in soybeans that is economically important pests in Brazil[1,2]. In our investigation on the chemical ecology related to the Pentatomidae family, we have started to elucidate the chemical communication system of *P. guildinii*[3] and we have reported previously that methyl 2,6,10-trimethyldodecanoate and methyl 2,6,10-trimethyltridecanoate are male-produced sex pheromones of this species[3,4]. Field experiments are now in progress to determine the potential for utilizing these compounds in an integrated pest management program.

An important characteristic of adult heteropterans is the fact that when molested, they usually retaliate by discharging volatiles secretions (defensive chemicals / alarm pheromones) from their ventral methatoracic glands (MTG)[5]. This intriguing mechanism has led to the isolation and identification of several defensive compounds released by a number of heteropteran species[5-8].

The *P. guildinii* metathoracic scent gland complex is similar of that reported by *Nezara viridula* by Gilby and Waterhouse[9]. The adult scent gland complex of both males and females consists of a median ventral metathoracic scent reservoir, which is orange-yellow in color, and paired colorless lateral glands sometimes called accessory glands. The lateral glands discharge through ducts into the reservoir, which also receives secretions from the gland cells which form its epithelium. The glands open to the exterior on the ventral surface[9].

The main purpose of the present work was to characterize the chemical composition of the metathoracic glands of *P. guildinii* and to identify the compounds that elicit dispersive behavior in other members of the species.

Experimental

Insects

Piezodorus guildinii and *Euschistus heros* were collected near Brasilia and raised continuously on soybean seeds (*Glycine max*), green beans (*Phaseolus vulgaris*), peanuts (*Arachis hypogaea*), and sunflower seeds (*Helianthus annuus*) at 26 ± 2 ºC, 70% relative humidity, and a 14:10 h light-dark cycle.

Extraction

An adult *P. guildinii* was pinned in a Petri dish with the dorsal side up. The dissection process consisted of cutting the dorsal abdominal edges of the insect cuticle up to the

*e-mail: pzarbin@quimica.ufpr.br

metathoracic region and under the scutelum. The dorsal abdominal cuticule was pulled back and the viscera were removed. The scent gland complex, located at the ventral abdominal metathoracic region, could be reached and removed with the aid of a small surgical scissors. The gland reservoir was removed and immersed in ~100 µL analytical grade hexane distilled from CaH_2 and stored at –20 ºC. One sample of dorsal abdominal gland (DAG) secretion of *E. heros* was prepared by immersing the second-instar nymphs in hexane (~100 µL) for 10 minutes and then transferring the extract directly to a clean conical bottom vial.

Chemical analyses

Samples were analyzed (~ 2 µL of the extract) by gas chromatography (GC) on a HP-5890 Series II (FID detector; 270 ºC) in splitless mode (250 ºC; 1.5 min). The HP-1 bonded methyl silicone column (25m x 0.25mm x 0.2µm) was operated at 50 ºC for 2 min to 220 ºC at 7 ºC/min, using helium as carrier gas (1.0 mL/min). Electron impact mass spectra were obtained at 70 eV using a Shimadzu QP-5000 GC-MS equipped with a DB-5 column (30m x 0.25 mm x 0.25 µm J & W Scientific) in splitless mode (250 ºC; 1.5 min) and with an interface temperature of 200 ºC. The temperature program and linear velocity of the carrier gas (He) was the same as described above. The IR refer to films and were measured on a Bomem M-102 spectrometer. The ^{1}H-RMN spectra were recorded at 200 MHz on a Bruker AC 200 spectrometer ($CDCl_3$). The ^{13}C-NMR spectra were recorded at 50 MHz on a Bruker AC 200. Column chromatography was carried out on columns packed with Merck Kieselgel 60, Art.-Nr 7734. (*E*)-2-hexenal, (*E*)-2-octenal, *n*-undecane, *n*-dodecane, *n*-tridec-1-ene, *n*-tridecane standards, and hexane were obtained from Aldrich Chemical Co.

Synthesis: (E)-4-(Tetrahydro-2'-pyranyloxy)-2-buten-1-ol **(9)**

A solution of *(E)*-1,4-butenediol **(8)** (2.14g; 24.3 mmol), dihydropyran (2.03g; 24.3 mmol), and *p*-TSA (70 mg) in dry THF (80 mL) was stirred for 6 h at –25 ºC. The product was extracted with ether, washed successively with saturated $NaHCO_3$ solution, water and dried under $MgSO_4$. Solvent removal and distillation under reduced pressure gave **9** in 60% yield (1.83 g); b.p. 133-37 ºC / 5-7 mm Hg; IR ν_{max} / cm^{-1} 3410, 2940, 2864, 1453, 1026; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.53-1.88 (m, 6H); 2.02 (s, 1H); 3.48-3.58 (m, 1H); 3.81-3.95 (m, 1H); 4.09-4.33 (m, 4H); 4.69 (d, *J* 4 Hz, 1H); 5.74 (dt, *J* 18 Hz ; 6 Hz, 1H); 5.83 (dt, *J* 18 and 6 Hz, 1 H); ^{13}C-NMR (50 MHz, $CDCl_3$) δ 19.4; 25.4; 30.6; 58.5; 62.2; 62.8; 97.6; 128.2; 132.3; GC-MS (70 eV) *m/z* 172 (M^+, 29%), 155 (6%), 101 (20%), 85 (100%), 67 (5%), 55 (3%), 39 (12%).

(E)-4-(Tetrahydro-2'-pyranyloxy)-2-butenal **(10)**

Pyridinium chlorochromate adsorbed in Al_2O_3 (29.9 g; 24.3 mmol) was suspended in dry CH_2Cl_2 (180 mL) at 25 ºC. Alcohol **9** (1.7 g; 9.9 mmol) in CH_2Cl_2 (5.0 mL) was added in one lot to the stirred solution. After 2h, the black reaction mixture was filtered through a short pad of SiO_2 and active charcoal. The solvent was removed under reduced pressure and the product obtained (1.35 g) was used in the next step without further purification; GC-MS (70 eV) *m/z* 169 (M-1, 3%), 101 (21%), 85 (100%), 67 (23%), 57 (22%), 41 (57%).

(E)-1-(Tetrahydro-2'-pyranyloxy)-2-hexen-4-ol **(11)**

To cold ethylmagnesium bromide prepared from magnesium (0.16g; 6.5 mmol) and bromoethane (0.73g; 6.5 mmol) in dry THF (30 mL), was added slowly and with constant stirring a soln. of aldehyde **10** (1.1 g; 6.40 mmol) in THF (5.0 mL). The mixture was left overnight at room temperature and quenched with an ice-cold solution of NH_4Cl and worked-up in the usual manner. The crude product was chromatographed over silica gel (ethyl ether: hexane / 2:1) and alcohol **11** was obtained in 70 % yield (0.92 g). IR ν_{max}/ cm^{-1} 3422, 2940, 2875, 1453, 1121, 1026; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.93 (t, *J* 7.4 Hz, 3H); 1.49-1.90 (m, 8H); 3.47-3.53 (m, 1H); 3.81-4.38 (m, 4H); 4.64 (t, *J* 3Hz, 1H); 5.76-5.80 (m, 2H); ^{13}C-NMR (50 MHz, $CDCl_3$) δ 9.64; 19.47; 25.4; 29.9; 30.6; 62.2; 67.0; 73.6; 98.0; 127.3; 135.0; GC-MS (70 eV) *m/z* 182 (M -18, 2%), 169 (1%), 98 (6%), 85 (100%), 67 (10%), 57 (23%), 41 (12%).

(E)- 2-Hexen-1,4-diol **(12)**

A solution of alcohol **11** (0.80 g; 4.0 mmol), methanol (12 mL) and *p*-TSA (40 mg) was stirred at room temperature for 4 h. The solvent was removed under reduced pressure and the residue was diluted in ether (15.0 mL), washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue obtained (0.40 g) was used in the next step without further purification. GC-MS (70 eV) *m/z* 98 (M -18, 33%), 87(16%), 85 (12%), 81 (100%), 69 (44%), 57 (90%), 55 (14%), 43 (46%).

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

As described above for the synthesis of aldehyde **10**, diol **12** (0.30 g; 2.60 mmol) was converted into compound **2** (77 % yield; 0.22 g) using PCC adsorbed in Al_2O_3 (12.7 g, 10.4 mmol) and after purification through column chromatography on silica gel (hexane : ethyl ether / 1:3). IR ν_{max} / cm^{-1} 2982, 1752, 1699, 1615, 1121, 980; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.16 (t, *J* 7 Hz, 3H); 2.73 (q, *J* 7 Hz,

2H); 6.71-6.94 (m, 2H); 9.78 (d, *J* 6 Hz, 1H); ^{13}C-NMR (50 MHz, $CDCl_3$) δ 7.5; 34.5; 137.2; 144.6; 193.3; 200.3; GC-MS (70 eV) *m/z* %: 112 (M^+, 16%), 97 (6%), 83 (100%), 69 (4%), 55 (77%), 39 (8%).

Results and Discussion

The chromatograms obtained by analyses of the crude hexane extract of the metathoracic glands secretions of *P. guildinii* showed seven peaks whose structures could be identified, three of those in higher amount than the other ones (Figure 1).

By comparison of these natural products with those on the computerized mass spectra data base, peaks **1**, **3-7** were tentatively identified as (*E*)-2-hexenal, (*E*)-2-octenal, *n*-undecane, *n*-dodecane, *n*-tridec-1-ene and *n*-tridecane, respectively. The identifications were confirmed by GC co-injection with commercial standards. EI-MS of compound **2** gave a base peak at *m/z* 83 along with the molecular ion $M^{+\cdot}$ 112 (11%). This structure was tentatively identified by comparison with spectra from natural products previously identified as pheromones and synthetic standards, as described below.

Defensive secretions from first and second-instar nymphs of stink bugs belonging to the same family as *P. guildinii* were described by Borges and Aldrich[6]. In immature heteropterans (nymphs), defensive secretions are produced in the dorsal abdominal glands (DAGs) instead of the metathoracic glands and the gland contents usually differ within a species[5,6]. DAG extracts of second-instar nymphs of *Euschistus heros* contained four compounds, and one of these had a MS consistent with the mass spectrum data of component **2**[6]. GC-MS co-injection of the MTG extract of *P. guildinii* with a DAG extract of *E. heros* showed that compound **2** had the same retention time and mass spectrum of the compound identified as (*E*)-4-oxo-2-hexenal [MS *m/z* (%): *112* (11), M^+; *97* (4), $CHOCHCHCOCH_2^+$; *83* (100), $CHOCHCHCO^+$; *55* (78), $CHOCHCH^+$].

To confirm this identification, we synthesized (*E*)-4-oxo-2-hexenal **2** in five steps, following the synthetic route shown in Scheme 1. The monoprotected alcohol **9** was obtained after protection of the diol **8** with DHP in 60 % yield[10]. Oxidation of **9** with PCC in methylene chloride afforded the aldehyde **10** in 80% yield[11]. Coupling of this compound with ethylmagnesium bromide[12] yielded the alcohol **11** (65 %) which was deprotected to give diol **12** (88%) by treatment with *p*TSA in methanol[10]. Oxidation of this compound with PCC supported on Al_2O_3 in CH_2Cl_2 afforded the desired product in 77% yield, which was found by GC-MS co-injection to be indistinguishable from the natural product.

Pinder and Staddon[13] described the first synthesis of **2** by employing an acid catalyzed hydrolysis of 2-ethyl-2,5-dimethoxy-2,5-dihydrofuran, which gives a mixture

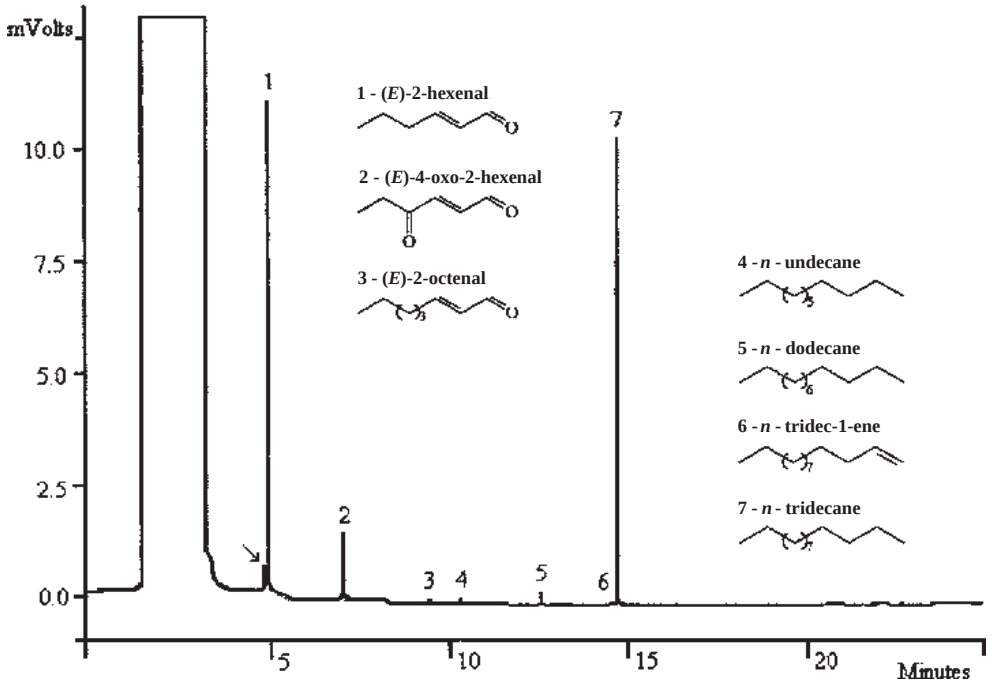
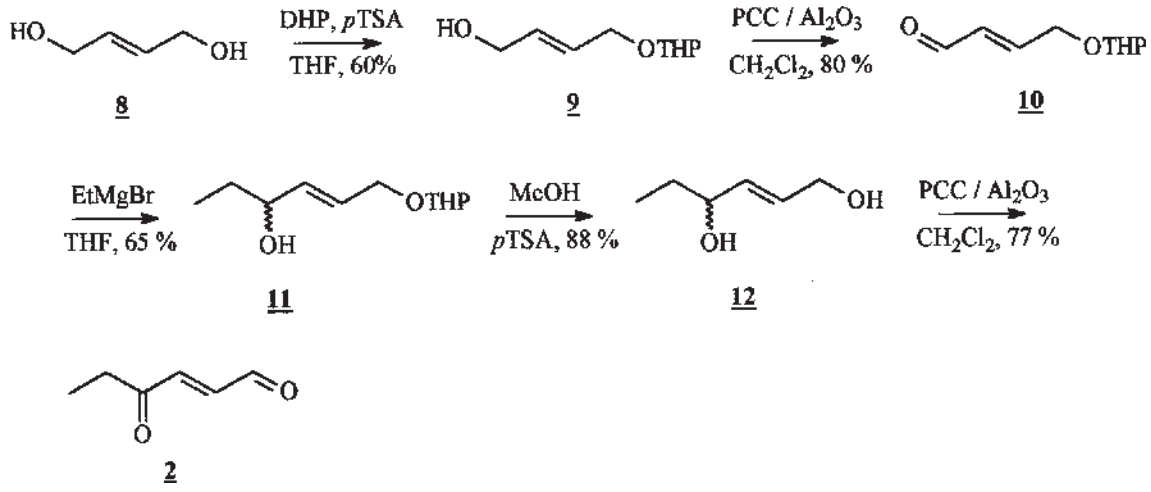


Figure 1. GC profile of a crude hexane extract sample from metathoracic scent gland of adult males *P. guildinii*. The peak labeled with an arrow is due to solvent impurity. Peak numbers correspond to compounds identified.



Scheme 1. Synthesis of *(E)*-4-oxo-2-hexenal **2**.

of the *cis* (major) and *trans* isomers. Later, Ward and Van Dorp[14] synthesized the same compound by reaction of propanal with the Grignard derivative of 1,1-diethoxy-2-propyne, followed by the reduction of the acetylenic bond with $Na/NH_3(l)$, oxidation with active manganese dioxide and acid hydrolysis. We now developed a synthesis of **2** in good overall yield by an operationally simple strategy. This synthesis offers a useful alternative to those described in the literature.

Therefore, the chemical composition of the metathoracic scent gland secretions of *P. guildinii* was identified as a mixture of (*E*)-2-hexenal, (*E*)-4-oxo-2-hexenal, (*E*)-2-octenal, *n*-undecane, *n*-dodecane, *n*-tridec-1-ene and *n*-tridecane (Figure 1). This composition is similar in both sexes indicating that these compounds do not play any role, as sexual attractant, together with the ones previously reported[3]. Compounds **1**, **4**, **5**, and **7** are known toxins, irritants, or repellents[15,16], and are released to the stink bugs in response to disturbance. These facts strongly suggest that they really acts as chemical defenses of the species. Further studies in field will clarify their role in the chemical ecology of *P. guildinii.*

It is not know how the various secretions components interact. However, it was reported for pentatomid that (*E*)-2-hexenal and *n*-tridecane were more effective as repellents to insects when combined than when individually tested[17]. Furthermore, other *n*-alkanes when combined with (*E*)-2-hexenal were not as effective deterrents towards other insects as *n*-tridecane. Hence, *n*-tridecane appears to be the optimal *n*-alkane to work synergistically with (*E*)-2-hexenal to repel insects[16]. The other secretions components of the multicomponent blend of *P. guildinii* may likewise function in an additive way.

(*E*)-2-hexenal **1** has been reported as active component for *Dysdercus intermedius*[18], *Cimex lectularius*[19], *Nezara viridula*[20] and *Elasmucha grisea*[21], while (*E*)-4-oxo-2-hexenal **2**, which appears to be biosynthesized via oxidation of **1** by enzymes secreted into the reservoirs of the glands[22], was described for *Euschistus heros*, *Euschistus tristigmus*, *Thyanta perditor*, and *Edessa meditabunda*[6].

We recently reported that the crude aeration extract of *P. guildinii* has attracted the egg parasitoids *Trissolcus teretis*, *Telenomus podisi*, and *Trissolcus urichi* to the baited traps[4]. The same phenomenon was observed when the synthetic sex attractant pheromone was employed[3,4]. Parasitoids have been reported to respond to the host's body odor; sex, and aggregation pheromones; salivary constituents; excretory products; webbing; honeydew; body scales; and eggs[7,23]. It has been reported that the egg parasitoid *Trissolcus basalis* utilizes a defensive substance produced by its host bug as a long-range attractant kairomone[24].

Therefore, these simple structures found on the metathoracic glands of adults *P. guildinii* could be used as kairomone to synchronize the parasitoid population at the beginning of the host flight season and pave the way for the development of invaluable tools in integrated pest management programs for this important soybean pest.

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