

Identification of (*Z*)-4- and 1-Tridecene in the Metathoracic Gland Secretions of Stink Bugs Employing the GC/FT-IR Technique

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Abstract The composition of stink bug allomones is similar for most species and includes hydrocarbons, as well as saturated and unsaturated aldehydes and esters. Analysis of extracts of the metathoracic glands of seven pentatomid species from different genera by gas chromatography/Fourier transform-infrared (GC/FT-IR) showed that the compound previously thought to be 1-tridecene actually showed characteristic bands of an internal as well as terminal double bond, the former compound having (*Z*)-configuration. The mass spectrum of an epoxy-derivative of the natural material demonstrated that the internal double bond was located at C4. GC/FT-IR and co-injections with synthetic standards on different capillary columns proved that all these stink bugs had 1-tridecene and (*Z*)-4-tridecene in their defensive secretions.

Keywords Heteroptera · Metathoracic gland · Microderivatization · Chemical defense

Introduction

Stink bugs (Heteroptera: Pentatomidae) are well known to produce large quantities of strong-smelling and irritating defensive chemicals (allomones), which are produced by nymphs and adults in the dorsal abdominal glands (DAG) and metathoracic glands (MTGs), respectively (Aldrich 1998). So far, more than 25 pentatomid species have had their defensive chemical compounds studied. The compounds found in all species are

quite similar, including aliphatic hydrocarbons, aldehydes, oxo-alkenals and esters (Fávaro et al. 2011, 2012), and some have already had their biological role determined. For example, the DAG secretion of first-instar nymphs of some pentatomid species contains (*E*)-4-oxo-2-decenal, which is totally absent in the secretions of later instars, and mediates aggregation (Fucarino et al. 2004). Also, *n*-tridecane, the major chemical defensive component in many pentatomid species, synergizes the longer chain oxo-alkenals and (*E*)-2-alkenals in deterring predators (Eliyahu et al. 2012).

The minor alkene component present in pentatomid secretions has always been tentatively identified as 1-tridecene (Fávaro et al. 2011, 2012; Krall et al. 1999; Moraes et al. 2008; Noge et al. 2012; Zarbin et al. 2000). However, due to the limited amount of natural tridecene in the extracts, none of these cited works could prove the double bond position unambiguously.

In this work, we reinvestigated the chemical structure of tridecene detected in the defensive blends of the stink bugs *Agroecus griseus*, *Pallantia macunaima*, *Pellaea stictica*, *Loxa deducta*, *Nezara viridula*, *Euschistus heros*, and *Piezodorus guildinii*, by employing gas chromatography coupled with Fourier transform infrared (GC/FT-IR) and mass spectrometry (GC/MS), as well as by microderivatizations.

Methods and Materials

Insects Stink bugs *Agroecus griseus*, *Pallantia macunaima*, *Pellaea stictica*, *Loxa deducta*, *Nezara viridula*, *Euschistus heros*, and *Piezodorus guildinii* were collected at EMBRAPA soybean fields in Londrina, Paraná State, Brazil (23°11' S, 51°11' W), and were maintained in the laboratory in plastic cages (35 × 20 × 20 cm) at 26 ± 2 °C with 70 % relative humidity and a 14/10 h L/D photoperiod. The colonies were reared

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on soybean seeds (*Glycine max*), green beans (*Phaseolus vulgaris*), peanuts (*Arachis hypogaea*), and glossy privet fruits (*Ligustrum lucidum*). The food was replaced every 3 d.

Extraction of Dorsal Abdominal Glands and Metathoracic Glands Contents Adults were killed by freezing, pinned dorsal-side-up through the prothorax in a Petri dish, and submerged in tap water. The dissection consisted of removing the wings, cutting the lateral margins of the abdomen anteriorly up to the metathorax, and transversely cutting the anterior margin of the scutellum. The tergal cuticle was pulled back, and the viscera were removed. Then, the scent gland complex, consisting of reservoir and lateral accessory glands, was removed, dried with tissue paper, immersed in 200 μl of analytical grade hexane (Aldrich, Milwaukee, WI, USA), and stored at $-20\text{ }^{\circ}\text{C}$ until analysis. Three extracts with at least three MTG's of both sexes of unknown age were prepared for each species (Fávaro et al. 2011, 2012; Zarbin et al. 1999).

Chemical Analysis Volatile extracts were analyzed by gas chromatography/Fourier transform-infrared spectroscopy (GC/FT-IR) with a Shimadzu GC2010 gas chromatograph coupled to a DiscovIR-GC infrared detector ($4,000\text{--}750\text{ cm}^{-1}$, resolution of 8 cm^{-1} , Spectra Analysis, Marlborough, MA, USA). The GC was equipped with a ECTM-5 and ECTM-WAX (Alltech, 30 m \times 0.25 mm \times 0.25 μm film thickness) capillary columns, helium as carrier gas (1 ml/min), and operated in splitless mode ($250\text{ }^{\circ}\text{C}$). The temperature program began at $50\text{ }^{\circ}\text{C}$ for 1 min, increasing at $7\text{ }^{\circ}\text{C}/\text{min}$ until reaching $200\text{ }^{\circ}\text{C}$, and maintaining this temperature for 10 min.

Gas chromatography/mass spectrometry (GC/MS) data were acquired using a Shimadzu QP2010-Plus electron ionization mass detector operating in the electron impact mode (70 eV) with an RTX-5[®] capillary column. The injector mode and program temperature were as described above.

Epoxidation of Carbon-Carbon Double Bonds A MTG extract in hexane (20 μl) of each species was filtered through a small silica column, made with a Pasteur pipette, with 0.5 ml of hexane to obtain a hydrocarbon extract. Then, the extract was added to an equal volume of *m*-CPBA in dichloromethane ($\sim 2\text{ mg}/\text{ml}$) in a glass vial. The mixture was stirred at room temperature for about 1 hr, and the product solution was washed with 20 μl of diluted sodium carbonate (Attygalle 1998). The same procedure was used with synthetic (*Z*)-4-tridecene (20 μl of 1,000 ppm solution in hexane).

Chemical Standards 1-Tridecene was purchased from Aldrich. (*Z*)-4-Tridecene was synthesized by coupling 1-decyne with 1-bromopropane (Kang and Parie 1988) followed by hydrogenation of the product with Lindlar catalyst (Pd/CaCO₃). The (*E*)-isomer was obtained by reacting the alkyne with LiAlH₄, under reflux of diglyme (Zarbin et al. 2007).

Results and Discussion

GC/FT-IR analysis of all MTG extracts showed similar infrared spectra for the natural tridecene (Fig. 1a). In addition to the absorptions between $2,960\text{ cm}^{-1}$ and $2,850\text{ cm}^{-1}$ relative to the C-H stretching mode characteristic of saturated carbon chains, the bands at $3,083\text{ cm}^{-1}$, $3,006\text{ cm}^{-1}$, 993 cm^{-1} , and 915 cm^{-1} also were present.

These results are not totally in agreement with those expected for 1-tridecene. Besides the absorptions related to vinyl groups at 915 cm^{-1} (=CH wag), 994 cm^{-1} (*trans* = CH wag) and $\sim 3,080\text{ cm}^{-1}$ (out-of-phase = CH stretch), it was also possible to recognize an internal double bond with *cis* configuration due to the band at $3,006\text{ cm}^{-1}$ of C-H stretching mode (Svatos and Attygalle 1997). These results suggested the coelution of compounds that have a vinyl and an internal double bond with *cis* configuration. Because of the absence of the band at 970 cm^{-1} , it was possible to affirm that the *trans*-isomer was not present.

To verify coelution of the two tridecenes on the nonpolar GC column, the extracts also were analyzed on a polar capillary column (ECTM-WAX). The chromatogram obtained showed that the single peak corresponding to the tridecene on a ECTM-5 column was now partially separated. In an attempt to obtain a baseline separation of these peaks, the rotation velocity of the ZnSe disk where the eluted compounds are deposited for IR analyses was increased (Spectra Analysis 2009), making it possible to observe the complete separation of tridecenes in question (Fig. 1b). The IR spectra of these peaks confirmed the presence of the two proposed tridecene isomers.

To determine the position of the internal double bond, a hydrocarbon fraction of a MTG extract was submitted to epoxidation (Attygalle 1998). The mass spectrum of the epoxidation product (Fig. 1c) exhibited a fragment at *m/z* 155, which indicated the presence of a double bond at C4. Thus, from the GC/FT-IR analysis of natural compound and GC/MS analysis of the epoxy-derivative, (*Z*)-4-tridecene was proposed as the chemical structure. To confirm the proposed structure, the synthetic (*Z*)-4-tridecene also was subjected to an epoxidation reaction, as done with the natural compound, and the product was identical to the natural epoxy-derivative. The retention indices of all of these compounds are given in Table 1.

The two isomers of tridecene were found in the MTG secretions of all seven species studied at different ratios ranging from 1:1.3 (*Z*)-4-tridecene : 1-tridecene in *L. deducta* to 1:15 in *E. heros*. As the extracts of glands were prepared from insects at different ages, it was not possible to conclude unambiguously that the different ratios are species-specific.

The (*Z*)-4-tridecene has been found previously in the chemical defensive secretions of other families, and has had its biological function proved. Due to mass spectra and

Fig. 1 a Infrared spectra of natural tridecene, 1-tridecene, (Z)-4-tridecene, and (E)-4-tridecene (resolution 8 cm^{-1}); **b** Chromatogram from GC/FT-IR analysis of metathoracic gland extract from *Loxa deducta* analyzed on $\text{EC}^{\text{TM}}\text{-5}$ and $\text{EC}^{\text{TM}}\text{-WAX}$ capillary column; the latter analyses was performed increasing the disk rotation of the DiscovIR-GC infrared detector; **c** mass spectrum of epoxy-derivative from (Z)-4-tridecene

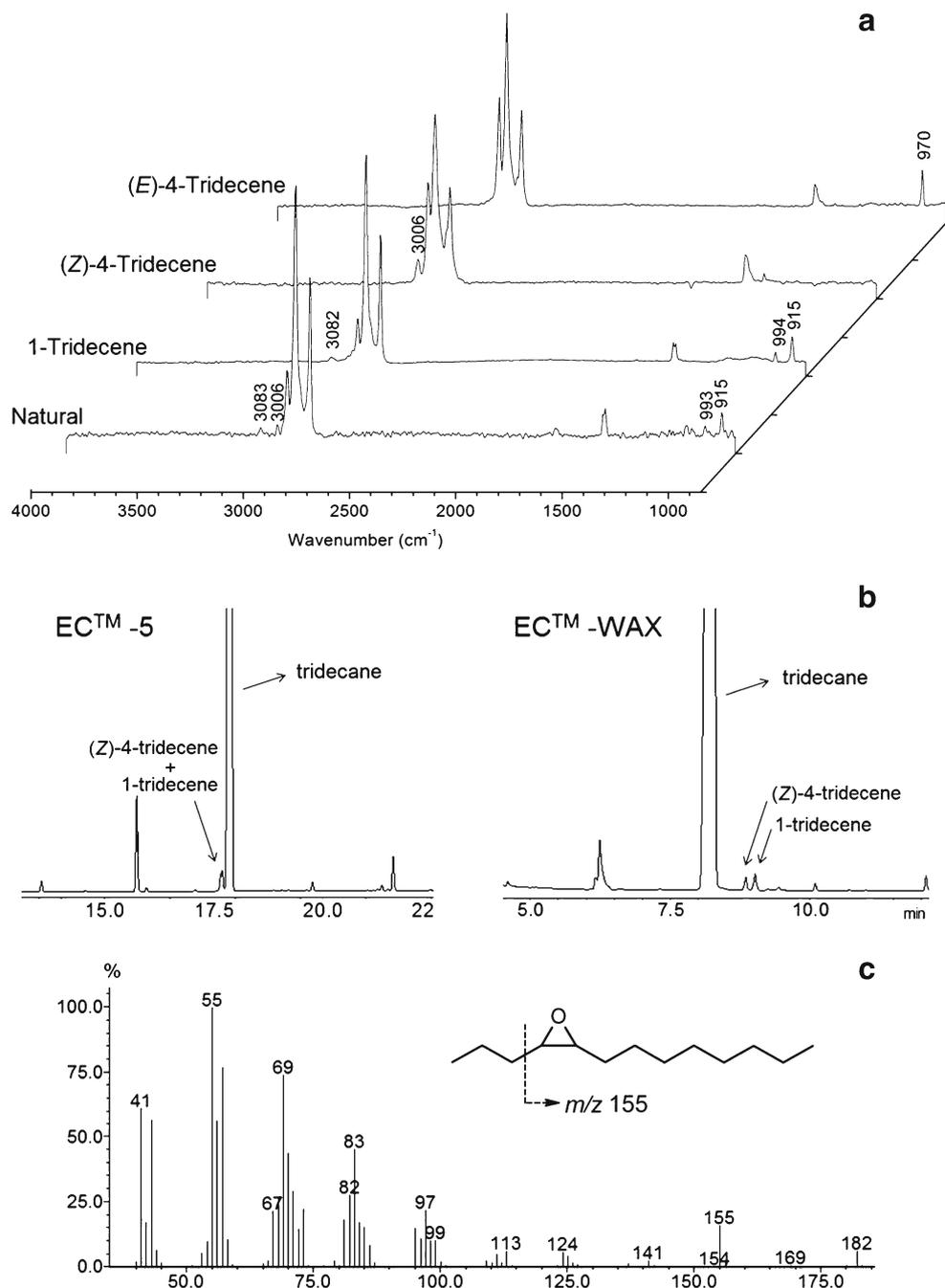


Table 1 Retention indices of the isomers of tridecene and the epoxy-derivative of (Z)-4-tridecene (relative to the retention times of *n*-alkanes)

	RTX-5 [®]	$\text{EC}^{\text{TM}}\text{-WAX}$	$\text{EC}^{\text{TM}}\text{-1}$
1-Tridecene	1295	1339	1292
(Z)-4-Tridecene	1296	1332	1292
(E)-4-Tridecene	–	1331	1293
Epoxy-derivative of (Z)-4-tridecene	1472	–	1454

retention times similarities, this compound was first identified as 1-tridecene in prothoracic gland (PG) extracts of *Chrysopa oculata* and *C. nigricornis* (Neuroptera: Chrysopidae), and further investigations showed that the correct structure was (Z)-4-tridecene (Aldrich et al. 2009). Zhu et al. (2000) identified (Z)-4-tridecene in defensive secretions of *Chrysoperla carea* and showed that this compound elicited arrestment behavior in Y-tube experiments, and that it was antagonist to attraction in field experiments. This compound also has been described as an alarm and defense component of several ant

species (Lanne et al. 1988). However, further studies will be necessary to clarify the exact function of these two isomers for pentatomids.

In summary, we described for the first time the identification of (*Z*)-4-tridecene as a component of the MTG secretion of several Pentatomidae species, in addition to the 1-tridecene. Moreover, this work showed the importance of using various analytical techniques such as GC/FT-IR, GC/MS, microderivatization, and coinjection with synthetic standards in order to reach the correct assignment of the chemical structure of a natural compound.

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