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Article

Identification and Synthesis of the Male-Produced Sex Pheromone of the Soldier Beetle *Chauliognathus fallax* (Coleoptera: Cantharidae)

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Chauliognathus fallax Germar 1824 (Coleoptera: Cantharidae) occurs in North and South America and Australia. Gas chromatographic (GC) analyses of volatiles released by adults showed the presence of a male specific compound. GC coupled with electroantennographic detection (GC-EAD) showed that this compound is exclusively bioactive on female antennae, suggesting it to be a sex pheromone. GC coupled with mass spectrometry (GC-MS) and Fourier transform infrared spectroscopy (GC-FTIR), as well as dimethyl disulfide (DMDS) derivatization and hydrogenation, suggested the target compound to be (*Z*)-tricos-11-ene. Unambiguous structural proof was achieved by independent synthesis, whereas the biological significance of the compound as a sex pheromone was confirmed by field bioassays.

Keywords: soldier beetle, GC-MS, GC-FTIR, pheromone synthesis, (*Z*)-tricos-11-ene

Introduction

Beetles belonging to the Cantharidae family are referred to as soldier or leatherwing beetles. They form a family containing 160 genera and 5100 species.[1] The genus *Chauliognathus*, consisting of more than 250 species, occurs in North and South America and Australia, mainly in the Neotropical ecozones.[2] Beetles of the specie *Chauliognathus fallax* are active during the day and are often found in large numbers on flowers and vegetation. They are important egg and larval predators of chrysomelid beetles defoliating eucalyptus, but also feed on pollen, nectar and new leaves.[3,4] Larvae occur on the ground and leaf litter and are predators and occasionally phytophagous.[5]

The specie *Chauliognathus fallax* frequently occurs in Southern Brazil, along with other species of the genus. The species shows a high degree of color polymorphism on the elytra and is a target of long-term studies, indicating that the origin of this polymorphism in species of the so called yellow-black complex goes back to a common ancestor, suggesting evidence of a Müllerian mimicry.[6] However, the explanation for the maintenance of the polymorphism is still unclear and could be caused by assortative mating, natural selection or other natural processes.[7] The study of chemical ecology as well as other ecological aspects of this species could help on the discovery and development of an environmentally friendly approach to the control of chrysomelid beetles.

The alkaloids senecionine, integerrimine, retrorsine and usaramine were previously identified as potential defensive compounds of *C. fallax*.[8] However, to the best of our knowledge, this paper describes the first sex pheromone identified in the family Cantharidae.

Experimental

Insects

Beetles of the specie *Chauliognathus fallax* were collected at the Universidade Federal do Paraná (UFPR) (Centro Politecnico) in Curitiba (Paraná State, Brazil, S25.45, W49.23). Adults (without age and mating status information) were sexed according to characters of the external genitalia,[9] and maintained in plastic cages (35 × 20 × 20 cm) at 25 ± 2 °C, 70 ± 5% humidity and L14:D10 (14 h light:10 h darkness) photoperiod. The colony was reared with 10% sucrose solution on cotton wad.

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Collection of volatiles

Volatiles were collected by aeration.[10] Ten males and ten females were placed separately in aeration chambers (33 × 3.5 cm ID), and a continuous flow of 1 L min^{-1} of humidified and charcoal-filtered air was pulled through each chamber, carrying the volatiles to glass tubes (6 × 0.3 cm) containing 20 mg HayeSep® (80/100 mesh, Sigma-Aldrich, Germany). After 24 h, the volatiles were eluted from the polymer with hexane (2 × 300 µL) and concentrated to 100 µL (10 µL *per* insect) under a gentle flow of argon. The collection of volatiles was continued for 2 weeks, and the extracts were stored at –20 °C until the use.

Chemical analyses

Gas chromatography (GC) was performed by using a Shimadzu GC2010 gas chromatograph equipped with a flame ionization detector (FID) and a RTX-5 column (Restek, 30 m × 0.25 mm × 0.25 µm film thickness). The injections were performed in splitless mode at 250 °C. The column temperature was programmed to start at 50 °C for 1 min and subsequently increased to 250 °C at a rate of 7 °C min^{-1} with a final hold of 10 min.

The retention indices (RI) were determined in a polar (RTX-WAX Restek, 30 m × 0.25 mm × 0.25 µm film thickness) and in a non-polar columns (RTX-5 Restek, 30 m × 0.25 mm × 0.25 µm film thickness). The column oven was programmed to start at 40 °C and subsequently increased to 250 °C at a rate of 3 °C min^{-1} with a final hold of 10 min.

GC coupled with mass spectrometry (GC-MS) was carried out on a Shimadzu QP2010-Plus mass spectrometer under the experimental conditions described above.

GC coupled with Fourier transform infrared spectroscopy (GC-FTIR) was carried out on a Shimadzu GC2010, coupled to a DiscovIR-GC infrared detector (DANI Instruments, Marlborough, Massachusetts, USA), with scan rage of 4000-750 cm^{-1} and resolution of 8 cm^{-1}, and again under the conditions described above.

GC coupled with electroantennographic detection (GC-EAD) was performed with a Shimadzu GC2010 coupled to a Syntech® electroantennographic detector (Hilversum, Netherlands).[11] GC was equipped with an RTX-5 capillary column (30 m × 0.25 mm × 0.25 µm) and operated in splitless mode (250 °C). The initial temperature was 50 °C, kept for 1 min and then increased to 250 °C at 10 °C min^{-1}, and hold at this temperature for 10 min. The column effluent was split in a ratio of 3:1, with one part going to the FID detector (270 °C) and three parts to the EAD. The electroantennograms were recorded using the Syntech GC-EAD32 program (version 4.6).

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra of the synthetic compounds were recorded on a Bruker ARX-200 spectrometer (200 and 50 MHz, respectively) as $CDCl_3$ solutions. Chemical shifts are expressed in ppm relative to TMS ($(CH_3)_4Si$).

Fractionation of extracts

Natural extracts (100 µL) were fractionated on a silica gel column (10 mm) using the tip of a Pasteur pipette, eluted with hexane (100 µL), hexane:diethyl ether ratio of 9:1 (100 µL), hexane:diethyl ether ratio of 6:4 (100 µL), hexane:diethyl ether ratio of 1:9 (100 µL) and diethyl ether (100 µL).

Micro-derivatizations

Pd/C catalyzed hydrogenation

The crude extract (100 µL) was put into a 2 mL vial containing 400 µL of hexane and a magnetic stirring device. The vial was flushed with Ar, and a catalytic amount of Pd/C (10%) was added. The vial was then flushed with hydrogen and maintained under stirring at 20 °C for 120 min. Subsequently, the catalyst was filtered off, and the extract was concentrated to 100 µL, followed by GC-MS analysis.

Methylthiolation

30 µL of DMDS (dimethyl disulfide, Sigma-Aldrich, USA), 30 µL of iodine in CS2 (5% m/m) and 30 µL of the crude natural extract were added to an ampoule. This was sealed and maintained at 30 °C for 12 h. The resulting solution was washed with a 10% solution of $Na_2S_2O_3$ and filtered over Na_2SO_4 prior to GC-MS analysis.[12]

Synthesis

Undecanal (**4**)

A pre-prepared solution of DMSO (0.87 mL) in 9 mL of dichloromethane (DCM) was added dropwise to a stirred solution of oxalyl chloride (0.6 mL, 6.3 mmol) in DCM (7.8 mL) at –78 °C. After 5 min, a solution of 1-undecanol (Sigma-Aldrich, USA) (**3**) (900 mg, 5.2 mmol) in DCM (7.5 mL) was added. The resulting mixture was stirred for 15 min at –78 °C, and triehtylamine was added (3.6 mL, 26 mmol). After 10 min, the temperature was increased to room temperature, and DCM (30 mL) was added. The resulting mixture was washed with saturated solutions of NH_4Cl and brine and dried with Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue was

purified by flash chromatography, furnishing undecanal (**4**) in 92% yield (816 mg, 4.8 mmol);[13] ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.91 (t, 3H, *J* 6.88 Hz), 1.25-1.36 (m, 14H), 1.54-1.74 (m, 2H), 2.45 (td, 2H, J_1 7.30 Hz, J_2 1.89 Hz), 9.79 (t, 1H, *J* 1.89 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.1, 22.6, 29.1, 29.3, 29.3, 29.4, 29.5, 31.9, 43.9, 202.9; MS *m/z* (%) 152 (1), 142 (5), 126 (23), 124 (15), 123 (8), 110 (17), 109 (16), 98 (15), 97 (36), 96 (48), 95 (41), 85 (25), 84 (28), 83 (37), 82 (84), 81 (47), 72 (11), 71 (49), 70 (40), 69 (48), 68 (54), 67 (54), 57 (100), 56 (57), 55 (80), 54 (18), 45 (14), 43 (99), 42 (25), 41 (91).

Dodecyltriphenylphosphonium bromide

The phosphonium salt was prepared by heating 1-bromododecane (Sigma-Aldrich, USA) (**5**) (2.00 g, 8.0 mmol) and triphenylphosphine (Sigma-Aldrich, USA) (2.16 g, 8.0 mmol) in a sealed ampoule at 90 °C for 24 h.[14] The resulting product was used in the next step without further purification.

(*Z*)-Tricos-11-ene (**1**) and (*E*)-tricos-11-ene (**2**)

A solution of dodecyltriphenylphosphonium bromide (2.52 g, 4.94 mmol) in dry THF (30 mL) was stirred for 20 min at room temperature under an argon atmosphere. The mixture was cooled to −78 °C, and *n*-BuLi (*n*-butyllithium, 3.3 mL, 5.14 mmol, 1.57 mol L^{-1}) was added dropwise. The solution was slowly warmed to 0 °C and stirred at this temperature for 30 min. The reaction was cooled again to −78 °C, and undecanal (**4**) (0.700 g, 4.11 mmol) in THF (5 mL) was added and stirred for 20 min at the same temperature. The mixture was warmed to room temperature and stirred for additional 12 h. The reaction was quenched with saturated NH_4Cl solution. The organic layer was separated, and the aqueous layer was extracted with hexane. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuum. The crude product was purified by flash chromatography (hexane) yielding a mixture of (*Z*)-tricos-11-ene (**1**) and (*E*)-tricos-11-ene (**2**) (1.137 g, 3.53 mmol, 86%).[14] The stereoisomers (*Z*)-tricos-11-ene (**1**) and (*E*)-tricos-11-ene (**2**) were separated by column chromatography employing $AgNO_3$ (5%) over silica flash (hexane as eluent).[15]

(*Z*)-Tricos-11-ene (**1**)

^{1}H NMR (200 MHz, $CDCl_3$) δ 0.88 (t, 3H, *J* 6.72 Hz), 1.21-1.59 (m, 34H), 1.93-2.08 (m, 4H), 5.26-5.44 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (2C), 22.9 (2C), 27.4 (2C), 29.4, 29.5 (2C), 29.6 (2C), 29.8 (2C), 29.9, 29.9 (2C), 30.0 (2C), 32.2 (2C), 32.8, 130.2 (2C); MS *m/z* (%): 322 (M^+, 12), 195 (5), 182 (4), 168 (6), 153 (9), 139 (17), 125 (35), 111 (59), 97 (100), 83 (87), 71 (44), 70 (40), 69 (67), 68 (12), 67 (21), 57 (73), 56 (33), 55 (66), 54 (14), 43 (57), 41 (38); IR (ZnSe) ν / cm^{-1} 723, 1471, 2853, 2922, 2958, 3005.

(*E*)-Tricos-11-ene (**2**)

^{1}H NMR (200 MHz, $CDCl_3$) δ 0.92 (t, 3H, *J* 6.71 Hz), 1.21-1.61 (m, 34H), 1.89-2.05 (m, 4H), 5.35-5.46 (m, 2H); ^{13}C NMR δ 14.1 (2C), 22.9 (2C), 27.4 (2C), 29.3, 29.5 (2C), 29.6 (2C), 29.7(2C), 29.9, 29.9 (2C), 30.0 (2C), 32.1 (2C), 32.8, 130.5 (2C); MS *m/z* (%) 322 (M^+, 13), 167 (8), 153 (13), 139 (15), 125 (32), 111 (56), 97 (100), 83 (88), 71 (44), 70 (42), 69 (70), 57 (80), 55 (73), 43 (59), 41 (42); IR (ZnSe) ν / cm^{-1} 723, 965, 1474, 2852, 2922, 2957.

Field experiments

Lures were prepared from 10 mm red rubber septa, loaded with hexane solutions (250 µL) of (*Z*)-tricos-11-ene (100 mg mL^{-1}) (3 lures) or with hexane (250 µL) only as control (3 lures). Field trials were conducted in January, 2015, at the UFPR. White Delta traps were used (Biocontrole, Indaiatuba-SP, Brazil). Treatment and control traps were randomly placed in the field, with a distance of 50 m between them. Traps were positioned in the lower branches of *Eucalyptus benthamii* trees, 2-2.5 m above ground. Counts were taken at any third day after initial setup for a total of 12 days. Captured insects were removed from traps and counted.

Results and Discussion

Comparison of GC chromatograms of aeration extracts from males and females *Chauliognathus fallax* showed the presence of a male specific compound (**1**) (Figure 1a), which proved to be active in GC-EAD assays, using the antennae of conspecific females (Figure 1b).

The following retention indices were calculated for compound **1**: 2268 (RTX-5) and 2313 (RTX-WAX). The difference of 45 units in the retention indices between the non-polar and the polar columns suggested low polarity of the target. This is corroborated by the fact that it was eluted with hexane during fractionation of the male extract on a silica gel column. The mass spectrum of compound **1** (Figure 2a) showed the typical fragmentation pattern of a hydrocarbon and indicated a possible molecular ion at *m/z* 322, suggesting a molecular formula of $C_{23}H_{46}$, which includes one degree of unsaturation. After hydrogenation of the natural extract, the formation of a product with a molecular ion at *m/z* 324 was observed, confirming the presence of a C–C double bond in compound **1**. This derivative showed a mass spectrum and retention index

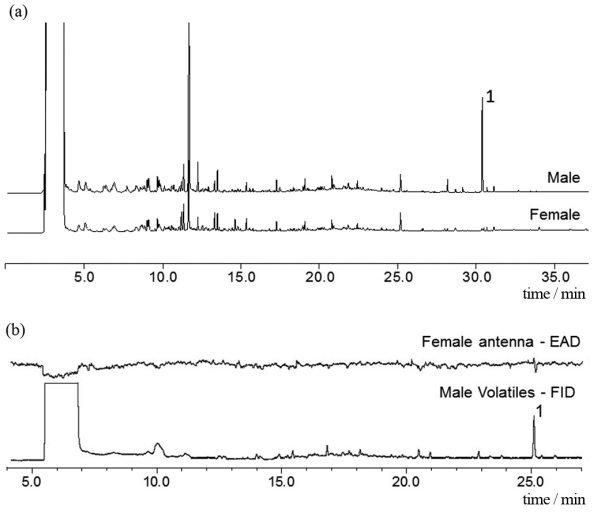


Figure 1. (a) Gas chromatograms of aeration extracts from males and females of *Chauliognathus fallax*, revealing a male specific compound **1**, and (b) electroantennographic response of a *Chauliognathus fallax* female antenna when exposed to male volatiles on a GC-EAD system.

identical to that one of tricosane (RI 2300), proving the natural product to be a tricosene.

The FTIR spectrum of compound **1** (Figure 2b) showed characteristic absorption bands of a hydrocarbon: aliphatic methylene and methyl axial deformations (υ) at 2853, 2921 and 2956 cm^{-1} and angular deformation of methyl groups (δ) at 1474 cm^{-1}. At 3005 cm^{-1}, a characteristic band of the C–H axial deformation of *Z* alkenes was clearly visible.

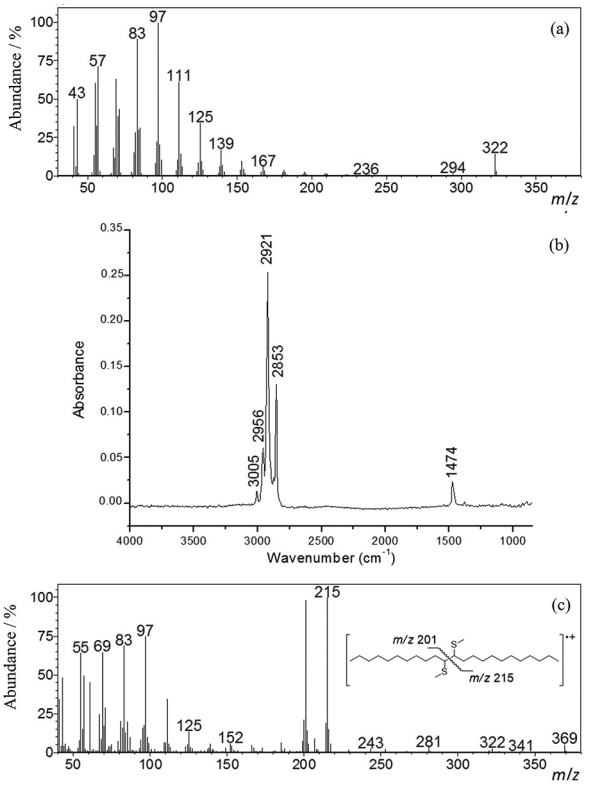


Figure 2. (a) Mass spectrum of the natural compound **1** by GC-MS, (b) FTIR spectrum of the natural compound **1** by GC-FTIR, and (c) mass spectrum of the DMDS adduct of the natural compound **1**.

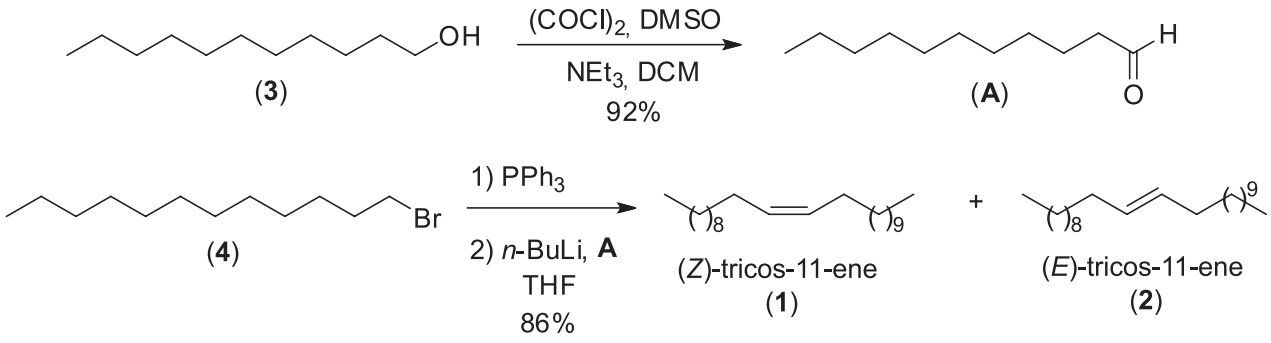
The double bond position of natural tricosene (**1**) was determined by derivatization of the natural extract with DMDS. GC-MS analysis of the DMDS adduct showed prominent fragments at *m/z* 369 (M^+-SCH_3), 201, and 215 which allowed the location of the C–C double bond on the parental alkene at C11 (Figure 2c), resulting in the assignment of (*Z*)-tricos-11-ene as the natural pheromone.

In order to obtain synthetic samples of (**1**) to confirm the proposed structure and to perform field bioassays, a synthetic route started with Swern oxidation of undecan-1-ol (**3**) to undecanal (**A**) (92%),[13] followed by Wittig reaction between the ylide generated from the phosphonuim salt of 1-bromododecane (**4**) and undecanal in 86% yield (Scheme 1).[14] As expected, the Wittig reaction resulted in a mixture of compound **1** and its (*E*)-isomer (**2**) in a ratio of 3:1 (due to the non-stabilized ylide as an intermediate),[16] which could be separated on silica gel containing $AgNO_3$ (5%).

Comparison of mass spectra of the natural compound and the synthetic tricos-11-enes showed no significant differences (Figure 3a). On the other hand, the stereoisomers could be easily distinguished by comparing their FTIR spectra, due to the characteristic bands at 3005 cm^{-1}, related to the (*Z*)-isomer and 965 cm^{-1} related to the (*E*)-isomer (Figure 3c). Comparison of GC retention indices (2268 for compound **1** and 2273 for compound **2** on a RTX-5 column), MS and FTIR spectra of the isomers confirmed the structure of the sex pheromone emitted by males *C. fallax* to be (*Z*)-tricos-11-ene (**1**) (Figure 3).

Unbranched long chain hydrocarbons are known to be related to chemical communication in both solitary and social insects.[17] One of the best-known examples of these naturally produced bioactive hydrocarbons is (*Z*)-tricos-9-ene which was described as a pheromone in dozens of species including the house fly (*Musca domestica*), acting in this case as a sex attractant.[18] On the other hand, there are only a few examples of natural occurrence of (*Z*)-tricos-11-ene (**1**) with only one example related to chemical communication. In this case, compound **1** was detected in a complex mixture of cuticular hydrocarbons which acts has a pheromone in *Drosophila birchii*.[19]

Preliminary field assays showed bioactivity of the synthetic (*Z*)-tricos-11-ene (**1**). A total of 12 *C. fallax* females was caught by the treatment traps, while no insect was captured in the control. However, additional field tests would be interesting by determining the most effective combination of trap characteristics such as design, color, placement and optimal amount of the synthetic compound *per* lure.[20]



Scheme 1. Synthesis of (*Z*)-tricos-11-ene (**1**) and (*E*)-tricos-11-ene (**2**).

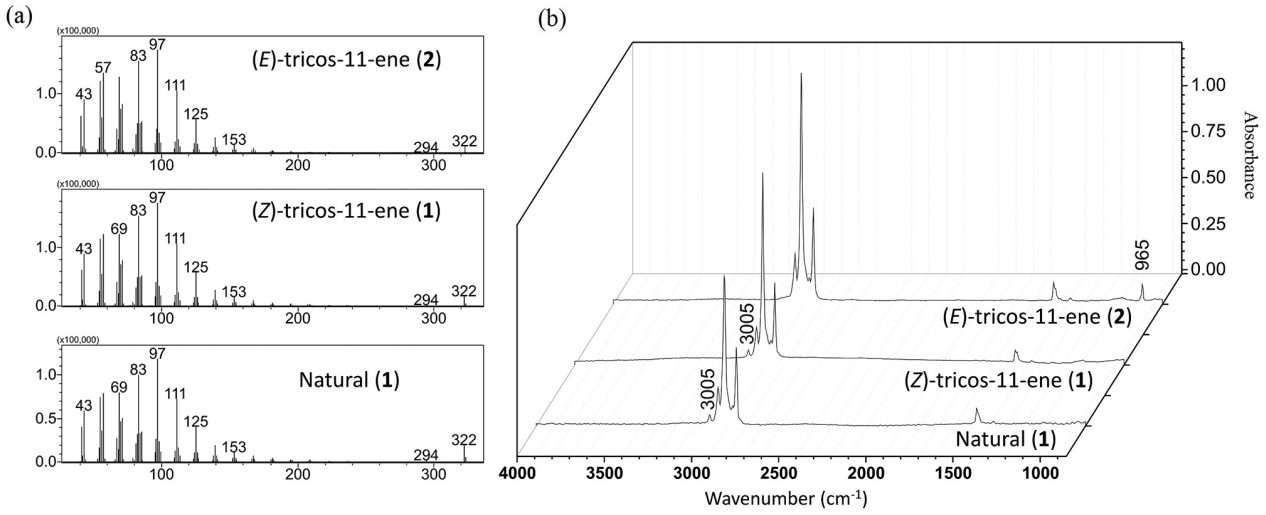


Figure 3. (a) Mass spectra and (b) FTIR spectra obtained for (*Z*)-tricos-11-ene (**1**), (*E*)-tricos-11-ene (**2**) and the natural compound.

Conclusions

In summary, the identification and synthesis of (*Z*)-tricos-11-ene (**1**) were described as the male-produced sex pheromone of the soldier beetle *Chauliognathus fallax*.

The family Cantharidae is one of the 165 families comprising the Coleoptera. Aggregation and sex pheromones have been described for several beetle species and have been found useful in traps for monitoring and mass trapping in pest management. The application of semiochemicals, particularly synthetic pheromones, has proven to be highly effective in pest management in Brazil, as shown by the commercial pheromones of *Anthonomus grandis* and *Rhyncophorus palmarum* (Coleoptera: Curculionidae), *Lasioderma serricorne* (Coleoptera: Anobiidae), as well as *Migdolus fryanus* (Coleoptera: Cerambycidae).[21] Despite the great importance of studies on insect chemical ecology and the use of semiochemicals in integrated pest management, our study is the first to identify the chemical structure of a pheromone in Cantharidae.

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References

1. Zhang, Z. Q.; *Animal Biodiversity: an Outline of Higher-Level Classification and Survey of Taxonomic Richness (Zootaxa 3148)*; Magnolia Press: Auckland, New Zealand, 2011.
2. Machado, V.; Galián, J.; Araújo, A. M.; Valente, V. L. S; *Hereditas* **2001**, *134*, 121.
3. Common Wealth Scientific and Industrial Research Organization, Division of Entomology, Australia; *The Insects of Australia: A Textbook for Students and Research Workers*, 2nd ed.; Cornell University Press: Ithaca, USA, 1991.

4. Shohet, D.; Clarke, A.; *Aust. J. Entomol.* **1997**, *36*, 37.
5. Costa, C.; Vanin, S. A.; Casari-Chen, S. A.; *Larvas de Coleoptera do Brasil*; Museu de Zoologia da Universidade de São Paulo (USP): São Paulo, Brazil, 1988.
6. Machado, V.; Araujo, A. M.; Serrano, J.; Galián, J.; *Genet. Mol. Biol.* **2004**, *27*, 55.
7. Machado, V.; Araújo, A. M.; *Rev. Bras. Entomol.* **2003**, *47*, 409.
8. Klitzke, C. F.; Trigo, J. R.; *Biochem. Syst. Ecol.* **2000**, *28*, 313.
9. Fender, K.; *Northwest Sci.* **1964**, *38*, 95.
10. Zarbin, P. H. G.; Ferreira, J. T. B.; Leal, W. S.; *Quim. Nova* **1999**, *22*, 263.
11. Zarbin, P. H. G.; Favaro, C. F.; Vidal, D. M.; Rodrigues, M. A. C. M.; *J. Chem. Ecol.* **2012**, *38*, 825.
12. Zarbin, P. H. G.; Lorini, L. M.; Ambrogi, B. G.; Vidal, D. M.; Lima, E. R.; *J. Chem. Ecol.* **2007**, *33*, 555.
13. Taillier, C.; Gille, B.; Bellosta, V.; Cossy, J.; *J. Org. Chem.* **2005**, *70*, 2097.
14. Favaro, C. F.; Soldi, R. A.; Ando, T.; Aldrich, J. R.; Zarbin, P. H. G.; *Org. Lett.* **2013**, *15*, 1822.
15. Yan, Y.; Wang, X.; Liu, Y.; Xiang, J.; Wang, X.; Zhang, H.; Yao, Y.; Liu, R.; Zou, X.; Huang, J.; Jin, Q.; *J. Chromatog. A* **2015**, *1425*, 293.
16. Maryanoff, B. E.; Reitz, A. B.; *Chem. Rev.* **1989**, *89*, 863.
17. Francke, W.; Schulz, S. In *Comprehensive Natural Products II*, vol. 4; Mander, L.; Liu, H., ed.; Elsevier: Oxford, UK, 2010, p. 153.
18. Carlson, D.; Mayer, M.; Silhacek, D.; James, J.; Beroza, M.; Bierl, B.; *Science* **1971**, *174*, 76.
19. Howard, R. W.; Jackson, L. L.; Banse, H.; Blows, M. W.; *J. Chem. Ecol.* **2003**, *29*, 961.
20. Crook, D. J.; Khrimian, A.; Cossé, A.; Fraser, I.; Mastro, V. C.; *J. Econ. Entomol.* **2012**, *105*, 429.
21. Zarbin, P. H. G.; Rodrigues, M.; Lima, E. R.; *Quim. Nova* **2009**, *32*, 722.

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