Tetrahedron Letters 56 (2015) 5353–5356

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Identification and synthesis of the male produced volatiles of the carrion beetle, *Oxelytrum erythrurum* (Coleoptera: Silphidae)

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ARTICLE INFO

Article history:
Received 10 July 2015
Revised 4 August 2015
Accepted 5 August 2015
Available online 8 August 2015

Keywords:
Coleoptera
Silphidae
Forensic entomology
Micro-derivatization
GC/FTIR

ABSTRACT

Necrophagous beetles belonging to the family Silphidae are recognized as potentially useful in forensic investigations (to estimate post mortem interval). Gas chromatography analyses of extracts of aerations of adult *Oxelytrum erythrurum* revealed the presence of two male-specific compounds. These compounds were identified as (*Z*)-1,10-nonadecadiene (major) and 1-nonadecene (minor) using microderivatizations of the natural male extract, such as hydrogenation, partial reduction and methylthiolation, mass spectrum comparisons, and co-injections with authentic standards. Both compounds might be components of a pheromone responsible for sexual communication in this species.

Through decomposition, the nutrients, and organic matter that are present in carcasses are recycled, and decomposing carcasses support a large and dynamic arthropod community.[1] Among members of this community, 'carrion beetles' (Coleoptera: Silphidae) are usually associated with vertebrate carcasses.[2,3] Due to this association, silphid beetles might be important tools to estimate the post mortem interval of corpses in criminal investigations. Species in the genus *Oxelytrum* are particularly useful because larvae are strictly necrophagous.[4]

Oxelytrum discicolle (Brullé) is the most commonly collected species of Silphidae in South America,[3] co-occurring with *Oxelytrum erythrurum* (Blanchard) in the southern regions of the continent. Not only is their distribution similar, but based on morphology it has been hypothesized that these species are closely related (evolutionary speaking).[3] Despite a few existing papers addressing their occurrence[5,6] and feeding habits,[7] only recently has the chemical ecology of *O. discicolle* been studied, revealing the existence of a male produced sex pheromone composed by a major and a minor component, (*Z*)-1,8-heptadecadiene and 1-heptadecene, respectively.[8] Because of the taxonomic proximity and the similarities concerning the feeding habits and the habitat of *O. discicolle* and *O. erythrurum*, the objective of this study was to identify and synthetize the chemical compounds produced by *O. erythrurum* using the same methodology applied by Fockink et al.[8] for *O. discicolle*.

In the current study, volatiles emitted by males and females of *O. erythrurum* were analyzed through gas chromatography[9,10] and two male-specific compounds were detected (Fig. 1), presenting the following Kovats Indexes (KI) on a RTX-5, RTX-WAX and EC-1 columns, respectively: (A) 1888, 1988, and 1896; (B) 1897, 1938, and 1932. The emission of the compounds started on the 15th day after the emergence of males, during the photophase and the scotophase. The ratio between the major (**A**) and the minor (**B**) compounds was of 92:8, respectively.

The GC/FTIR and the GC/MS spectra of *O. erythrurum* male-specific components are shown in Figure 2. For the major compound (**A**), the infrared spectrum (Fig. 2a) showed characteristic bands in the region of hydrocarbons between 2800 cm^{-1} and 3000 cm^{-1}, beyond of bands at 3089 cm^{-1}, 3002 cm^{-1}, 1640 cm^{-1}, 995 cm^{-1}, and 911 cm^{-1} indicating a terminal and Z-configuration double bond.[11–16] The mass spectra (Fig. 2b) exhibited molecular ion at *m*/*z* 264 and through these data, the compound **A** may have the molecular formula $C_{19}H_{36}$.

The infrared spectrum of the minor compound (**B**) also revealed bands characteristic of hydrocarbons (Fig. 2c). However, it is possible to observe only one characteristic band of the double bond at 3084 cm^{-1}, and along the bands at 1645 cm^{-1}, 992 cm^{-1}, and 916 cm^{-1} indicating a single terminal double bond. The mass spectra of the compound **B** (Fig. 2d) revealed a molecular ion (*m*/*z* 266) with two mass units more than compound **A**, confirming the existence of only one double bond. The empiric formula proposed was $C_{19}H_{38}$.

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http://dx.doi.org/10.1016/j.tetlet.2015.08.004
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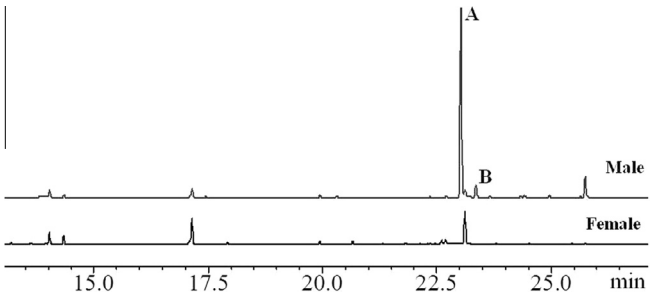


Figure 1. Gas chromatographic analyses of volatiles obtained from male and female *Oxelytrum erythrurum* (Coleoptera: Silphidae) evidencing male-specific major (**A**) and minor (**B**) compounds.

To determine the structures of these compounds several microderivatizations were carried out with the natural compounds (hydrogenation, partial reduction, and methylthiolation). First, the compounds were submitted to a catalytic hydrogenation over Pd/C[17] to prove the presence of double bonds. The single product formed revealed a M^+ at *m*/*z* 268, as a result of the insertion of four hydrogens in the compound **A**, and two hydrogens in **B**. The hydrogenated product showed a fragmentation pattern of a linear chain, which suggested it to be a *n*-nonadecane. This was confirmed by co-injections with authentic samples. Besides, the identity of the minor compound (**B**) was confirmed by co-injecting the extract of males with an authentic sample of 1-nonadecene.

For position determination of double bonds of the major compound (**A**), direct reactions of *O. erythrurum* male extracts with dimethyl disulfide intended to identify the DMDS derivatives, however they did not succeed. Although in such reaction products of dimethyl disulfide generated several peaks on GC/MS analyses, mass spectra were difficult to interpret. Several conditions of reactions were evaluated without success. In contrast, the DMDS derivatives of compounds with one double bond produced very characteristic mass spectra.[18] Because of that, the strategy for the determination of double bond positions was carried out first in a partial reduction employing NH_2NH_2,[17] to produce the respective monoenes, followed by the DMDS reaction.[17,19] This procedure was also applied to determine the complete structure of the major component (a triene acetate) of the sex pheromone of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on a 100 ng scale.[19]

The reactions of the major compound (**A**) with NH_2NH_2 followed by DMDS showed in the mass spectra for nonterminal adduct monoene intense fragments at *m*/*z* 173 and *m*/*z* 187, indicating a double bond at carbon 9 or 10 (Fig. 3). Based on these data, (*Z*)-1,10-nonadecadiene (**1**) or (*Z*)-1,9-nonadecadiene (**2**) were proposed as the identity of the major compound. Both compounds were synthetized using the same methodology to confirm the proposed structures (Scheme 1).

The first proposed structure, (*Z*)-1,10-nonadecadiene (**1**) was obtained according to Scheme 1a. 2-(9-bromononyloxy)-tetraydro-2H-pyran (5) was prepared from 1,9-nonanediol (**3**), which suffered a monobromination[20] and was subsequently protected with DHP.[21] The ether (**5**) was alkylated with the anion generated with *n*-BuLi from 1-decyne (**6**), providing the intermediate **7**,[22] which was de-protected to yield the alcohol (**8**).[23] The stereoselective reduction of the alcohol (**8**) with H_2 over $Pd/CaCO_3$ (Lindlar's reagent) resulted in the alcohol (**9**).[24] The alcohol (**9**) was

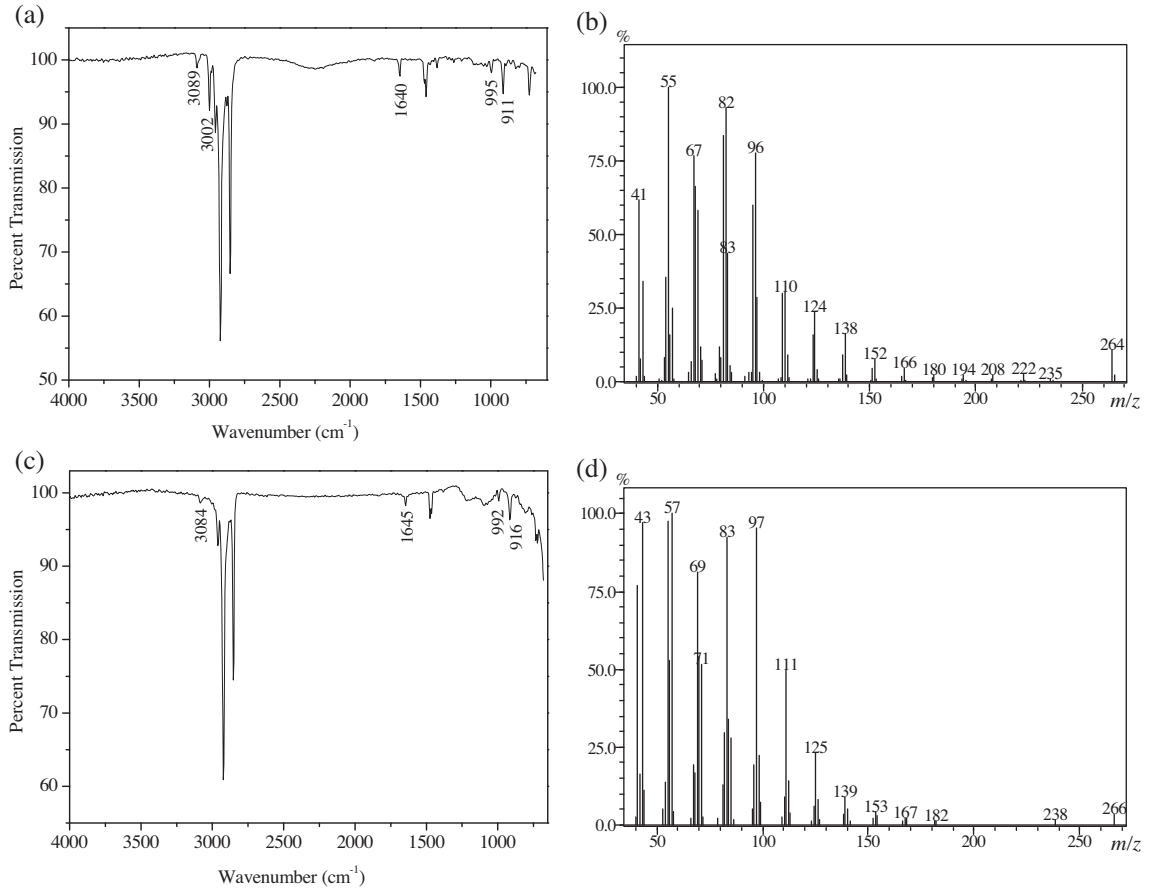


Figure 2. Infrared and electron impact mass spectra of the male-specific major (a and b) and minor (c and d) compounds of *Oxelytrum erythrurum* (Coleoptera: Silphidae).

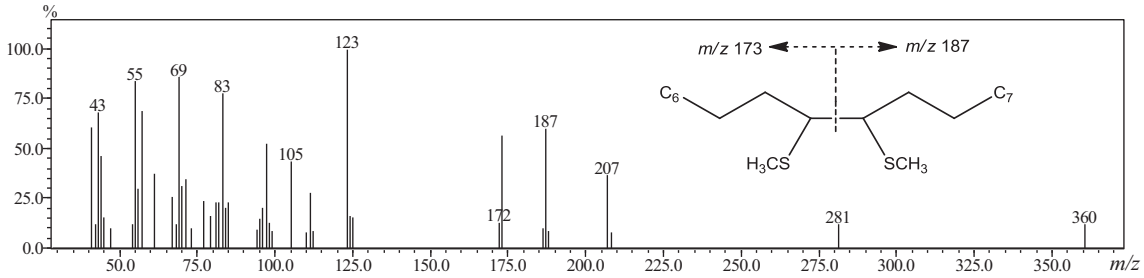
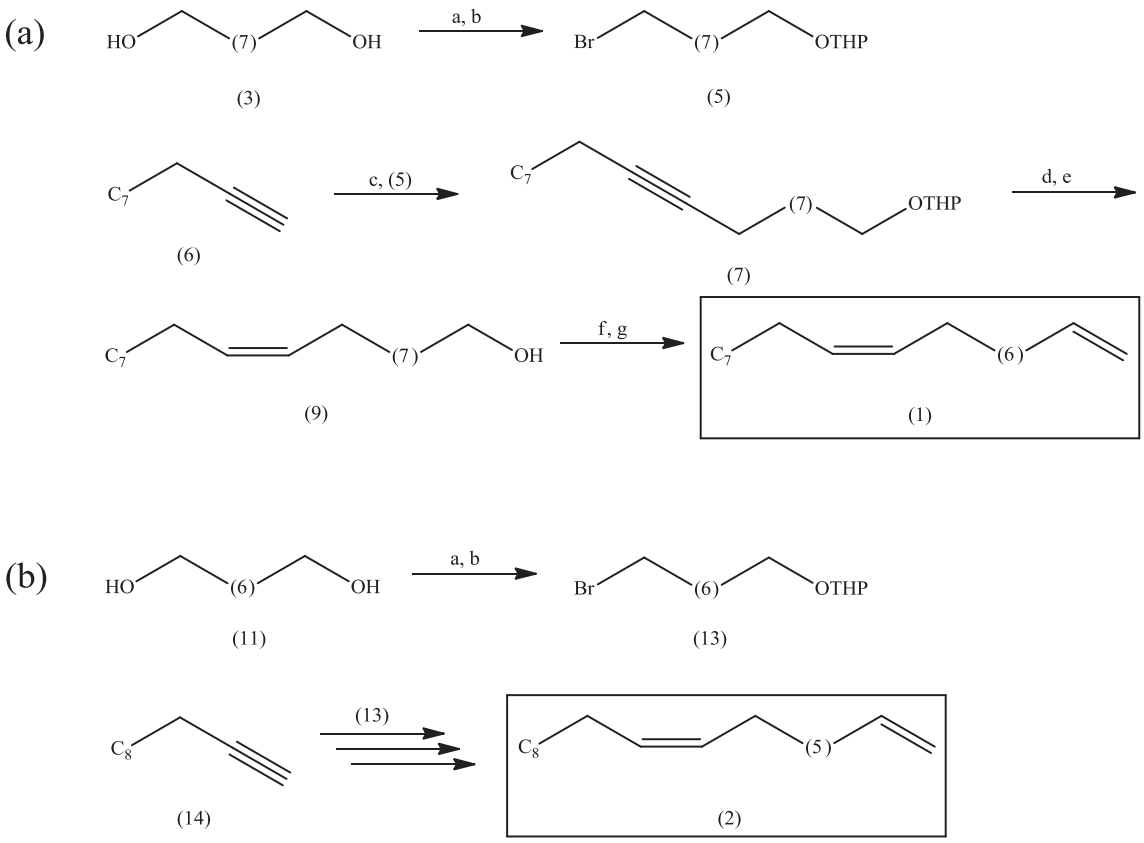


Figure 3. Electron impact mass spectra of DMDS adduct formed for the double bond in the carbon 9 or 10 of the major compound (**A**) emitted by males of *Oxelytrum erythrurum* (Coleoptera: Silphidae).



Scheme 1. (a) Synthesis of (*Z*)-1,10-nonadecadiene (**1**): (a) 48% HBr, 83%, (b) DHP, *p*TSA, 89%, (c) *n*-BuLi, **(5)**, 90%, (d) *p*TSA, 92%, (e) H_2, Lindlar, 83%, (f) CBr_4, Ph_3P, 87%, (g) *t*-BuOK, 79%. (b) Synthesis of (*Z*)-1,9-nonadecadiene (**2**): (a) 48% HBr, 83%, (b) DHP, *p*TSA, 89%, (c) *n*-BuLi, (**13**), 89%, (d) *p*TSA, 91%, (e) H_2, Lindlar, 65%, (f) CBr_4, Ph_3P, 75%, (g) *t*-BuOK, 79%, tentative components of the major compound (**A**) of *Oxelytrum erythrurum* (Coleoptera: Silphidae).

converted to bromide (**10**),[25] and an elimination reaction of this bromide (**10**) afforded the diene (**1**).[26] The yield of each step is shown in the legend of Scheme 1 and the overall yield of this synthetic route was 35%.

The second proposed structure, the (*Z*)-1,9-nonadecadiene (**2**), was synthesized based on the same route starting from 1,8-octanediol (**11**), with an overall yield of 23% after seven steps (Scheme 1b).

The two synthetic compounds were co-injected with the natural extracts of males and co-eluted on all three different GC columns tested (RTX-5, EC-1, and RTX-WAX). The mass and infrared spectra of the (*Z*)-1,10-nonadecadiene (**1**) were indistinguishable from the spectra of the natural compound (see Fig. 4a and c). However, for the (*Z*)-1,9-nonadecadiene (**2**) small differences in intensity of some fragments (*m*/*z* 81/82, *m*/*z* 109/110, *m*/*z* 235/236) could be detected (Fig. 4b).

In conclusion, all of the analytical data confirmed that the major and the minor male-produced compounds from *O. erythrurum* are (*Z*)-1,10-nonadecadiene and 1-nonadecene, respectively. Both structures are very similar to male produced sex pheromone components of the congener, *O. discicolle*' ((*Z*)-1,8-heptadecadiene and 1-heptadecene). Because of difficulty obtaining adult *O. erythrurum*, bioassays to test the attraction of both sexes to these compounds were not performed. However, we hypothesize that the chemical mediated sexual communication and the biosynthetic pathways[27] in *O. erythrurum* and *O. discicolle* are alike because of the close relationship and the similar natural history of both species. In *O. discicolle*, females are not attracted to the male pheromone alone, but only in the presence of a carcass, apparently revealing an association of reproduction and oviposition site.[8] So, we expect that the two compounds herein identified are the male produced sex pheromone of *O. erytrhurum*.

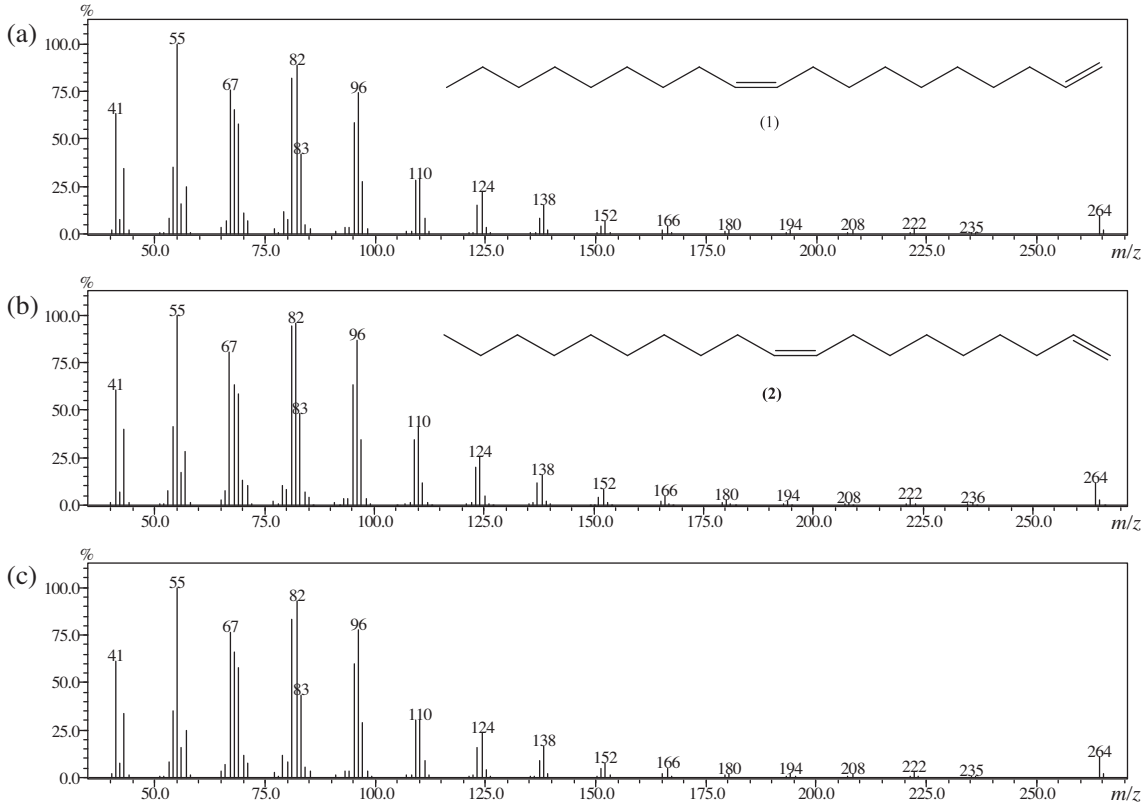


Figure 4. Comparisons of the electron impact mass spectrum of (a) (*Z*)-1,10-nonadecadiene (**1**), (b) (*Z*)-1,9-nonadecadiene (**2**) and (c) natural major component (**A**) of *Oxelytrum erythrurum* (Coleoptera: Silphidae).

Thus, studying the chemical ecology of necrophagous species creates an important chemotaxonomic identification tool, which could be easier than comparing the external morphology of closely related species, such as *O. discicolle* and *O. erythrurum*.

Acknowledgments

The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Instituto Nacional de Ciências e Tecnologia de Semioquímicos na Agricultura (INCT).

References and notes

1. Anderson, G.; VanLaerhoven, S. *J. Forensic Sci.* **1996**, *41*, 617–625.
2. Moura, M. O. *Rev. Bras. Zool.* **2004**, *21*, 409–419.
3. Peck, S. B.; Anderson, R. S. *Quaest. Entomol.* **1985**, *21*, 247–317.
4. Oliva, A.; Di Iorio, O. R. In *Silphidae*; Claps, L. E., Debandi, G., Roig-Junent, S., Eds.; Editorial Sociedad Entomologica Argentina: Mendoza, 2008; Vol. 2, pp 461–470.
5. Carvalho, L. M. L.; Thyssen, P. J.; Linhares, A. X.; Palhares, F. A. B. *Mem. Inst. Oswaldo Cruz* **2000**, *95*, 135–138.
6. Mise, K. M.; Almeida, L. M.; Moura, M. O. *Rev. Bras. Entomol.* **2007**, *51*, 358–368.
7. Ururahy-Rodrigues, A.; Rafael, J. A.; Pujol-Luz, J. R.; Henriques, A. L.; Queiroz, M.; Barbosa, R. R.; Baroni, M. N. *EntomoBrasilis* **2010**, *3*, 45–48.
8. Fockink, D. H.; Mise, K. M.; Zarbin, P. H. *J. Chem. Ecol.* **2013**, 1–10.
9. Zarbin, P. H. G.; Ferreira, J. T. B.; Leal, W. S. *Quím. Nova* **1999**, *22*, 263–268.
10. Zarbin, P. H. G.; Rodrigues, M. A. C. M.; Lima, E. R. *Quim. Nova* **2009**, *32*, 722–731.
11. Attygalle, A. B. *Pure Appl. Chem.* **1994**, *66*, 2323–2326.
12. Smith, B. C. *Infrared Spectral Interpretation: A Systematic Approach*; CRC Press: New York, 1999.
13. Pouchert, C. J. *The Aldrich Library of FT-IR Spectra: Vapor Phase*; Aldrich Chemical Company: Milwaukee, 1989.
14. Nyquist, R. A. *The Interpretation of Vapor-Phase Infrared Spectra: Group Frequency Data*; Sadtler Research Laboratories: Philadelphia, 1984.
15. Attygalle, A. B.; Svatos, A.; Wilcox, C.; Voerman, S. *Anal. Chem.* **1994**, *66*, 1696–1703.
16. Attygalle, A. B.; Svatos, A.; Wilcox, C.; Voerman, S. *Anal. Chem.* **1995**, *67*, 1558–1567.
17. Attygalle, A. B. In *Methods in Chemical Ecology*; Millar, J. G., Haynes, K. F., Eds.; Chapman & Hall: New York, 1998.
18. Attygalle, A. B.; Jham, G. N.; Svatoš, A.; Frighetto, R. T. S.; Ferrara, F. A.; Vilela, E. F.; Uchôa-Fernandes, M. A.; Meinwald, J. *Bioorg. Med. Chem.* **1996**, *4*, 305–314.
19. Jham, G. N.; Attygalle, A. B.; Meinwald, J. *J. Chromatogr. A* **2005**, *1077*, 57–67.
20. Chong, J. M.; Heuft, M. A.; Rabbat, P. *J. Org. Chem.* **2000**, *65*, 5837–5838.
21. Santangelo, E. M.; Coracini, M.; Witzgall, P.; Correa, A.; Unelius, C. R. *J. Nat. Prod.* **2002**, *65*, 909–915.
22. Kang, S. K.; Park, S. K. *Bull. Korean Chem. Soc.* **1988**, *9*, 149–152.
23. Zarbin, P. H. G.; Lorini, L. M.; Ambrogi, B. G.; Vidal, D. M.; Lima, E. R. *J. Chem. Ecol.* **2007**, *33*, 555–565.
24. Overman, L. E.; Brown, M. J.; Mccann, S. F. *Org. Synth.* **1993**, *8*, 609–613.
25. Hu, T. S.; Yu, Q.; Wu, Y. L.; Wu, Y. *J. Org. Chem.* **2001**, *66*, 853–861.
26. Manabe, Y.; Minamikawa, M. J.; Otsubo, J.; Tamaki, Y. *Agric. Biol. Chem.* **1985**, *49*, 1205–1206.
27. Leal, W. S.; Zarbin, P. H. G.; Wojtasek, H.; Ferreira, J. T. *Eur. J. Biochem.* **1999**, *259*, 175–180.