



A new sesquiterpenoid produced by female *Callosobruchus rhodesianus* (Pic): a possible component of the sex attractant pheromone

Kenji Shimomura^{a,*}, Hiroyuki Koshino^b, Arata Yajima^c, Noriko Matsumoto^a, Shunsuke Yajima^a, Kanju Ohsawa^a

^a Department of Bioscience, Faculty of Applied Bioscience, Tokyo University of Agriculture, Setagaya-ku, Tokyo 156-8502, Japan

^b Chemical Biology Core Facility, Advanced Science Institute, RIKEN, Wako, Saitama 351-0198, Japan

^c Department of Fermentation Science, Faculty of Applied Bioscience, Tokyo University of Agriculture, Setagaya-ku, Tokyo 156-8502, Japan

ARTICLE INFO

Article history:

Received 2 September 2010

Revised 14 October 2010

Accepted 20 October 2010

Available online 26 October 2010

Keywords:

Callosobruchus rhodesianus

(*E*)-6-Ethyl-2,10-dimethyl-5,9-undecadienal

GC-EAD

Sex attractant pheromone

Seed beetle

ABSTRACT

(*E*)-6-Ethyl-2,10-dimethyl-5,9-undecadienal was identified from female seed beetle, *Callosobruchus rhodesianus* (Pic), as an electroantennographic-detection (EAD) active compound in the male. The ratio of the (*E*)-6-ethyl-2,10-dimethyl-5,9-undecadienal and (3*S*,6*E*)-2,3-dihydrohomofarnesal, previously identified as a sex attractant pheromone, was estimated at approximately 1:4.5. In combination with the stereoisomer-activity relationship of 2,3-dihydrohomofarnesal, we suggest that this new compound might function as a minor sex attractant pheromone in *C. rhodesianus*.

© 2010 Elsevier Ltd. All rights reserved.

Commonly in the insect world, sex pheromones are significant signals involved in successful mating, and their composition varies enormously between species. Furthermore, insects tend to use multi pheromone components; closely related species use the same major component, but differ in the complement or ratio of minor components in the blend.¹ The seed beetle, *Callosobruchus rhodesianus* (Pic), is an economic pest of stored legumes, especially cowpeas, in Africa. It is distributed in the local Afrotropical region, mainly found in southern Africa, and has spread around the equator, where it has been sporadically-reported.² Because of the narrow distribution and limited damage to legumes, *C. rhodesianus* has been seldom studied. We recently reported the presence of female produced sex attractant pheromone component and identified it as 2,3-dihydrohomofarnesal, that is, (3*S*,6*E*)-7-ethyl-3,11-dimethyl-6,10-dodecadienal (**1**) (Fig. 1).³ In the course of the study, we also found the presence of a gas chromatography coupled electroantennographic-detection (GC-EAD) active compound affecting male antennae in the headspace volatiles collected from virgin females.³ Although the compound exhibited large response from antennae of the male beetle, it had no attractive activity.³ To clarify this inconsistency, we report herein the characterization of the chemical properties of the component.

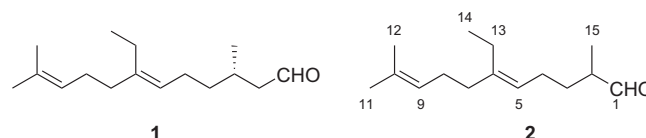


Figure 1. The structures of the pheromone components of the seed beetle, *Callosobruchus rhodesianus*.

The headspace volatiles were collected from virgin females housed in glass container using adsorbent trap containing Tenax. Gas chromatography–electron ionization mass spectrometry (GC–EI–MS) analysis of the EAD active compound showed a base peak at m/z 69 (Fig. 2). Gas chromatography–chemical ionization mass spectrometry (GC–CI–MS) analysis of the compound showed sets of characteristic fragments at m/z 205 $[M+1-H_2O]^+$ (100), 223 $[M+1]^+$ (44), indicating a molecular weight of 222 (Fig. 2). The high-resolution MS spectrum of the compound was consistent with a molecular formula of $C_{15}H_{26}O$ (observed m/z : 222.1989; calculated m/z : 222.1984). This molecular formula indicated three degrees of unsaturation. Since GC–MS analysis suggested that this component was novel, crude volatile collections were accumulated from approximately 37,000 virgin females for structural analysis. The crude extracts were purified with chromatography techniques

* Corresponding author.

E-mail address: k3shimom@nodai.ac.jp (K. Shimomura).

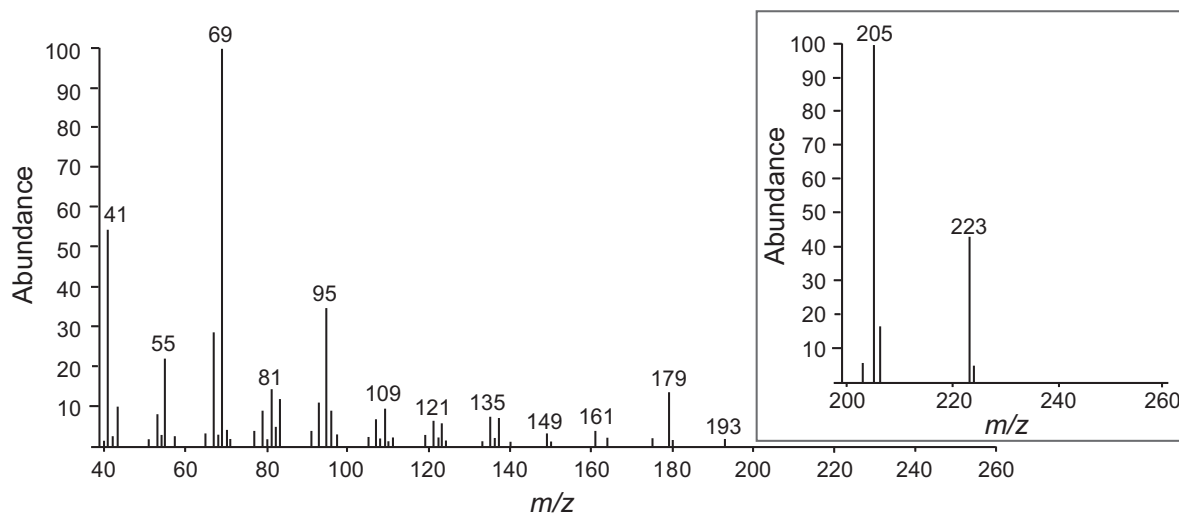
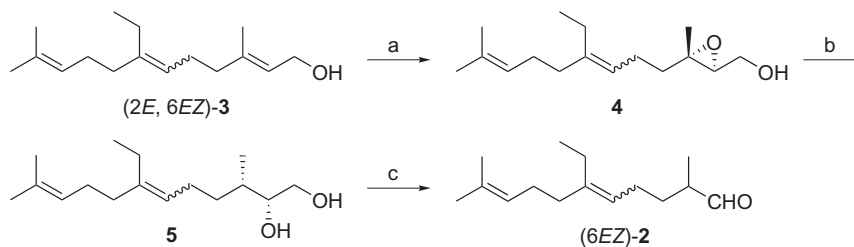


Figure 2. EI-MS spectrum and CI-MS spectrum (inlet) of the male EAD active compound collected from headspace volatiles of female *Callosobruchus rhodesianus*.



Scheme 1. Synthesis of a stereoisomeric mixture of **2**. Reagents and conditions: (a) *L*-diethyl tartrate, Ti(OiPr)₄, *t*-BuOOH, CH₂Cl₂ (65%); (b) NaBH₃CN, BF₃·OEt₂, THF (41%); (c) NaIO₄, THF, H₂O (59%).

as previously reported,³ and isolated compound by a micro-preparative GC system was analyzed with ¹H-NMR.³ Because of the limited quantities of the isolated compound (approximately 15 μg/C₆D₆ 0.2 ml), it was difficult to interpret all signals. The presence of an aldehyde group adjacent to a methine group was evident at 9.31 (d, 1H, *J* = 2.0 Hz) ppm. Two distinct olefinic protons were detected at 5.22 (1H) and 5.04 (1H) ppm. Two methylene groups at allylic positions (CH₂-C=C) were detected at 2.15 (m, 2H) and 2.08 (m, 2H) ppm. Obscure signals were also detected from 1.89–1.93 (3H) ppm; these signals could be assigned to one methylene group at another allylic position and one methine proton at the α-position of the aldehyde group. Three distinct methyl signals were clearly observed; two signals at 1.68 (s, 3H) and 1.56 (s, 3H) ppm derived from two methyl groups on the double bond (CH₃-C=C), and one signal adjacent to the methine group (CH₃-CH) at 0.78 (d, 3H, *J* = 6.9 Hz) ppm. The presence of one ethyl group on the double bond (CH₃-CH₂-C=C) was evident from the signals at 0.93 (t, 3H, *J* = 7.6 Hz) and 1.97 (q, 2H, *J* = 7.6 Hz) ppm for a terminal methyl and an allylic methylene group with an appropriate coupling constant. In light of the structural information and the similarity of the MS fragment pattern to that of 2,3-dihydrohomofarnesal (**1**), previously identified as a sex attractant pheromone of *C. rhodesianus*,³ the compound was suggested to be a 6-ethyl-2,10-dimethyl-5,9-undecadienal (**2**).

To confirm the structural identification, a stereoisomeric mixture of **2** was synthesized by starting from a stereoisomeric mixture of homofarnesol (**3**)⁴ according to the reported methods by Yamazaki et al.⁵ (Scheme 1). Briefly, (2*E*,6*EZ*)-homofarnesol (6*E*/6*Z* = 2:3) (**3**) was epoxidized, and reductive opening of the

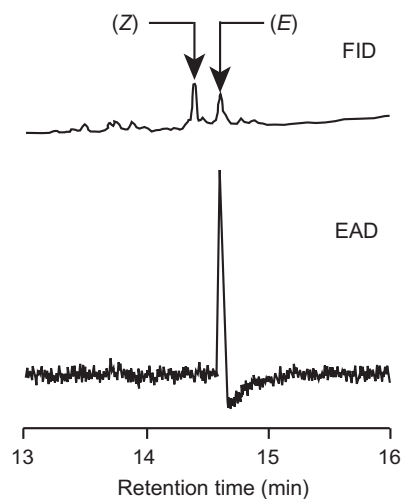


Figure 3. GC-EAD responses of male *Callosobruchus rhodesianus* antennae to synthetic *E/Z* isomers of 6-ethyl-2,10-dimethyl-5,9-undecadienal (**2**).

epoxide to give diol (**5**).⁶ Oxidative cleavage of 1,2-diol gave **2** as an inseparable geometric isomeric mixture and the geometric ratio was *E/Z* = 2:3 in ¹H NMR.⁷ Unfortunately, the obtained **2** was not optically active, which indicates that the C-2 of synthetic **2** epimerized during purification processes.

The GC-EAD analysis of the synthetic mixture of the *E/Z* isomers showed that one of the two geometric isomers elicited responses in

male antennae, and the retention time on two different columns and MS fragment pattern of the isomer matched that of the natural compound. By referring to the retention order on GC columns of the *E/Z* isomers of 2,3-dihydrohomofarnesal and a ratio of the synthesized geometric isomers, the EAD active compound was confirmed to be (*E*)-6-ethyl-2,10-dimethyl-5,9-undecadienal [(*E*)-**2**] (Fig. 3). The natural ratio of the (*E*)-**2** and (3*S*,6*E*)-2,3-dihydrohomofarnesal (**1**) was estimated at approximately 1:4.5 in GC.

Regarding the sex-attractive activity of (*E*)-2,3-dihydrohomofarnesal (**1**), only the (*S*)-enantiomer is active and (*R*)-enantiomer antagonistically inhibits the attractive activity of (*S*)-enantiomer.³ In combination with this result, we suggest that the chirality of (*E*)-**2** might be important to function as a minor sex attractant pheromone. To clarify this suggestion, stereoselective synthesis and rapid behavioral analysis are in progress.

Acknowledgments

We thank Dr. Takane Fujimori (Tokyo University of Agriculture) for his advice on the structural analysis. This study was supported in part by a Grant-in-Aid for Scientific Research from Ministry of Education, Science, Sports, and Culture of Japan.

References and notes

- Smadja, C.; Butlin, K. R. *Heredity* **2009**, *102*, 77–97.
- (a) Southgate, B. J. *Annu. Rev. Entomol.* **1979**, *24*, 449–473; (b) Giga, D. P.; Smith, R. H. *J. Stored Prod. Res.* **1983**, *19*, 189–198.
- Shimomura, K.; Koshino, H.; Yajima, A.; Matsumoto, N.; Kagohara, Y.; Kamada, K.; Yajima, S.; Ohsawa, K. *J. Chem. Ecol.* **2010**, *36*, 824–833.
- Shimomura, K.; Nojima, S.; Yajima, S.; Ohsawa, K. *J. Chem. Ecol.* **2008**, *34*, 467–477.
- Yamazaki, N.; Suzuki, T.; Yoshimura, Y.; Kibayashi, C.; Aoyagi, S. *Heterocycles* **2008**, *75*, 285–290.
- Because the synthetic intermediates **4** and **5** were geometric isomeric mixtures, it is difficult to determine the enantiomeric purities of them. Physical properties of synthetic **4** and **5**. (a) Compound **4**: $[\alpha]_D^{24}$ –5.7 (c 1.1, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 0.96 and 0.98 (t, *J* = 7.8 Hz, 3H), 1.31 (s, 3H), 1.48 (m, 1H), 1.65–1.74 (m, 1H), 1.60 (s, 3H), 1.68 (s, 3H), 1.95–2.15 (m, 8H), 2.97 (m, 1H), 3.69 (m, 1H), 3.82 (m, 1H), 5.04–5.13 (m, 2H). (b) Compound **5**: $[\alpha]_D^{24}$ –11.1 (c 1.1, CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 0.94 (d, *J* = 6.9 Hz, 3H), 0.96 and 0.98 (t, *J* = 7.8 Hz, 3H), 1.15–1.56 (m, 3H), 1.60 (s, 3H), 1.68 (s, 3H), 1.95–2.08 (m, 8H), 3.55 (dd, *J* = 8.2, 10.1 Hz, 1H), 3.60 (ddd, *J* = 2.3, 4.6, 8.2 Hz, 1H), 3.66 (dd, *J* = 2.3, 10.1 Hz, 1H), 5.06 (m, 2H).
- Spectra data for synthetic **2** (as a mixture of *E/Z* = 2:3). Complete ¹H and ¹³C NMR signals of each geometrical isomer were assigned by means of 2D DQF-COSY, NOESY, HSQC, and HMBC experiments. (a) (*E*)-Isomer: ¹H-NMR (600 MHz, C₆D₆): δ 0.78 (d, *J* = 6.9 Hz, H-15), 0.93 (t, *J* = 7.6 Hz, H-14), 1.12 (m, H-3a), 1.53 (m, H-3b), 1.56 (s, H-12), 1.68 (s, H-11), 1.90 (m, H-4), 1.92 (m, H-2), 1.97 (q, *J* = 7.6 Hz, H-13), 2.08 (m, H-7), 2.14 (m, H-8), 5.04 (br t, *J* = 6.9 Hz, H-5), 5.22 (m, H-9), 9.31 (d, *J* = 2.0 Hz, H-1). ¹³C NMR (150 MHz, C₆D₆): δ 13.26 (C-15), 13.34 (C-14), 17.72 (C-12), 23.41 (C-13), 25.15 (C-4), 25.82 (C-11), 27.27 (C-8), 31.03 (C-3), 36.89 (C-7), 45.76 (C-2), 123.62 (C-5), 124.91 (C-9), 131.44 (C-10), 141.81 (C-6), 203.17 (C-1). GC-El-MS: *m/z* 222 (M⁺, 0.4), 193 (2), 179 (13), 137 (7), 121 (6), 109 (8), 95 (32), 81 (13), 69 (100), 55 (25), 41 (55). (b) (*Z*)-Isomer: ¹H NMR (600 MHz, C₆D₆): δ 0.79 (d, *J* = 7.6 Hz, H-15), 1.00 (t, *J* = 7.6 Hz, H-14), 1.12 (m, H-3a), 1.53 (m, H-3b), 1.56 (s, H-12), 1.66 (s, H-11), 1.92 (m, H-2), 1.94 (m, H-4), 2.00 (br q, *J* = 7.6 Hz, H-13), 2.08 (m, H-7), 2.09 (m, H-8), 5.06 (br t, *J* = 7.2 Hz, H-5), 5.21 (m, H-9), 9.31 (d, *J* = 2.0 Hz, H-1). ¹³C NMR (150 MHz, C₆D₆): δ 13.08 (C-14), 13.26 (C-15), 17.64 (C-12), 25.37 (C-4), 25.82 (C-11), 27.42 (C-8), 29.86 (C-13), 30.71 (C-7), 31.07 (C-3), 45.87 (C-2), 123.15 (C-5), 124.78 (C-9), 131.22 (C-10), 141.68 (C-6), 203.11 (C-1). GC-El-MS: 222 (M⁺, 0.3), 193 (2), 179 (10), 137 (6), 121 (9), 109 (8), 95 (27), 81 (12), 69 (100), 55 (28), 41 (59).