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(6*R*,10*S*)-Pallantione: The First Ketone Identified as Sex Pheromone in Stink Bugs

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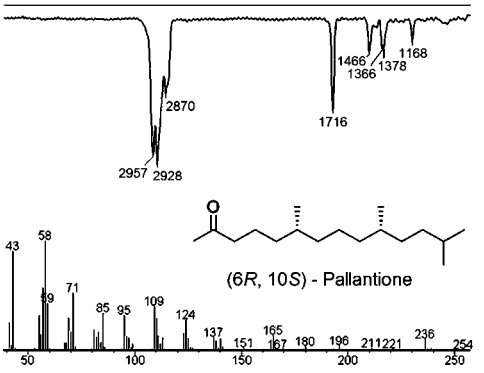
ABSTRACT



This work describes the structural elucidation of the sex pheromone of the soybean stink bug, *Pallantia macunaima*. The biological activity of the synthetic pheromone was demonstrated by behavioral and EAD experiments. Furthermore, the absolute configuration of the natural pheromone was determined as (6*R*,10*S*)-6,10,13-trimethyltetradecan-2-one. This is the first ketone identified as a male-produced sex pheromone in *Pentatomidae*, and the trivial name, pallantione, was assigned to this novel pheromone molecule.

To date, pheromones identified for pentatomid species (stink bugs) exhibit a wide range of molecular structures, such as saturated hydrocarbons, terpenes and terpenoids, and methyl esters.[1−6]

Stink bugs produce two types of volatile chemicals, defensive compounds (allomones) and pheromones. Previously, we identified the allomones produced by adults and nymphs of *Pallantia macunaima*, an important soybean pest in Brazil.[7] We now report the structural elucidation, synthesis, and absolute configuration of the sex pheromone for this species, including electrophysiological and behavioral activity.

The GC analysis of volatiles produced by males and females revealed the presence of a male-specific compound that elicited a strong electrophysiological response only from the antennae of females of the species (Figure 1). Kovats indices for this compound were calculated on three different chromatographic columns, RTX-5, EC-WAX, and EC-1, with values of 1754, 2035, and 1735, respectively.

Bioassays showed that male aeration extracts are significantly more attractive to females than were the controls [treatment 30 (65%), control 16 (35%), $N = 46$, $P < 0.05$]; however, the same extract was not attractive to males, suggesting the existence of a male-produced sex pheromone.

The male-specific compound was analyzed by GC-FTIR, and the infrared spectrum (Figure 2A) showed

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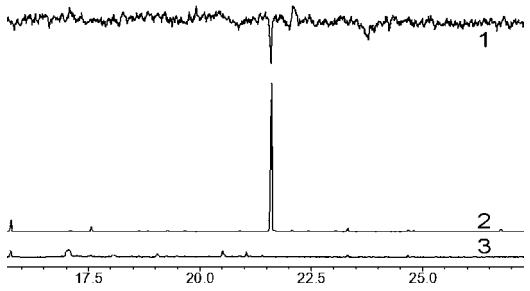

Figure 1. Gas chromatograms of volatiles produced by *P. macunaima* males (**2**) and females (**3**) and the antennal response of a female to the male-specific compound (**1**) (~50 ng).

absorption at 1716 cm^{-1} and 1168 cm^{-1}, related to C=O and the C–C–C stretching characteristic of a ketone functionality. The intense symmetrical (1378 cm^{-1}) and asymmetrical (1466 cm^{-1}) bands indicative of methyl groups were also observed, suggesting a chemical structure containing at least two methyl branches. The mass spectrum (Figure 2B) had a molecular ion at *m*/*z* 254, and a fragment at *m*/*z* 236 ($M^+ - 18$) due to loss of a water molecule. The base peak at *m*/*z* 58, attributed to McLafferty rearrangement,[8] suggested the presence of a carbonyl group at C-2. Neither mass nor infrared spectra showed any indication of carbon–carbon double bonds in the molecule. Therefore, the empirical formula of the molecule is $C_{17}H_{34}O$.

GC–MS analysis of a silylated alcohol derivative (obtained by reaction with $LiAlH_4$ followed by TMSCl) established the carbonyl position at C-2, since a base peak was observed at *m*/*z* 117 due to a cleavage α to the siloxy group (Figures S1 and S2, Supporting Information [SI]).[9]

To further verify the structural elucidation, a catalytic hydrogenation of geranyl acetone (6,10-dimethylundecan-2-one) was performed because the product would, theoretically, exhibit a structural pattern similar to that of the natural pheromone (a carbonyl at C-2 and two methyl branches). As expected, the hydrogenated geranyl acetone exhibited a base peak at *m*/*z* 58, and a fragment at *m*/*z* 180 due to loss of a molecule of water ($M^+ - 18$). A similarity in the intensity of fragments at *m*/*z* 113 and *m*/*z* 95 was also observed for the geranyl acetone product, comparable to the corresponding fragments in the mass spectrum of the natural product for a methyl branch at C-6 in the carbon chain. This strong similarity suggested the chemical structure of the male-specific compound also has a methyl branch at the same position as that in the hydrogenation product of geranyl acetone.

The relative intensity of the fragment at *m*/*z* 165 (*m*/*z* 183; $M^+ - 18$) in the MS of the natural compound, suggested the presence of a second methyl branch at C-10. On the basis of the above data, the proposed chemical structure was determined to be 6,10-dimethylpentadecan-2-one (**1**).

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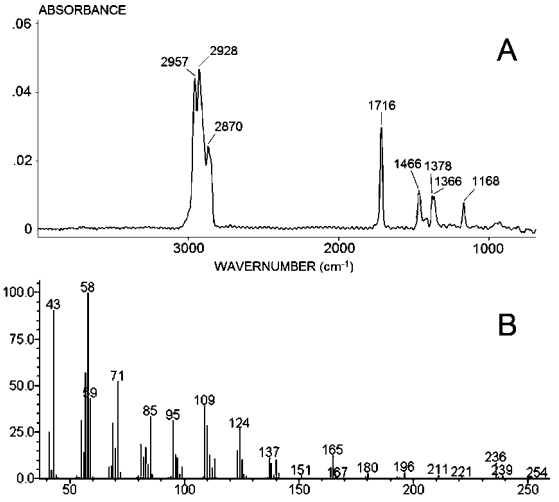

Figure 2. Infrared (A) and electron impact mass spectra (B) of the male-specific *P. macunaima* compound.

Compound **1** was synthesized as a mixture of all possible stereoisomers, employing geranyl acetone (**2**) as the starting material (Figure 3). The initial step was carbonyl protection with ethylene glycol, which led to the ketal **3**. An allylic oxidation of ketal **3** was performed using a mixture of selenium dioxide (SeO_2) and *tert*-butylhydroperoxide (*t*-BuOOH) resulting in the allyl alcohol, which was readily oxidized with PCC to the aldehyde **4**.[10]

The product of the Wittig reaction of phosphonium salt **5** (obtained from *n*-butyl bromide and triphenylphosphine) with the aldehyde **4** led to formation of compound **6**.[11] Ketal **6** was submitted to catalytic hydrogenation over Pd/C, and the hydrogenated product was deprotected with oxalic acid as catalyst,[12] resulting in the desired ketone **1** in 30.3% overall yield.

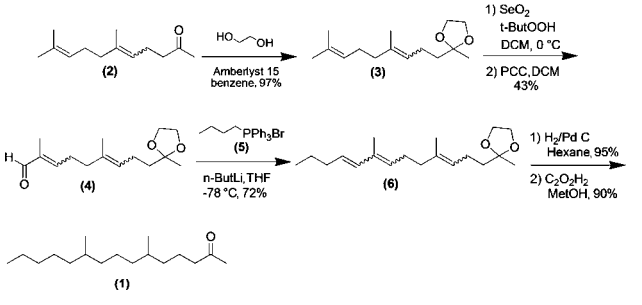

Figure 3. Synthetic route for the 6,10-dimethylpentadecan-2-one (**1**).

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The fragmentation pattern of the mass spectrum of synthetic **1** was very similar to that of the natural product (Figure S3 in SI). However, there were huge differences in the Kovats indices (1790/RTX-5; 2085/EC-WAX; 1772/EC-1) of synthetic **1** vs those of the natural product.

The differing retention times of the synthetic standard and natural compound suggested that the natural product should contain three methyl branches in a C_{14} carbon chain instead of two methyl branches in a C_{15} carbon chain.

To better evaluate the branch positions, a derivatization of the natural product was performed with tosylhydrazine to obtain the tosylhydrazone product. Subsequently, the product was reduced with $LiAlH_4$ and $LiAlD_4$ to obtain the basic carbon skeleton with two hydrogens or deuterons in place of oxygen.[13] In addition, the deuterated and hydrogenated products were analyzed by GC−MS with lower ionization energy (20 eV); at lower energies, the more stable fragments (which are the diagnostic fragments from cleavage of the bonds adjacent to branches) are usually enhanced.[8] The resulting hydrocarbon mass spectrum (Figure 4A), showed that the relative intensities of the fragments *m*/*z* 99 and *m*/*z* 169 are quite prominent; these fragments were associated with the methyl branches at positions C-6 and C-10. The deuteride spectrum confirmed the methyl branch at the C-6 position, since an increase in the intensity of the fragments at *m*/*z* 100/101 was observed.[1] Also, in the deuteride spectrum, the relative intensity of the *m*/*z* 169 fragment decreased by about 50% relative to that of the corresponding hydrogenated product, plus an *m*/*z* 171 ion was present, confirming the C-10 branch position (Figure 4B).

Another fragment of notable relative intensity in the hydrocarbon spectrum was the *m*/*z* 57 of much higher intensity than that for unbranched hydrocarbons. However, this fragment did not differ from the deuteride spectrum, indicating that this ion is derived from the part of the molecule that does not have deuterium.

The *m*/*z* 57 ion could be associated with a *sec*- or an *iso*-butyl fragment, both of which are more stable than *n*-butyl and, thereby, justify the large relative intensity (Figure 4A and B). From this observation, we deduced the presence of a third methyl branch at positions C-12 or C-13. Thus, two other chemical structures for the natural product were proposed: 6,10,12-trimethyltetradecan-2-one (**7**) and 6,10,13-trimethyltetradecan-2-one (**8**).

Compounds **7** and **8** were synthesized as a mixture of all possible isomers using the same methodology previously employed in the synthesis of ketone **1**. In these two synthetic routes, the phosphonium salts **9** and **11** were employed to obtain the ketals **10** and **12** by a Wittig reaction with aldehyde **4**. After hydrogenation and deprotection, ketones **7** and **8** were obtained from **4** in 64.0% and 69.9% yield, respectively (Figure 5).

Ketone **7** eluted as two barely resolved peaks by GC due to separation of the diasteroisomers, with Kovats indices close to those for the natural product. The mass spectrum of **7** was also similar to that for the natural product (Figure S4, SI). However, when synthetic standard **7** was co-injected with the natural product, the natural compound eluted between the synthetic diasteroisomers (Figure S5A in SI).

On the other hand, synthetic ketone **8** and the natural product co-eluted on three different GC columns tested (Figure S5B, SI; EC-WAX and EC-1 data are not shown). In addition, mass and infrared spectra of the synthetic compound (p S16, SI) were identical to those for the natural product, confirming the chemical structure of the *P. macunaima* male-specific compound as that of 6,10,13-trimethyltetradecan-2-one (**8**).

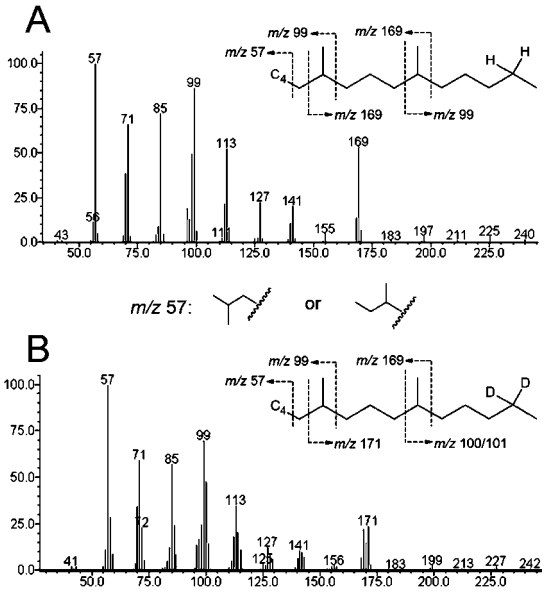

Figure 4. Mass spectra (20 eV) of $LiAlH_4$ (A) and $LiAlD_4$ (B) hydrocarbon derivatives from the natural *Pallantia macunaima* male-specific compound.

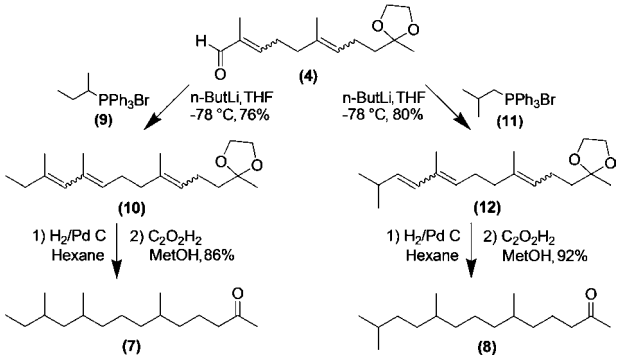

Figure 5. Synthetic route for the 6,10,12-trimetyltetradecan-2-one (**7**) and 6,10,13-trimetyltetradecan-2-one (**8**).

Synthetic ketone **8** showed the same bioactivity for antennae of females by GC-EAD as that of the natural compound. In addition, of 34 females tested in a olfactometer, 25 (76%) chose the source containing **8** ($P < 0.001$). However, only

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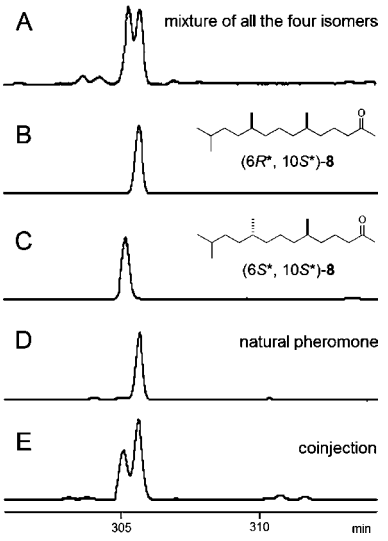

Figure 6. GC partial separation of all the four stereoisomers of 6,10,13-trimethyltetradecan-2-one (**8**).

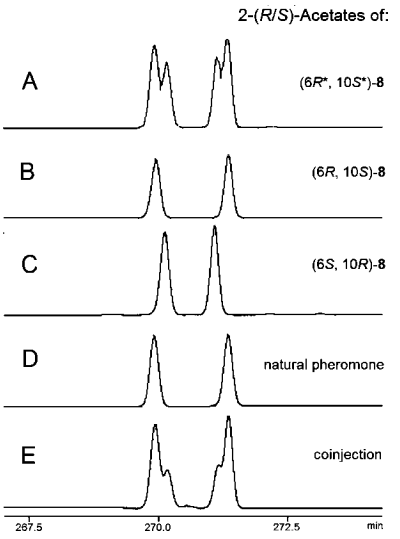

Figure 7. GC partial separation of the (6*R*,10*S*)- and (6*S*,10*R*)-6,10,13-trimethyltetradecan-2-one (**8**) enantiomers by their acetylated derivatives.

36% of males tested chose the odor source containing the synthetic pheromone, meaning that 6,10,13-trimethyltetradecan-2-one (**8**) is a male-produced sex pheromone of the stink bug *Pallantia macunaima* (Figure S6 in SI).

To determine the chirality of the natural product, the four stereoisomers of synthetic ketone **8** were synthesized by coupling with two chiral blocks, which were synthesized starting from (*S*)- and (*R*)-propylene oxides, by applying stereospecific inversion of secondary tosylates as a key reaction.[14]

The resolution of all stereoisomers were tested by employing several chiral GC and HPLC stationary phases; the β-DEX 325 column (Supelco) was partially effective, separating a mixture of (6*R**,10*S**)- from (6*S**,10*S**)-**8**. (Figure 6A−C). By comparing the retention times of natural pheromone and synthetic stereoisomers, it was clear that the natural configuration was restricted to the (6*R*,10*S*)- or (6*S*,10*R*)-**8** isomers, which was confirmed by co-injection (Figure 6D−E).

Due to the difficulty in separating these enantiomers, we decided to introduce an acetate group at C-2, generating a third chiral center in the molecule, since it is well-known that secondary acetates usually promote resolution on chiral GC columns.[15] So, the enantiomers (6*R*,10*S*)- and (6*S*,10*R*)-**8** and the natural pheromone were reduced to the corresponding alcohols, employing $LiAlH_4$, and acetylated with Ac_2O and pyridine.

The 2-(*R*/*S*)-acetate obtained from a mixture of the (6*R**,10*S**)-**8** resulted in four peaks upon chiral GC, but the enantiomers were also resolved (Figure 7A). A baseline separation was achieved for the 2-(*R*/*S*)-acetates of (6*R*,10*S*)- and (6*S*,10*R*)-**8** isomers (Figure 7B,C).

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By comparison of the retention time of the 2-(*R*/*S*)-acetylated pheromone with the isomers discussed, the absolute configuration of the sex pheromone is the enantiopure (6*R*,10*S*)-6,10,13-trimethyltetradecan-2-one, which was confirmed by co-injection (Figure 7D,E).

In summary, the structural elucidation for the *P. macunaima* sex pheromone was determined. The biological activity of the racemic synthetic pheromone was demonstrated by behavioral and EAD experiments. Furthermore, the absolute configuration of the natural pheromone was determined.

To date, attractant pheromones have been identified for more than 30 stink bug species, but this is the first ketone identified as a male-produced sex pheromone in *Pentatomidae*. The trivial name, pallantione, was assigned to this novel pheromone molecule. Further experiments employing enantiopure, as well as racemic, pallantione are underway in the laboratory and the field; these results will be described in due course.

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Supporting Information Available. Experimental procedures, characterization data, chiral chromatograms, and copies of ^1H and ^{13}C NMR. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.