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Ab Initio Calculations on (Z)-5-Decenyl Acetate, a Component of the Pheromone Complex of *Agrotis segetum* (Lepidoptera: Noctuidae) and Electrophysiological Studies with Chain Elongated Analogues†

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Abstract—Conformational analyses of (*Z*)-5-decenylacetate, a sex pheromone component of the turnip moth, *Agrotis segetum*, and double unsaturated pheromone analogues **4** and **5** have been performed by ab initio calculations using Gaussian 92. Two minima were found for a *cisoid* and a *transoid* conformer, differing for 0.03 kcal/mol only. Conformational energies of diene analogues (5*Z*,7*E*)-5,7-decadienyl acetate (**4**) and (3*E*,5*Z*)-3,5-decadienyl acetate (**5**) were determined for conformers required to mimic spatial relationships of the *cisoid* conformation of the natural pheromone **2**. Finally, single sensillum recording studies were carried out with chain elongated C_{11}- to C_{16}-pheromone analogues **6**. Copyright © 1996 Elsevier Science Ltd

Introduction

Recent investigations indicate that second messenger cascades are involved both in olfactoric signal perception in insects and in vertebrates. It is generally assumed that they are triggered by an interaction between the signal molecule and a receptor situated at the sensilla of the insect's antennae. Structure–activity studies[2,3] have demonstrated that these interactions are determined by stereospecific factors as well as by electronic properties[4] and dipole interactions.[5] Hence, the receptors of the lepidoptera antennae tolerate a series of structural variations in the aliphatic moiety of the semiochemical quite well, as shown in Scheme 1.

Thus, structurally varied pheromone molecules are still able to elicit olfactoric sensory cells, as can be seen from electrophysiological measurements. On the other hand, changes in the functional moiety A of **1** causes in most cases an almost complete loss of activity. However, even then a distinct structural variation can still evoke an excitation of the nerve cell.[4] In this connection it should be pointed out that activities determined by electrophysiological recordings do not allow any conclusions with respect to a behavioral release. Systematic investigations on the relationships between the molecular structure of pheromones and their biological behavioral activities have not yet been carried out.

For ants' antennae, which have several olfactoric receptors responsible for different behavioral patterns, the steric specificity of the interactions between receiver and signal are more pronounced than with Lepidoptera. This especially is valid for behavioral responses.[6,7] In this case, the large difference between electrophysiological activity and behavioral response can be demonstrated.[7]

We have proposed a flexible model for the interactions between pheromones of Lepidoptera and their corresponding receptors.[2c,d,e,f,g,h,6] According to this model, the interaction does not follow a rigid key-lock principle, but rather the 'zipper principle'.[8] The signal molecule fits the receptor site in a conformatively flexible way and is recognized by its haptophoric group. Subsequently, a stepwise imbedding into the complementary receptor sphere follows, in the course of which the insertion rate steadily increases because of decreasing degrees of freedom of the pheromone molecule. During this process conformational changes will also occur synchronously in the receptor region (induced fit).[9] Finally, the signal molecule and the corresponding receptor region will be in a defined conformation determined by non-covalent interactions.[2g] This model has been supported by electro-

$$CH_3-(CH_2)_m-CH=CH-(CH_2)_n-CH=CH-(CH_2)_o-A$$

A = CHO, CH_2OH, CH_2-O-CO-CH_3 **1**

Scheme 1.

†Dedicated to Professor Ivar Ugi on the occasion of his 65th birthday.

Pheromones, Part 102. For Part 101, see ref. 1.

physiological recordings with chiral pheromone derivatives.[2d,e,f,h,i]

Results and Discussion

T. Liljefors et al. recently reported a model for the interactions of (*Z*)-5-decenyl acetate (**2**), a component of the pheromone complex of *A. segetum* (Lepidoptera, Noctuidae, Noctuinae),[10] with its receptor at the antenna of the male moths of this species.[3]

This model is essentially based on the assumption that an energy poor, all-*anti*-conformation of the signal molecule represents the biologically active conformation at the receptor site. The basis for this postulate are MM2 force-field calculations with (*Z*)-4-octene (**3**), from which a global minimum for a *cisoid* conformation was obtained, represented by **2a** (*cisoid* 1) in Figure 1.[3b] Additional minima are found with the conformations **2b** (*cisoid* 2) and **2c** (*transoid*) (see Fig. 1). The conformational energy difference calculated between **2a**, **2b** and **2c** is smaller than 0.06 kcal/mol. This model has been used in additional studies of the Swedish group to explain the results of electrophysiological recordings with structurally varied derivatives of **2**.

For this calculation, the two terminal C atoms of the configurations **2a-c** were fixed in their positions in the energy minimum of **2**. Corresponding pheromone derivatives were thus structurally fitted to these fixed points, so that they could adopt the conformations of **2a-c** as closely as possible. The energy of the conformations thus derived subsequently were calculated as well as the one of the global minimum of the 'nonfixed' molecule. The difference of these two conformations was termed the 'conformational energy', which has to be gained by the pheromone derivative to adopt the assumed conformation of the true pheromone molecule at the receptor site as closely as possible. These energy differences were correlated with electrophysiological single cell recordings (ESG, electrosensillogram). Calculations with diene analogues **4** and **5** led to the conclusion that the *cisoid* 2 conformation (**2b**) is the biologically most active[3c] since the calculated conformation energies for *cisoid* 1 (**2a**) and *cisoid* 2 (**2b**) differed partly by a factor of two.

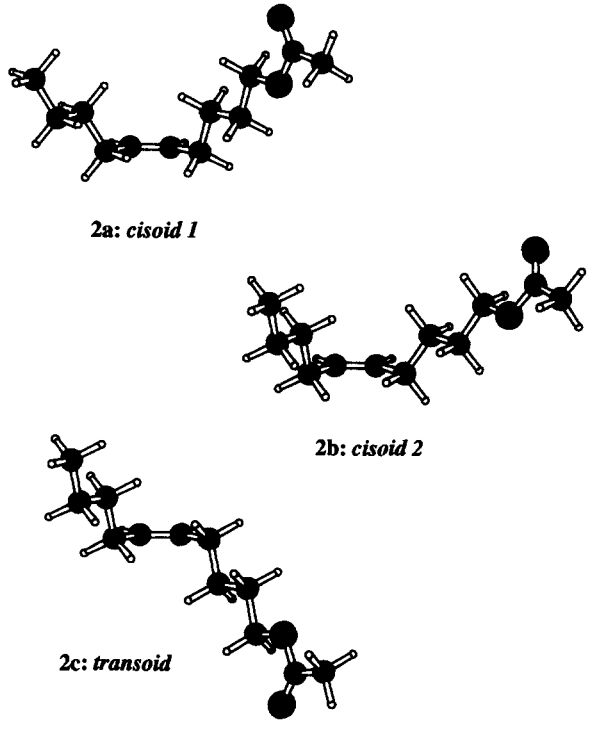
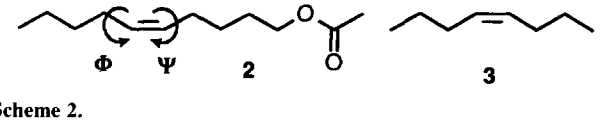


Figure 1. Minimum conformations [MM2] of (*Z*)-5-decenyl acetate (**2**), calculated by Liljefors et al.:[3] **2a** (*cisoid* 1): $\phi = 116.1°$, $\psi = -87.2°$, $H_{rel} = +0.06$ kcal/mol; **2b** (*cisoid* 2): $\phi = 88.4°$, $\psi = -114.9°$, $H_{rel} = +0.06$ kcal/mol; **2c** (*transoid*): $\phi = 88.2°$, $\psi = -87.9°$, $H_{rel} = 0.00$ kcal/mol.

Our investigations of chiral pheromone derivatives, however, led to the conclusion that the biologically active conformations occuring in the receptor region represent the thermodynamically most stable[2c,d,e,f,h,6] in a few cases only.

Since calculational techniques have improved drastically during the past years we have repeated a series of the Liljefors et al. calculations[3] using ab initio methods with the Gaussian 92 program package. All the geometry optimizations proceeded at the Hartree–Fock level (closed shell) using a 6-31-G* basis set (RHF/FOPT). Starting geometries for the calculations with (*Z*)-5-decenyl acetate were the conformations *cisoid* 1 (dihedral angle $\phi = 116.1°$ and $\psi = -87.2°$), *cisoid* 2 (dihedral angle $\phi = 88.4°$ and $\psi = -114.9°$) and *transoid* (dihedral angle $\phi = 88.2°$ and $\psi = -87{,}9°$), the minima conformations determined by Liljefors, as well as a completely stretched conformation (dihedral angle $\phi = 180.0°$ and $\psi = 180.0°$, for the definition of dihedral angles see formula Scheme 2). The optimized geometries, as expected, showed C_1 symmetry and are minima (NIMAG = O). Frequency calculations have been carried out in all cases to correct the enthalpies of formation for the zero-point energy. Nevertheless, these corrections in all cases have no influence on the stability series of the conformers. The geometries calculated with ab initio differ significantly from those of Liljefors et al.:[3] both *cisoid* starting conformations lead to the same conformer, of which the dihedral angle ϕ of the *n*-chain corresponds to that of the Liljefors' *cisoid* 1 geometry, whereas the dihedral angle ψ of the *m*-chain deviates from the starting value by about 33° (see Table 1 and Fig. 2). Furthermore, the completely stretched starting geometry also gives exactly the same *cisoid* conformer as the *cisoid* starting geometries. The *transoid* starting geometry, however, after optimization yields a conformation with both dihedral angles enlarged by ca. 28° (see Table 1 and Fig. 2).



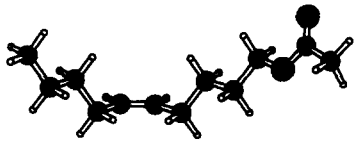
Scheme 2.

Table 1. Ab initio calculations on (*Z*)-5-decenyl acetate (**2**) (Gaussian 92, 6-31G*, RHF/FOPT)

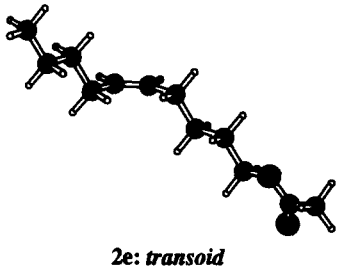
Starting geometry dihedral angle (°)	Optimized geometry dihedral angles (°)	Relative formation enthalpy ΔHF_{rel} (kcal/mol)
cisoid 1	*cisoid*	
$\phi = 116.1$, $\psi = -87.2$	$\phi = 119.2$, $\psi = -119.8$	+0.03
cisoid 2	*cisoid*	
$\phi = 88.4$, $\psi = -114.9$	$\phi = 119.2$, $\psi = -119.8$	+0.03
transoid	*transoid*	
$\phi = 88.2$, $\psi = 87.9$	$\phi = 116.4$, $\psi = 116.6$	0.00
linear	*cisoid*	
$\phi = 180.0$, $\psi = 180.0$	$\phi = 119.2$, $\psi = -119.8$	+0.03

Alltogether, the *transoid* conformation reflects the most stable conformer, nevertheless, the relative formation enthalpies of the *cisoid* conformers are only 0.03 kcal/mol higher, i.e. the differences are negligable.

Furthermore, to compare with the results in ref. [3c] calculations were carried out with diene analogues of (*Z*)-5-decenyl acetate, namely (5*Z*,7*E*)-5,7-decadienyl acetate (**4**) and (3*E*,5*Z*)-3,5-decadienyl acetate (**5**) at the same level. First, geometry optimization was performed for both molecules without any restriction. In subsequent calculations, the carbon atom of the terminal methyl group (C-1) and the three atoms of the acetate group (C-11,O-1,O-2) were fixed in positions corresponding to those of the same atoms of the optimized geometry of (*Z*)-5-decenyl acetate (see above) during the geometry optimization. As a consequence, only the carbon chain between these terminal 'fix points' was allowed to move. The results are shown in Table 2 and Figure 3. It can be seen that the differences of the heats of formation between the completely optimized and the terminally fixed optimized conformers are only 0.19 kcal/mol and 0.06 kcal/mol, respectively, and thus negligable. This means that both diene analogues of (*Z*)-5-decenyl acetate can adopt a conformation almost the same as that of the energy minimum of the pheromone at essentially no energetic cost (see Scheme 3 and Fig. 4).



2d: *cisoid*



2e: *transoid*

Figure 2. (*Z*)-5-Decenyl acetate (**2**), Gaussian 92 optimized conformations: **2d** (*cisoid*): $\phi = 119.2°$, $\psi = -119.8°$, $H_{rel} = +0.03$ kcal/mol; **2e** (*transoid*): $\phi = 116.4°$, $\psi = 116.6°$, $H_{rel} = +0.00$ kcal/mol.

The results suggest that based on ab initio calculations, the existence of two *cisoid* conformations (*cisoid* 1 and *cisoid* 2) with a global energy minimum cannot be proven. There is only one *cisoid* energy minimum. In addition, it is shown that for the two diene analogues **4** and **5** practically no conformational energy has to be spent to adopt the thermodynamically most stable conformation of **2** (*cisoid*) at the receptor site. This is demonstrated in Figure 5, where the most stable conformations of **2** and **4** are superimposed.

We have furthermore remeasured the dependence of the electrophysiological activities from the chain length in molecule **6** for $n = 3$, 4, 5, 7 and 9 with *A. segetum*. In doing so, the measuring technique reported by Kaissling and Thorson[11] was used and recorded from a sensory cell selectively responding to **2**[12] (see Scheme 4).

A defined air stream of 1 s length was directed over a filter paper with a distinct amount of test molecule **6** and onto the isolated insect antenna. The resulting receptor potential amplitudes (RP) and the number of action potentials (AP) per second were determined.

Table 2. Results of ab initio calculations on pheromone analogues (5*Z*,7*E*)-5,7-decadienyl acetate (**4**) and (3*E*,5*Z*)-3,5-decadienyl acetate (**5**) (Gaussian 92, 6-31G*, RHF/FOPT)

Pheromone analogues	Optimized geometry dihedral angles (°)	Enthalpy of formation (*HF*, a.u.) and energy difference (ΔHF, kcal/mol)
(5*Z*,7*E*)-5,7-decadienyl acetate (**4**) completely optimized	$\phi = 118.1$, $\psi = 243.4$	$HF = -616.7819039$
(5*Z*,7*E*)-5,7-decadienyl acetate (**4**) terminally fixed	$\phi = 109.0$, $\psi = 240.5$	$HF = -615.7815937$ $\Delta HF = 0.19$
(3*E*,5*Z*)-3,5-decadienyl acetate (**5**) completely optimized	$\phi = 119.8$, $\psi = 242.4$	$HF = -615.7819489$
(3*E*,5*Z*)-3,5-decadienyl acetate (**5**) terminally fixed	$\phi = 117.7$, $\psi = 249.9$	$HF = -615.7818557$ $\Delta HF = 0.06$

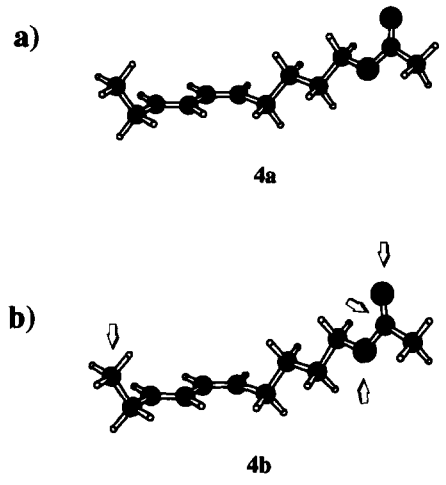
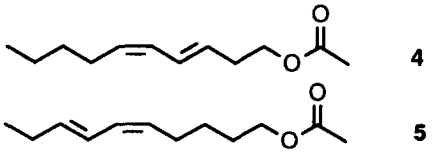


Figure 3. (5*Z*,7*E*)-5,7-Decadienyl acetate (**4**), Gaussian 92 optimized conformations: **4a** (not fixed): $\phi = 118.1°$, $\psi = -116.6°$; **4b** (marked atoms fixed): $\phi = 109.0°$, $\psi = -119.5°$.



Scheme 3.

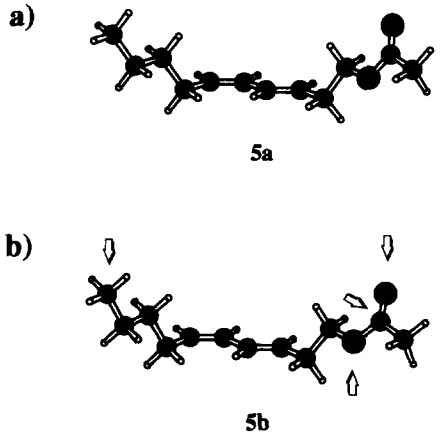


Figure 4. (3*E*,5*Z*)-3,5-Decadienyl acetate (**5**), Gaussian 92 optimized conformations: **5a** (not fixed): $\phi = 119.8°$, $H = -117.6°$, **5b** (marked atoms fixed): $\phi = 117.7°$, $\psi = -117.6°$.

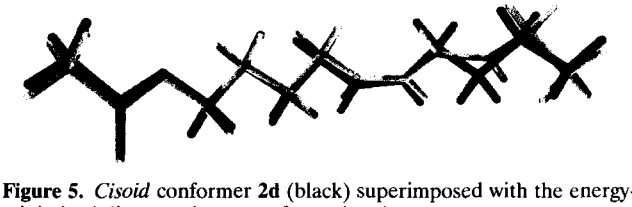
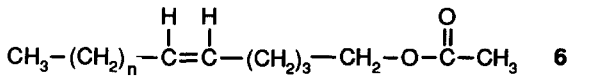


Figure 5. *Cisoid* conformer **2d** (black) superimposed with the energy-minimized diene analogue conformation **4a**.



Scheme 4. $n = 3$: **2** (*Z*5-10:Ac); $n = 4$: *Z*5-11:Ac; $n = 5$: *Z*5-12:Ac; $n = 7$: *Z*5-14:Ac; $n = 9$: *Z*5-16:Ac.

To equalize variations within a recording series it was processed according to ref. 13; for the 'Kennlinien', the amplitudes of each of the corresponding stimuli of the test substances to be compared were always determined starting from the lowest to the highest concentration. The amplitude of the receptor potential RP and the number of action potentials AP were normalized to the maximum value of each recording series (RP/RP_{max} and AP/AP_{max}, respectively).

The compounds tested were GC-pure, the relative volatilities of substances **6** were taken into consideration with a corresponding factor[14] in the evaluation of the results.

Figure 6 shows the result of receptor potential determination with the compounds **6**, representing an elongation of the *n*-chain of **6** ($n = 3 = \mathbf{2}$) for 1, 2, 4 and 6 CH_2 groups. From 20 'Kennlinien' measured, the means of the normalized RP value at 2 µg stimulus loading were taken for the graphical presentation. A graduated decrease of the electro-physiological responses from the natural pheromone *Z*-5-10:Ac (**2**) over *Z*-5-11:Ac, *Z*-5-12:Ac, *Z*-5-14:Ac to the *Z*-5-16:Ac can be seen. The same sequence of activity is found for all stimulus loadings, i.e. from 0.002 to 200 µg. The difference of the loss of activity between **2** and the chain length 12 over 14 to 16 are not as significant as that from C-10 via C-11 to C-12. An increase of the receptor potential from *Z*-5-14:Ac to *Z*-5-16:Ac, reported in ref. 3a,b, was not observed in our experiments.

Therefore, we again carried out 20 recordings, this time taking the number of action potentials per second as a basis. The results are presented in Figure 7. A drastic decrease of the receptor response is found from C-10 over C-11 to C-12. The response then slightly increases from C-12 to C-14 and does not change significantly to C-16.

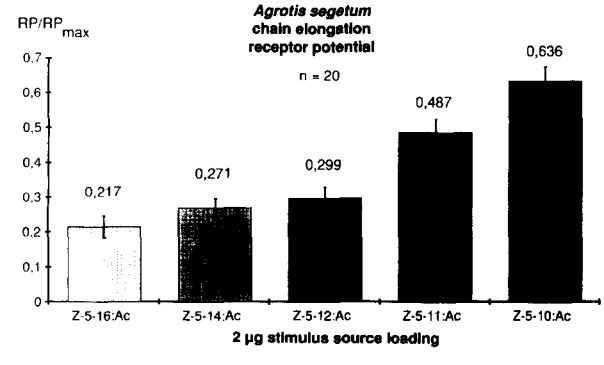


Figure 6. Single sensillum recordings, receptor potentials, of male *A. segetum* antennae, stimulated with chain elongated pheromone analogues **6**, 2 µg stimulus source loading.

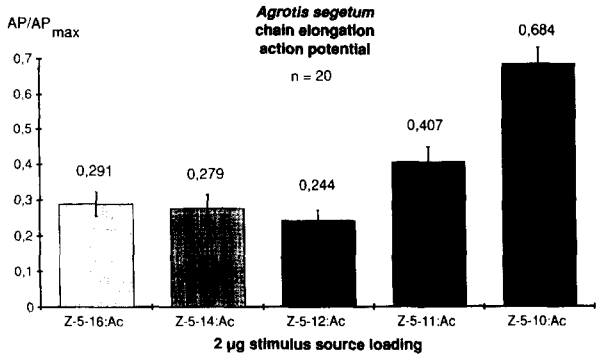


Figure 7. Single sensillum recordings, action potentials, of male *A. segetum* antennae, stimulated with chain elongated pheromone analogues **6**, 2 µg stimulus source loading.

The deviations of our measuring results from those of the authors of ref. 3a,b may be due to the fact that the *A. segetum* race[15] used in our experiments was different from that of the other group. To the best of our knowledge there have been no investigations to clarify whether the activity spectrum with respect to antennal responses for a set of structural analogues of pheromones must be the same for receptors of different geographical races of a species.

Our improved calculations show how decisive the method used for the calculation of conformational energies may be and how carefully one must be with the interpretation of biological measuring results correlated with calculated conformational energies. The question still arises whether a global minimum conformation will really be adopted at the receptor. As already mentioned above, doubts about this hypothesis arise from our investigations with chiral pheromone derivatives.

The almost rigid key-lock principle of E. Fischer will come nearest to the assumption that the thermodynamically most stable form of the signal molecule will exist at the receptor site.[9,16] Our flexible model,[2c-,d,e,f,h,6] which can be seen as a combination of the zipper[8] and the induced fit models,[9] however, allows any plausible conformation at the final state of imbedding of the pheromone molecule into the flexibly reacting receptor region and thus comes, in our opinion, nearest to the experimental results.[2]

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