Possible Origin of Modified EAG Activity by Point-Fluorination of the Insect Pheromone Eldanolide

Shuichi Hayase,*[a] Michel Renou,[b] and Toshiyuki Itoh*[a]

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Conformational analysis with B3LYP/6-31G* calculations was carried out on the insect pheromone eldanolide and its fluorinated analogues in order to clarify the origin of the differences in pheromone activity between these compounds, as measured by electroantennography (EAG). The calculations indicate that some conformers have a distinctively higher energy than their fluorinated analogues. Significant differences were found between the populations of the preferable conformers of EAG-active and EAG-inactive compounds. We suggest that the differences in the populations of the favored conformers of the eldanolide analogues best explain the differences in biological activity according to the position of fluorination.

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Introduction

In spite of the growing attention on chemical communication among insects, the molecular basis of its outstanding specificity is far from being totally understood. The most striking example of specificity is the ability of the insects' olfactory receptors to discriminate between enantiomers. Mori established that there are at least ten categories of stereochemistry–pheromone activity relationships, so the biological activity of unnatural enantiomers may be unpredictable.[1] Many types of fluorinated pheromone molecules have been synthesized to determine the origin of such complicated pheromone–receptor interactions.[2,3,4] Schlosser and coworkers first synthesized the fluorinated analogue of an insect pheromone[3] and concluded that the fluorine-modulated olfactory response can be rationalized without involving steric factors.[2a,3] We synthesized eldanolide (**1**),[5] which is a sex pheromone of the male African sugarcane borer *Eldana saccharina*,[5] and all enantiomers of its partially fluorinated analogues **2**,[6] **3**,[7] and **4**[7] and tested their pheromone activity by electroantennogram (EAG) response.[8] Interestingly, both the (+)-**2** and (–)-**2** enantiomers were as active as the natural eldanolide (+)-**1** with the insect olfactory receptors,[6b] whereas none of the enantiomers of **3** and **4** showed EAG activity.[7b]

Michel and Schlosser proposed that fluorine affects odor perception as a consequence of conformational change. However, they provided no model to explain how the conformation changes, following the fluorination of the parent molecules, affect odor activity.[9] It has also been suggested that inhibition of the pheromone catabolism is dependent on the conformations of the pheromone molecule, on the basis of computational analysis of these molecules.[10] The first step in pheromone detection by insects is known to start by the recognition of a molecule by pheromone-binding proteins (PBPs) followed by its interaction with an olfactory receptor (OR) belonging to the G-protein-coupled receptor family.[11] Functional data are missing for insect ORs, but binding experiments and structural studies indicate that ligands induce conformational changes in the 3D structure of the PBP.[12] Therefore, it appears particularly relevant to look at the conformational changes in the pheromone molecule caused by fluorine atom substitution, be-

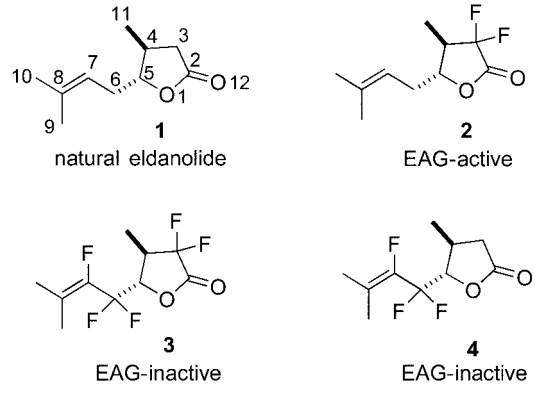

Figure 1. Eldanolide (**1**) and its fluorinated analogues **2**–**4**.

[a] Department of Materials Science, Faculty of Engineering, Tottori University,
4-101 Koyama-Minami, Tottori 680-8550, Japan
E-mail: shu@chem.tottori-u.ac.jp
titoh@chem.tottori-u.ac.jp
[b] INRA, Unité de Phytopharmacie et Médiateurs Chimiques, Route de Saint Cyr, 78026 Versailles, France

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cause they directly affect the shape of the molecule and alter its biological activity. Liljefos proposed a bioactive conformation of a pheromone component using a 3D-QSAR study,[13] and Hoffmann and Rychlewski reported that the substitution of hydrogen by fluorine caused a significant modification of the favored conformer.[14] Here we report the results of our computational study and show that the modified conformational preference caused by the point-fluorination of eldanolide is consistent with a loss of EAG activity (Figure 1).

Results and Discussion

Energy and Conformation in vacuo

Initially we searched for stable conformers of compounds **1**, **2**, and **4**. The results are shown in Figures 2, 3, and 4.[15] The calculation suggested six stable conformers for the natural pheromone **1**: T-1, T-2, G1-1, G1-2, G2-1, and G2-2. Since each conformer exists in two forms, axial and equatorial, with respect to the γ-lactone ring, a total of twelve stable conformers were suggested by the calculation (Figure 2). Here, "T" indicates the *trans* and "G" indicates the *gauche* conformer. The conformational energy map of **1** (Figure 2) clearly shows that six conformers (G1-1eq, T-1eq, G2-1eq, G1-2eq, T-2eq, and G2-2eq) in particular have lower energy because they are located deep in potential valleys. Similar results were obtained for α,α-difluoroeldanolide **2** (EAG-active) as shown in Figure 3. It was thus found that the relative energies of the favored conformers of **2** were similar to those of the natural pheromone **1**.

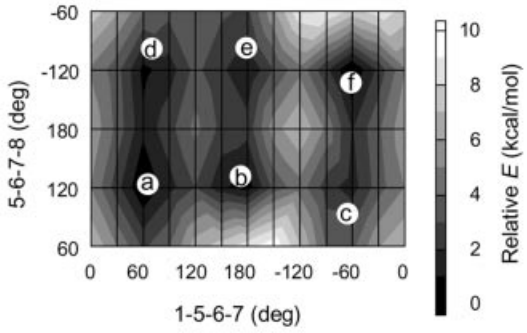


Figure 2. B3LYP/6-31G* relative energy as a function of the dihedral angles, 1-5-6-7 and 5-6-7-8, in **1**. Calculations were performed for molecules in vacuo. Open circles show the favored conformers: (a) G1-1eq, (b) T-1eq, (c) G2-1eq, (d) G1-2eq, (e) T-2eq, and (f) G2-2eq.

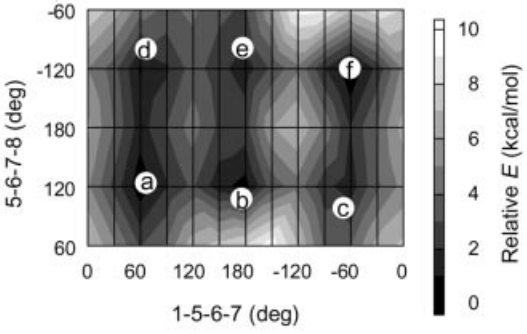


Figure 3. B3LYP/6-31G* relative energy as a function of the dihedral angles, 1-5-6-7 and 5-6-7-8, in **2**. Calculations were performed for molecules in vacuo. Open circles show the favored conformers: (a) G1-1eq, (b) T-1ax, (c) G2-1eq, (d) G1-2eq, (e) T-2eq, and (f) G2-2eq.

Contrary to this, there were great differences in energy between the stable conformers of the fluorinated analogue **4** (EAG-inactive). Thirteen stable conformers around the C5–C6 and C6–C7 bonds were found, and eight favored forms were suggested: G1-1ax, G1-2ax, T-1ax, G2-1eq, T-2ax, G2-2eq, T-3ax, and G2-3eq (Figure 4 and Figure 5).[15]

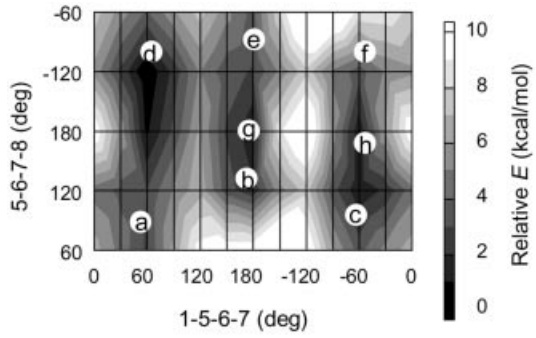
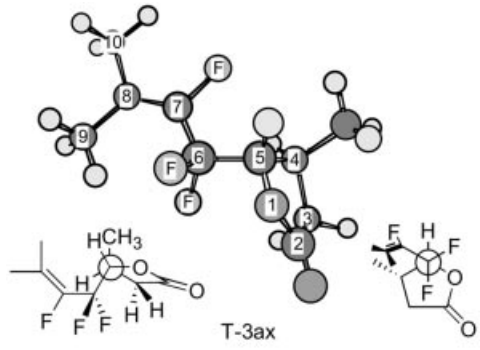


Figure 4. B3LYP/6-31G* relative energy as a function of the dihedral angles, 1-5-6-7 and 5-6-7-8, in **4**. Calculations were performed for molecules in vacuo. Open circles show the favored conformers: (a) G1-1ax, (b) T-1ax, (c) G2-1eq, (d) G1-2ax, (e) T-2ax, (f) G2-2eq, (g) T-3ax, and (h) G2-3eq.



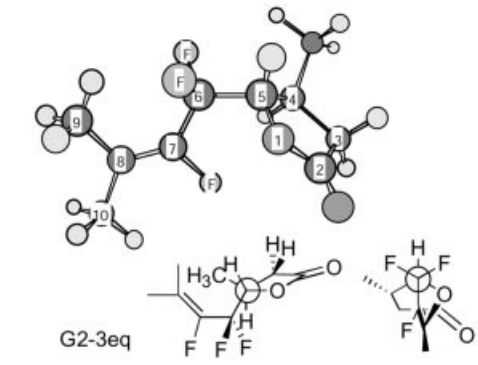


Figure 5. T-3ax and G2-3eq conformations in **4**.

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The conformational energy map in Figure 4 shows that the eight most favored conformers are located deep in the valleys of energy. Two of them, T-3ax and G2-3eq, were not found among the stable conformers of the EAG-active compounds **1** and **2**. Furthermore, the five conformers, G1-1ax, T-1ax, G2-1ax, T-2eq, and G2-2eq were also not favored for compounds **1** and **2**.

It should be noted that the relative energy values of the favored conformers of compounds **1** and **2** are quite similar, but completely different from those of the favored conformers of **4**. Furthermore, the difference between the relative energies of all favored conformers of **1** or **2** is at most 1.0 kcal/mol, whereas that of **4** reaches 3.2 kcal/mol.[15]

Energy and Conformation in Water

As in vivo interactions between pheromones and their receptor molecules take place in hydrated fluid, the sensillum lymph, we next estimated the stable conformers for **1, 2**, and **4** in water. Like the results in vacuo, a completely different pattern of stable conformers was suggested for the EAG-inactive **4**. Eight favored conformers were determined among the thirteen stable conformers: T-1ax, T-2ax, T-3ax, G1-1ax, G1-2ax, G2-1eq, G2-2eq, and G2-3eq. The last one was not found among the conformers of **1** and **2**. It should be noted that the five conformers with axial configuration (T-1, T-2, T-3, G1-1, and G1-2) were more stable than the equatorial ones for compound **4**, as calculated in vacuo.

In water, the differences in energy between the favored conformers of compounds **1** and **2** were not significant; the difference between all favored conformers in **1** or **2** was at most 1.5 kcal/mol.[15] However, a remarkable difference was again obtained between the conformers of EAG-inactive **4**: the difference between the relative energies of the favored conformers of **4** reached 2.9 kcal/mol. The relative energies of conformers **1, 2**, and **4** could be related to the contributions of the conformers to their populations by the thermostatistic principle.

Population Ratio of Stable Conformers in vacuo and in Water

We estimated the populations of all stable conformers of compounds **1, 2**, and **4** using the relative Gibbs energies and the Boltzmann factor, and the results are shown in Figure 6. Since the energy difference between the axial and equatorial forms of each conformer is not very significant, we compared the ratio of stable conformers as "population" values and discussed the differences between the three compounds; the "population" indicates the total values of both axial and equatorial forms.

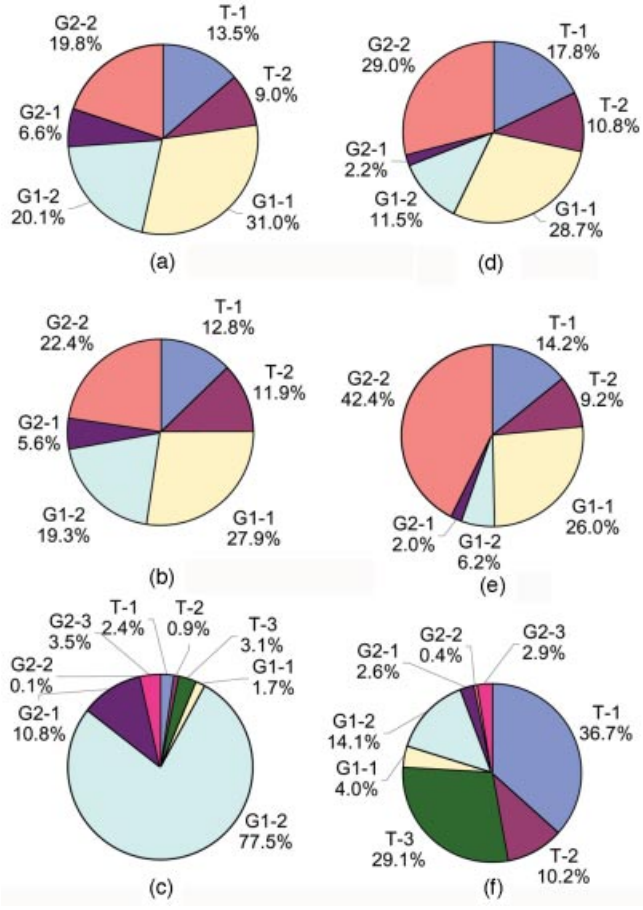

Figure 6. Populations calculated from the relative Gibbs energies for stable conformers of **1**, **2**, and **4** in vacuo and in water: (a) **1** in vacuo, (b) **2** in vacuo, (c) **4** in vacuo, (d) **1** in water, (e) **2** in water, and (f) **4** in water.

As clearly shown in Figures 6 (parts a and b) the population pattern of the stable conformers of natural pheromone **1** is similar to that of compound **2**, whereas it is completely different from that of compound **4** (Figure 6, part c). Compound **2** is EAG-active whereas compound **4** is inactive; this suggests that the pattern of population for the stable conformers predicts EAG activity. In particular, it is assumed that EAG activity is dependent on the population of G2-2: the population of G2-2 (22.4%) in **2** (EAG-active fluorinated analogue) is quite similar to that of pheromone **1** (19.8%), whereas it is only 0.1% for the EAG-inactive compound **4**. This is believed to result from the higher relative energy of the G2-2ax conformer of **4**. It should also be pointed out that the populations of G1-1 and G1-2 seem to be important for EAG activity, because the population pattern of EAG-active **2** is quite similar to that of pheromone **1**, although it is completely different from that of EAG-inactive **4**: G1-2 accounts for 77.5% of the conformer population in **4**, compared to only 20.1% in pheromone **1** and 19.3% in compound **2**.

The population pattern of T conformers also seems important for EAG activity; conformers T-1 and T-2 account for a significant population in pheromone **1** or compound

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2 (ca. 20%), compared to only 7% in compound **4**, where T-3 accounts for 3.1%.

Interestingly, the population pattern of the stable conformers in compound **4** is strongly dependent on the medium: G1-2 accounts for 77.5% in vacuo, but only 14.1% in water. T-1, T-2, and T-3 are the major conformers for compound **4** in water (see parts c and f in Figure 6). In particular, 29.1% of T-3 is present in compound **4** in water, while none of it is found in **1** or **2** under the same conditions. Furthermore, there were small differences between the distribution of the populations in vacuum and in water for compounds **1** and **2**, except for G2-2; G2-2 accounts for 22.4% in vacuo in compound **2**, whereas it is almost double that (42.4%) in water, as shown in parts b and e of Figures 6. As T-1 or T-2 has a larger dipole moment than G1-2 or G2-1, the presence of water seems to neutralize the differences in energy for highly polar molecules, whereas it has no effect on nonpolar molecules; rather it enhances the steric effect of nonpolar compounds by hydrophobic packing. This seems to reflect the great differences in the population patterns.

Conclusions

The results of the B3LYP/6-31G* calculations for the natural pheromone component **1** and its fluorinated analogues show that biological activity is correlated to the population pattern of the stable conformers. The G2-2 conformer is significantly unfavorable for compound **4** (EAG-inactive), whereas it is favored for both pheromone **1** and the EAG-active fluorinated analogue **2**. We have also established that the fluorinated points of the molecules change the species of stable conformers and their populations. EAG activity is therefore critically controlled by the position of the fluorinated point of the mother molecule. Up to now, much emphasis has been given to the 3D structure of the acceptor proteins and the conformational changes they undergo upon ligand binding.[16,17] The conformation of the ligand molecules has, in turn, received less attention. We believe that our findings will contribute to the understanding of the molecular mechanisms involved in odor recognition, at least in the case of eldanolide. In particular, our calculations of the energy-minimized conformers, associated with the measure of their activity, might be of great interest in docking studies aimed at unraveling the mechanisms of binding and activation of the receptors.

Computational Methodologies

Geometrical optimization and vibrational analysis were performed at the B3LYP/6-31G* level of theory. Vibrational analysis was used to characterize each stationary point as an energy minimum and to obtain the harmonic frequencies without scaling for calculations of zero-point and thermal corrections. All of the calculations were carried out with the Gaussian 98 suite of programs.[18] We used compounds **1**, **2**, and **4** for the present study because the same low EAG activity was recorded for the fluorinated eldanolides **3** and **4**. We chose several stable conformers of **3** and compared them with those of **4**; no significant difference was found between them.[19] Therefore, we decided to perform a detailed calculation study focusing on compound **4**. It was anticipated that the conformation of the isobutenyl side chain group at position 5 of the γ-lactone ring might play an important role in the recognition of the pheromone molecule by PBPs or ORs. Therefore, compounds **1**, **2**, and **4** were optimized by starting from the six conformers around the C5–C6 and C6–C7 bonds, including two kinds of twist conformation (axial and equatorial) of the γ-lactone moiety as shown in Figure 7. Only the staggered conformation was considered for the methyl group at the β-position of the γ-lactone ring, and the eclipsed form for the

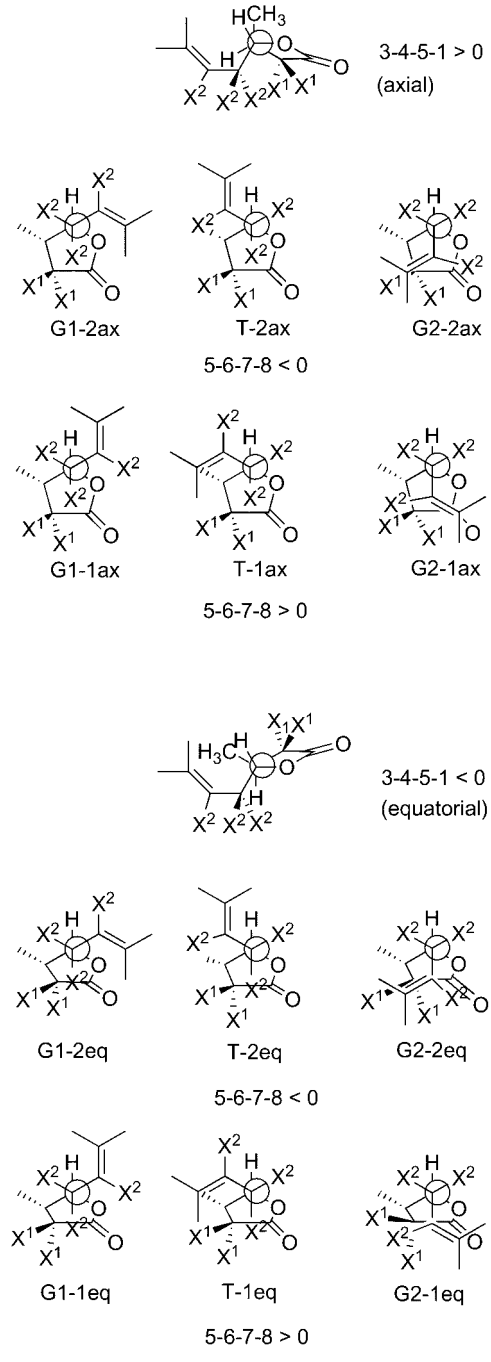

Figure 7. Starting conformations for the optimization of **1** (X^1 = X^2 = H), **2** (X^1 = F, X^2 = H), and **4** (X^1 = H, X^2 = F). Here, "T" means *trans* conformer and "G" means *gauche* conformer.

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terminal methyl group on the side chain was counted as a stable conformer.

The hydration effect was estimated by the Onsager model[20] with a dielectric constant, ε_r, of 78.39 for water, which is available from the Gaussian 98 suite of programs. Geometrical optimization and vibration analysis in water were also performed at the B3LYP/6-31G* level of theory.

Supporting Information Available (see also the footnote on the first page of this article): Tables for the results of the B3LYP/6-31G* calculations for compounds **1**–**4** and full details of the calculations.

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Acknowledgments

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