Chinese Science Bulletin 2005 Vol. 50 No. 22 2587—2591

An analysis of structure fitting and bioactivity between sex pheromone of cotton bollworm, *Helicoverpa armigera* (Hübner) and its fluorinated analogs

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Abstract **A study on the structure-activty relationship between (Z)-hexadec-9-enal (Z-9-16:Ald) and its analog was conducted by comparing the structures of the sex pheromone of cotton bollworm, *Helicoverpa armigera* (Hübner) with its fluorinated analog using computer molecular fitting. It is demonstrated that the structure of analog substituting for hydrogen atom on the terminal carbon atom is similar to Z-9-16:Ald. The EAG result showed that there is no significant difference in activities between Z-9-16:Ald and its fluorinated analog synthesized.**

Keywords: ***Helicoverpa armigera*** **(Hübner), sex pheromone, fluorinated analogs, structure-activity, EAG.**

DOI: 10.1360/982005-860

Cotton bollworm, *Helicoverpa armigera* (Hübner), a worldwide pest, distributes in the vast area between N50° and S50°, including Europe, Asia, Africa, and islands in the southwest Pacific Ocean. It occurred very seriously many times in the last century and brought about great losses to cotton and the other economic crop yields in China. Pesticides such as organochlorine, organophosphorus, carbamate and pyrethroid have been widely applied to control the pest in cotton fields since the 1950s. But unrestricted, unplanned and excessive use of pesticides has induced great resistance of the moth to the chemicals and caused environmental pollution and damage to the natural enemies of pests. Therefore, alternative measures such as the trap-killing method of using sex attractant were developed to control the pest species[1].

For cotton bollworm, sex pheromone is released from sex glands of virgin females and attractive to males. Kehat and Dunkelblum[2] discovered the components of sex pheromone by chemical analysis and behavioral test, which are composed of (Z)-hexadec-9-enal (Z-9-16:Ald), (Z)-hexadec-11-enal (Z-11-16:Ald), (Z)-hexadec-11-en-1-ol (Z-11-16:OH), (Z)-hexadec-7-enal (Z-7-16:Ald), and (Z)-tetradec-9-enal (Z-9-14:Ald). The main constituents are (Z)-hexadec-9-enal (Z-9-16:Ald) and (Z)-hexadec-11-enal (Z-11-16:Ald).

However, further fundamental research is needed, though there have been so much achievements in synthesis and field application of sex pheromone of the cotton bollworm, to answer the following questions: what is the relationship between the molecular structure and bioactivity of Z-9-16:Ald as a primary component of sex pheromone? Is it true that the molecular structure of Z-9-16:Ald cannot be decorated or changed because of its specificity? The studies about these are important not only to the elucidation of the mechanism of interaction between sex pheromone and their receptors but to the exploration of new type of sex attractants and their field application.

The study of structure-activity relationship can be traced back to the 1880s. Since 1980s, with the rapid development of the computational chemistry, scientists have engaged to study the structure-activity of insect pheromone at three-dimensional structure level, and made some progresses. Liljefors et al.[3,4] researched the sex pheromone of turnip moth, *Agrotis segetum* by single-cell measurements and molecular mechanics calculations (MM2), and presented a new model of ligand-receptor. Bengtsson et al.[5], Jönsson et al.[6−9] and Wu et al.[10] synthesized a series of alkylation and halogenated compounds, investigated the structure-activity relationship among these compounds by single-cell measurements and molecular mechanics calculations (MM2), and verified the model further. Bykhovskaia and Zhorov[11] calculated all the minimum energy conformations (MECs) of two sex pheromones of the American cockroach, *Periplaneta americana*, and their 11 structural analogs (seven agonists, two antagonists, and two inactive compounds) using the molecular mechanics method. The MECs of the analogs were compared with the most populated MECs of the pheromones. An atomic model complementary to the bioactive conformation of one of the pheromones was constructed. Warthen et al.[12] made the structure-activity relationship observations for the pheromone of Z-type European corn borer moth, *Ostrinia nubilalis*, and a series of analogs with fluorination in the alcohol portion of the molecular. The results demonstrated that a critical range of electrostatic potential on the protons of the double-bond appears to be essential for optimal acceptor fit and attractiveness. In addition, Warthen et al.[13] also studied the structure-activity relationship from the pheromone of bagworm moth, *Thyridopteryx ephemeraeformis*, and a series of analogs with modification in the alcohol portion of the molecule. It was demonstrated that the attractiveness of these analogs was related to molecular structure

and their physical attributes, such as size, shape, charge distribution, and chirality.

Some strict limitation existed in rebuilding the molecular structure of sex pheromone because bioactivity of the pheromone depends on their molecular structure specifically. For example, there was a tight relationship between bioactivity and molecular qualities such as shape, position of double-bond and functioning group, spatial configuration, and conformation. The fluorine atom is only 10% larger than the hydrgen atom, with a Van der Waals radius of 1.65 Å vs. 1.50 Å for hydrogen. Substitution of fluorine for hydrogen would not induce a significant change in molecular volume or surface area[10]. The bioactivity of fluorinated pheromone analogs has been studied by many scientists[10,12,14−27].

We calculated the fitting energy of Z-9-16:Ald and its fluorinated analogs based on molecular mechanics using computer-assisted methods, and then tested EAG responses of male moths to the analogs. These will make some contribution to explanation of the questions above.

1 Materials

1.1 PowerFit

PowerFit is a computer-assisted molecular fitting program designed by Microsimulation Company. The computation engine of the PowerFit is based on the steric and electrostatic alignment which was advanced by Kearsley and Smith[28] has been improved by many research groups[29].The sum of steric overlap, electrostatic matching, atom type matching, distance constraints and conformational energy is fitting energy.

1.2 Insects

The cotton bollworm was maintained in the laboratory at (26±1)℃ and R.H. 60%－70% under a light-dark photoregime of 16L∶8D h. Larvae were reared in the laboratory on a modified semiartificial diet as described by Wu and Gong[30]. Pupae were separated by sex and kept in wooden boxes with screened tops until emergence. Adults were supplied with 10% honey water solution.

1.3 Chemicals

(Z)-16-fluorohexadec-9-enal (16-F-Z-9-16:Ald) (pure> 95%) was synthesized in the laboratory in the following manner. (Z)-hexadec-9-enal (Z-9-16:Ald) (pure>95%) and (Z)-hexadec-11-enal (Z-11-16:Ald) (pure>95%) were purchased from Shin-Etsu, and hexadecanal (16:Ald) (pure>95%) was purchased from Aldrich.

Each compound was diluted with hexane to desired concentration and then stored at −20℃ until further use (Table 1).

Table 1 The solution for test

Materials	Concentration of solution /ng · mL^{-1} hexane
16-F-Z-9-16:Ald	1, 10, 100
Z-9-16:Ald	1, 10, 100
Z-11-16:Ald	1, 10, 100
16-F-Z-9-16:Ald + Z-11-16:Ald (5∶95)	1, 10, 100
Z-9-16:Ald + Z-11-16:Ald (5∶95)	1, 10, 100
16:Ald	1

1.4 EAG test instrument

Electrode: Ag-AgCl wires was inserted into a capillary filled with saline solution; DC/AC amplifier: Syntech UN-06; stimulating amplifier: syntech CS-05; fine truning: syntech MP-15; binocular microscope: syntech WILD M3Z; snalysis Program: syntech software.

2 Experimental procedure

2.1 Fitting computational procedure

There are 16 carbon atoms in the Z-9-16:Ald. By replacing one hydrogen atom in each carbon with one fluorine atom except the atom conjugation carbonyl, we got 15 fluorinated analogs. In computational procedure, the Z-9-16:Ald was first fitted with itself, the fitting energy of Z-9-16:Ald was regarded as normal fitting energy. Then, each of the 15 fluorinated analogs was fitted with Z-9-16:Ald. Then the corresponding Fitting Energy was obtained.

2.2 Synthesis of (Z)-16-fluorohexadec-9-enal

By referring to the synthesis of Z-11-16:Ald[31], the chlorofluodrocarbon and alkynol were synthesized first, then they were joined by BuLi, and finally hydrogenated and oxidated. The final product was (Z)-16-fluorohexadec-9-enal (16-F-Z-9-16:Ald.) (Fig. 1).

IR: 3434 cm^{-1} (—C═O), 3005 cm^{-1} (H—C═C-H), 2929 cm^{-1}, 2856 cm^{-1} (—CH_2—), 2717 cm^{-1} (H—C═O), 1728 cm^{-1} (—C═O), 1462 cm^{-1} (—CH_2—), 1391 (H—C═O), 1047 cm^{-1} (—C—F), 725 cm^{-1} (*cis* H—C═C—H).

H^1 NMR: (δ, 10^{-6}) 1.35－1.44 (m, 14H), 1.71 (m, 2H), 1.82 (m, 2H), 2.09 (t, 4H), 2.51 (t, 2H), 4.45 (t, 1H), 4.60 (t, 1H), 5.44 (m, 2H), 9.85 (s, 1H).

2.3 Electroantennogram sesponses

Antennal receptivity of male cotton bollworm to the tested compounds was determined by EAG. An antenna was excised from its base, and the distal part of the terminal segment was cut off. The capillaries were filled with saline solution into which Ag-AgCl wires were inserted. The signals generated by the antenna were passed through a high-impedance amplifier and displayed on a monitor. EAG result of 16:Ald was used as a standard for normalizing all responses so that responses within an individual and between individuals could be compared. Stimulation with the standard preceded and followed by every two test

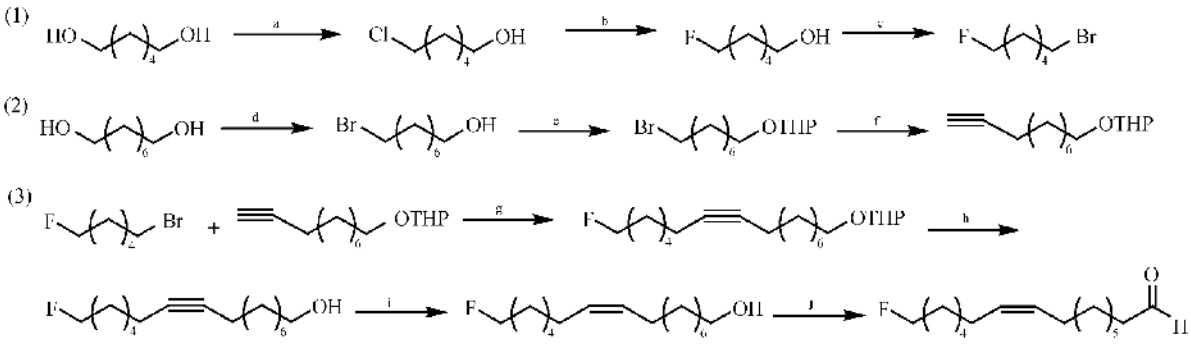

Fig. 1. Scheme of the preparation of (Z)-16-fluorohexadec-9-enal. a, HCl, heptane, 90℃; b, KF, diethyleneglycol, 125℃; c, PBr_3; d, HBr, heptane, 90℃; e, THP, CH_2Cl_2; f, acetylene, Na/NH_3, –30℃; g, BuLi, THF, –80℃; h, CH_3OH, heat; i, H_2, $Pd/CaCO_3$, pentane; j, PCC, CH_2Cl_2.

stimulations.

In the test, the odor delivery system was similar to what was previously described by Wu et al.[10]. The stimulus (1-s duration) was delivered into a purified airstream (1 L/min) flowing continuously over the preparation. Samples (25 μL) of the standard solutions of test compounds were applied to filter paper strips (6 cm × 0.5 cm), and the solvent was allowed to evaporate (30 s). The paper strip was placed in the clean pipet and left for 40 s prior to use. The vapor from the pipet was injected into the airstream passing over the antennal preparation. Each stimulation was in 0.1 s duration and followed by a 30-s purge period of filtered air to ensure recovery of antennal receptors. For each compound tested, EAG were recorded from 30 male moths. One antenna was tested for less than 10 times.

2.4 Data processing

EAG responses to sex pheromone and fluorinated analogs were compared statistically using Duncan's multiple comparison. Student's t test was invoked to compare EAG responses of males using different doses of each compound.

3 Result and discussion

3.1 Result of fitting

From computing result, it was found that Fitting energy values varied regularly with fluorinated substitution at different sites. Normally, when hydrogen atom adjacent to carbon of double-bond was replaced with fluorine atom, the energy was the highest. When the replacement occurred at other carbons, the energy decreased gradually. When terminal alkyl hydrogen atom was substituted, the energy was the lowest.

According to fitting principle, the lower the fitting energy, the more the similarity of molecular structures. The fitting energy of fluorinated analog, (Z)-16-fluorohexadec-9-enal (16-F-Z-9-16:Ald), substituting for hydrogen atom on the terminal carbon atom was the most similar to (Z)-hexadec-9-enal (Z-9-16:Ald). Therefore, perhaps, the bioactivity of this analog was similar to (Z)-hexadec-9-enal. Analogs, having a single fluorine at other carbon atoms, were inactive because of their higher fitting energy. For European corn borer (*Ostrinia mubilalis*)[19] and western spruce budworm (*Choristoneura occidentalis*)[21], the bioactivity of terminal fluorinated analogs of their pheromones had been confirmed.

3.2 Discussion for EAG responses

In experiments, EAG responses of males for Z-9-16:Ald and 16-F-Z-9-16:Ald were tested in different doses individually. EAG responses for Z-9-16:Ald increased with increasing doses. When dose was ⩾10 ng/mL, the difference was significant (p<0.05). EAG responses for 16-F-Z-9-16:Ald was similar to for Z-9-16:Ald (Talble 2).

EAG responses of males for 16-F-Z-9-16:Ald was lower than that for Z-9-16:Ald in the same dose, but the difference was not significant (p<0.05) (Table 2).

In experiments, EAG responses of males for mixtures containing Z-9-16:Ald, Z-11-16:Ald and containing 16-F-Z-9-16:Ald, Z-11-16:Ald were also tested individually at different doses. EAG responses for blends of Z-9-16:Ald and Z-11-16:Ald increased with increasing doses; the difference was significant (p<0.05). EAG responses for blends of 16-F-Z-9-16:Ald and Z-11-16:Ald increased with increasing doses of components also. However, when dose was ⩾10 ng/mL, the difference was significant (p<0.05) (Table 3).

EAG responses for blends of 16-F-Z-9-16:Ald and Z-11-16:Ald was lower than for blends of Z-9-16:Ald and Z-11-16:Ald in the same dose, but the difference was not significant (p<0.05) (Table 3).

Table 2 EAG responses of male for one component[a)]

Component	Relative EAG responses		
	1 ng/mL	10 ng/mL	100 ng/mL
Z-9-16:Ald	1.30±0.12b	2.62±0.30b	5.99±0.76a
16-F-Z-9-16:Ald	1.22±0.08b	2.17±0.37b	4.73±0.76a

a) Different letters indicated significantly different values at the 95% confidence level.

Table 3 EAG responses of male for two mixtures[a)]

Mixtures	Relative EAG responses		
	1 ng/mL	10 ng/mL	100 ng/mL
Z-9-16:Ald + Z-11-16:Ald	3.64±0.53b	6.65±1.12b	10.47±1.32a
16-F-Z-9-16:Ald + Z-11-16:Ald	3.02±0.36b	3.98±0.78b	7.55±0.98a

a) Different letters indicated significantly different values at the 95% confidence level.

The recent hypotheses presented by Prestwich[32] concerning mechanisms involved in moth olfactory sense of pheromones propose that lipophilic pheromone enters olfactory sensilla on the male`s antenna through pores in the cuticular wall for sensilla and that the pheromone is solubilized in the hydrophilic lumen of the sensilla by binding to pheromone-binding protein[33]. The bound pheromone is transported across the sensillar lumen and transferred to a membrane-bound pheromone receptor protein[34] on sensory dendrites within the sensillum. The binding of pheromone with the receptor causes generation of an electrophysiological response in the dendrites, making direct input into the olfactory glomeruli of the moth central nervous system[35] and resulting in the display of complex behavior reactions by the male. The receptor-bound pheromone is then removed from the receptor and catabolized to make the system ready for a fresh incoming stimulus[36,37].

The structure-activity relationship of pheromones of the turnip moth, *Agrotis segetum*, was studied by single-cell measurements and molecular mechanics. Base on this, a new model for the interactions between pheromone and its receptor was presented. The general hypothesis for pheromone binding involves a three-pronged interaction of the pheromone with the acceptor: (i) by hydrogen bonding to and/or electrostatic interaction with the polar functional group, (ii) electrostatic interaction with the double-bond, and (iii) much weaker dispersion forces than are responsible for binding the terminal alkyl group[3,4].

The replacement of hydrogen atoms with fluorine atoms in pheromone components will change the properties of the pheromone molecules, including polarity, flexibility, and hydrophobicity[10]. The part of the receptor interacting with the terminal alkyl chain was most probably highly lipophilic. The most likely reason for the reduced electrophysiological activity of fluorinated analogs is the low affinity of fluorinated hydrocarbons for a lipophilic environment[26]. In addition to a low affinity between a flurinated analog and the pheromone component receptor, the low lipid solubility of anlogs may decrease their adsorption on the lipophilic antennal surface and their solubilization in the sensillum lymph via complex equilibria involving lipophilic pheromone binding protein[26]. This may lead to a reduction in the number of molecules reaching the receptor. It can decrease the activity response for fluoro analogs[10].

Although the steric effects of fluorine substitute in the allylic positions are small, the strong permanent dipole of the C-F bond may interfere with the proper binding of the double bond to its receptor binding site. This may be an additional activity-decreasing factor for such fluoro analogs[10].

In our experiments, EAG responses provoked by fluorinated analogs were lower than that by sex pheromone at the same concentrations. We think that the above statement is a reasonable elucidation about our results.

Acknowledgements The work was supported by the National Key Basic Research Project (Grant No. G20000162) and the Key Program of the Chinese Academy of Sciences (Grant No. KSCX2-1-02).

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(Received May 31, 2005; accepted August 9, 2005)