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INHIBITION OF ANTENNAL ESTERASES OF THE EGYPTIAN ARMYWORM *Spodoptera littoralis* BY TRIFLUOROMETHYL KETONES[1]

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Abstract. A series of aliphatic and aromatic trifluoromethyl ketones has been tested as inhibitors of the antennal esterases of the Egyptian armyworm *Spodoptera littoralis*, by evaluation of the extent of hydrolysis of [1-^{3}H]-(*Z,E*)-9,11-tetradecadienyl acetate (**1**), a tritiated analog of the major component of the sex pheromone. The most active compounds with a long chain aliphatic structure were 3-octylthio-1,1,1-trifluoropropan-2-one (**2**) (IC_{50} 0.55 μM) and 1,1,1-trifluorotetradecan-2-one (**4**) (IC_{50} 1.16 μM). The aromatic compounds were generally less potent inhibitors than the corresponding aromatic ones, although β-naphthyltrifluoromethyl ketone (**10**) exhibited a remarkable inhibitory activity (IC_{50} 7.9 μM). Compounds **2**, **4** and **10** exhibit a competitive inhibition with K_i values of 2.51×10^{-5} M, 2.98×10^{-5} M and 2.49×10^{-4} M, respectively. Some of the trifluoromethyl ketones tested were slow-binding inhibitors and compounds **2** and **10** are described as inhibitors of the antennal esterases of a moth for the first time.

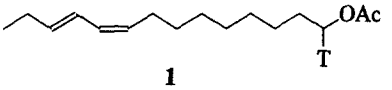
A very important aspect of insect physiology which has received an increasing interest in the last years concerns the study of pheromone-degrading enzymes.[2] These enzymes may inactivate the incoming pheromone molecules after the stimulatory interaction with the receptor molecules has occurred. In this regard two possible inactivation mechanisms have been postulated, either by binding of the stimulus molecules to the pheromone binding proteins located in the sensillum lymph[3] or through enzymatic degradation of the pheromone molecules by esterases located in the hairs[4].

Trifluoromethyl ketones have been proven to inhibit the action of a variety of serine esterases[5], juvenile hormone esterase (JHE)[6], or mammalian carboxylesterases[7]. Some of them have been tested in a limited number of insects as inhibitors of the antennal esterases[2a] and their activity reasoned in terms of the tetrahedral geometry of the geminal diol produced by hydration of the ketones in aqueous solution. In the presence of esterases, trifluoromethyl ketones may form hemiketals with a serine residue present at the active site of the enzyme, acting therefore as reversible inhibitors. In this paper we report the inhibition of antennal esterases of the Egyptian armyworm *Spodoptera littoralis* by a variety of aliphatic and aromatic trifluoromethyl ketones **2-13**, most of them previously synthesized in our laboratory[8], by evaluating the extent of hydrolysis of the major component of the sex pheromone, (*Z,E*)-9,11-tetradecadienyl acetate, labeled with tritium at C-1 (compound **1**). To our knowledge, no study has been undertaken so far on this polyphagous pest and some of the trifluoromethyl ketones have been tested for the first time.

Tritium-labeled pheromone **1** was prepared from the unlabeled alcohol **14** through oxidation to the

corresponding aldehyde **15** followed by reduction with $NaBT_4$ (469 mCi/mmole) and acetylation (Scheme 1) The resulting solution had a specific activity of 137 mCi/mmole and the radiochemical yield was 77 6%. To carry out the experiments a stock solution of **1** ($5.64x10^{-2}$ M in ethanol) was prepared.

Preliminary trials to separate pheromone-specific sensory hairs from the antennal branches, following a protocol previously established for other insects, i.e. *Agrotis segetum*[9], were unsuccessful. Therefore, the enzyme preparation was obtained from the crude homogenate of antennae of freshly emerged males, as follows. Antennae from 1-2 day old anesthetized males were removed, immediately frozen and kept at -80°C. When required, the antennae were homogenized at 680 rpm, on an ice bath and in the presence of 75 mM phosphate buffer (pH 7.4), and centrifuged at 12000 g for 2 min and 4°C to remove the cuticular debris. The inhibition assays were carried out by placing 1-10 μl of several concentrations of the inhibitor (0.03 to 3 mM in ethanol) in borosilicate tubes, the solvent was evaporated and the required amount of phosphate buffer to get a total volume of 100 μl subsequently added. After vortexing for 30 sec. and cooling on an ice bath, an aliquot of the



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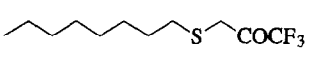
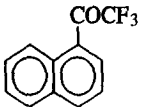


2

$CH_3(CH_2)nCOCF_3$

n = 9: **3** n = 13: **6**
n = 11: **4** n = 15: **7**
n = 12: **5** n = 16: **8**



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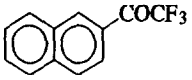

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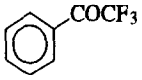

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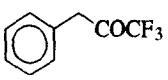

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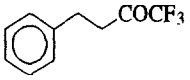

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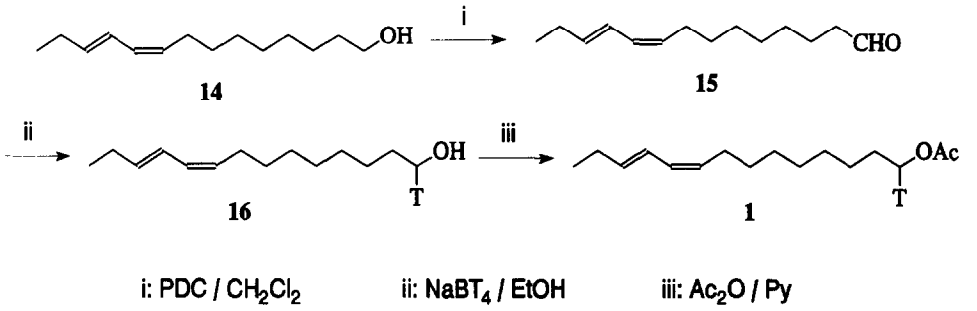
esterase extract corresponding to 2 antennae was added to the tubes and vortexed for 5 sec. In some cases a preincubation period of 10 min was used. The labeled substrate (2 μl of a $5.64x10^{-4}$ M soln. in ethanol, 11.3 μM final concentration) was added and the mixture immediately incubated on a thermostatized water bath at 25°C for 30 min. The tubes were cooled on an ice bath and treated with 100 μl of ethyl acetate. The phases were separated after vortexing for 1 min and 5 μl aliquots of the organic solution were spotted on 10 x 5 cm TLC plates, upon which unlabeled solutions of the synthetic pheromone acetate and alcohol had been previously pre-spotted. The plates were eluted with hexane:ethyl acetate 5:1, the spots visualized with iodine, and the radioactive zones cut into polyethylene scintillation vials. The vials were counted using Unisolve as scintillation liquid in a Kontron Betamatic scintillation counter, and the extent of hydrolysis was calculated as the ratio of dpm of the tritiated resulting alcohol to the total amount of dpm of the alcohol plus the unreacted labeled acetate. Three replicates for each inhibitor concentration were carried out.

Firstly, we calculated the K_m and V_{max} of the enzyme by using homogenates of 2 antennae and final

concentrations of substrate ranging from 0.56 to 45.1 μM. The corresponding values turned out to be 13.9 μM and 4.66×10^{-9} M.sec^{-1}, respectively, according to the double reciprocal plot of Lineweaver and Burk[10]. Secondly, the potential inhibitors **2-13** were tested at 0.6, 6 and 60 μM final concentrations in the presence of 11.3 μM concentration of the substrate. The compounds exhibiting the highest inhibitory potency were 3-octylthio-1,1,1-trifluoropropan-2-one (OTFP, **2**), β-naphthyltrifluoromethyl ketone (**10**) and 1,1,1-trifluotetradecan-2-one (**4**). Compound **2**, which has been shown to be a highly effective slow tight-binding inhibitor of JHE from *Trichoplusia ni*[11] and *Manduca sexta*[6], has proved useful as ligand for the affinity purification of JHE. Compound **10** has been found to display a good inhibition of the pheromone reception in EAG, although in the field showed a moderate disruptant effect of the pheromone action[12].



Scheme 1

1,1,1-Trifluorotetradecan-2-one (**4**), in turn, has been found to be highly active against JHE ($IC_{50} 1 \times 10^{-7}$ M), binding almost irreversibly to the active site of the enzyme[13] and as potent inhibitor of the sensillar esterase in *Antheraea polyphemus*[14]. However, and taking into account the isosteric replacement of the acetate group by the trifluoroacetonyl moiety CH_2COCF_3, the fact that the chain length of compound **4** is three and five carbons shorter than the pheromonal structures of *S. littoralis* and *A. polyphemus*, respectively, point out to a lack of specificity in the inhibition induced by this trifluoromethyl ketone. This assumption is confirmed by the very modest inhibition of the antennal esterases of *Plutella xylostella* displayed by (Z)-1,1,1-trifluoro-14-nonadecen-2-one, a mimic of the natural pheromone[15]. It is likely that formation of stable tetrahedral hemiketals with a serine residue of the enzyme may not be the only mechanism involved, and that other stereoelectronic and stereochemical effects (see below) might be also considered for a successful interaction with the enzyme. The compound is also a good blocker of the pheromone action in EAG[12].

To determine the mode of binding of the most promising inhibitors to the active site, experiments in the presence or not of a preincubation period of 10 min were carried out. This time was selected since previous assays with 0, 3, 5, 7, 10 and 15 min of preincubation time of OTFP (0.6 μM) with the esterase extract corresponding to 2 antennal equivalents, followed by addition of the substrate (11.3 μM final concentration),

revealed higher inhibition values with increasing preincubation periods up to 5-10 min. To our knowledge, this is the first observation wherein OTFP behaves not only as a potent inhibitor of JHE[6,11] but as a slow-binding inhibitor of antennal esterases of an insect as well. The IC_{50} values of the trifluoromethyl ketones **2-13** were calculated by plotting the percentage of inhibition against logarithm of inhibitor concentration and are shown in the Table. Within the aliphatic series and with preincubation, the inhibition activity could be arranged following the order **2** > **4** > **5** > **6** ≈ **3**. Compound **2** showed, therefore, the most potent inhibition followed by compound **4**, two fold less active, and by compound **5** which presented a lower IC_{50} by one order of magnitude. Without preincubation, the inhibitors displayed less potency, the inhibition being three to nine-fold lower than those obtained with preincubation. Compounds **7** and **8** were less effective. Our results show that compounds **2**, **4** and **5** behave as tight slow-binding inhibitors of the antennal esterases of *S. littoralis* and that the activity of linear aliphatic trifluoromethyl ketones *vs* number of carbon atoms of the chain followed a parabolic curve with an inflexion point for n=11 (compound **4**). Compounds with longer or shorter aliphatic chain led to lower inhibitory potency. The aromatic compounds were much less potent inhibitors, except β-naphthyltrifluoromethyl ketone (**10**), which exhibited IC_{50} values of 11.4 and 7.9 μM with and without preincubation, respectively. This result shows that compound **10** binds to the active site of the enzyme in a time-independent manner and that the stereochemical requirement is also a factor to consider in the interaction

Table. Inhibition of antennal esterases of *S. littoralis* by trifluoromethyl ketones **2-13**.

Compound	IC_{50} values (μM)	
	With preincubation	Without preincubation
2	0.55	3.7
3	19.3	ND[a]
4	1.16	10.6
5	4.4	11.0
6	18.4	ND[a]
7	ND[a]	(50%)[b]
8	ND[a]	(44%)[b]
9	ND[a]	(38%)[b]
10	11.4	7.9
11	40.7	(31%)[b]
12	ND[a]	(36%)[b]
13	ND[a]	(43%)[b]

[a]ND = Not determined.
[b]Values between parenthesis represent the inhibition values at 63 μM inhibitor concentration.

process with the enzyme[7]. In this context, taking into account the reasonable correlation between the hydration constants (K_D) of several types of trifluoromethyl ketones and their inhibitory effect,[16] and assuming that the K_D of compounds **9** and **10** should be very similar, the clear difference in activity between these two ketones might be attributed to the higher accessibility of the carbonyl group and/or the corresponding hydrate in compound **10** for a successful interaction with a serine residue present at the active site of the enzyme.

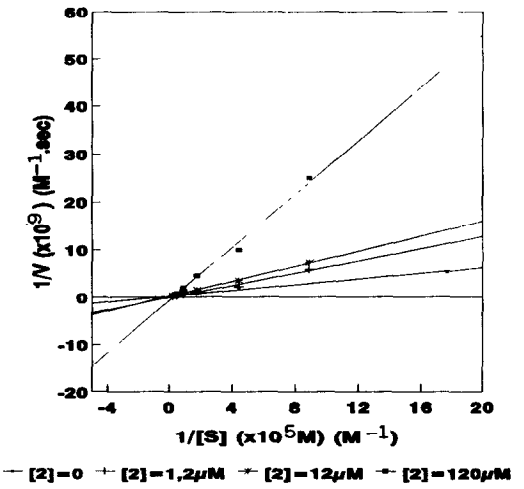


Figure 1. Inhibition of antennal esterases of *S. littoralis* by OTFP (2) after a 10 min preincubation using compound **1** as substrate.

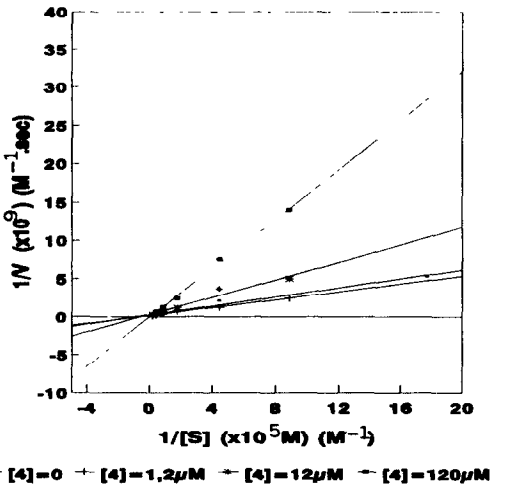


Figure 2. Inhibition of antennal esterases of *S. littoralis* by TFT (4) after a 10 min preincubation using compound **1** as substrate.

In order to assess the type of inhibition displayed by the most active compounds **2**, **4** and **10**, experiments were carried out to know the effect of several concentrations of the substrate on the velocity of the process at several concentrations of the inhibitor (0, 1.2 μM, 12 μM, 120 μM). Prior to the addition of the labeled substrate, compounds **2** and **4** were preincubated for 10 min with the esterase extract, as cited above. When we plotted 1/V *vs* 1/[S] a typical pattern of competitive inhibition was obtained (Figs. 1-2 for compounds **2** and **4** only shown), after regression analysis (r^2=0.99). A replot of the slope of each reciprocal plot *vs* the corresponding inhibitor concentration gave a straight line, whose interception with the abscissa afforded the following inhibition constants: K_i 2.51×10^{-5} M for **2**, 2.98×10^{-5} M for **4** and 2.49×10^{-4} M for **10**. The competitive mode of action was confirmed when we plotted [S]/V *vs* [I] for each substrate concentration, according to Cornish-Bowden[17]. As expected, straight parallel lines were obtained for the three inhibitors, thus indicating that the inhibition activity displayed by **2**, **4** and **10** was competitive.

In summary, we have found that some aliphatic and aromatic trifluoromethyl ketones behave as good inhibitors of the antennal esterases of *S. littoralis*, being worth of note the activity of 3-octylthio-1,1,1-

trifluoropropan-2-one (**2**) and 1,1,1-trifluorotetradecan-2-one (**4**), two well-known inhibitors of JHE, and β-naphthyltrifluoromethyl ketone (**10**). The activity of compounds **2** and **10** as inhibitor of the antennal esterases of an insect is described for the first time.

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