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Chapter 15

Inhibition of Juvenile Hormone Esterase by Transition-State Analogs

A Tool for Enzyme Molecular Biology

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A summary of Transition State Theory is presented, as it applies to the design of trifluoromethyl ketone esterase inhibitors. Possible mechanisms for the inhibition of the enzyme are discussed. A new series of compounds, α,α'-alkanebis-thiotrifluoro-propanones was synthesized and showed excellent *in vitro* and moderate *in vivo* inhibition of the insect juvenile hormone esterase from the fifth instar larvae of *Trichoplusia ni* (cabbage looper). The potency of the above series was also screened for its ability to inhibit other esterases of toxicological and pharmacological significance. Trifluoroketones are discussed as an example of the importance of chemistry in biotechnology approaches.

It is generally recognized that the widespread use of insecticides has two serious side-effects: evolution of insecticide resistance in insects and danger to environmental and human safety. The continued use of insecticides requires stricter agricultural practices as well as the improvement of the chemicals themselves. Equally as important, however, new strategies for development of insecticidal agents are needed (1-3).

The elucidation of enzyme-substrate interactions has established new paradigms leading to the discovery of biologically active compounds. One such paradigm is the "Transition State Theory" as it applies to the mechanism of enzymatic reactions. Based on this theory, series of transition state analog inhibitors known as trifluoromethyl ketones have been synthesized in our laboratory. Our target has been an insect enzyme of developmental and reproductional importance, juvenile hormone esterase (4-12). The development of those extremely potent inhibitors served several aims: in addition to providing "traditional" inhibitors that can be used to block the enzyme and study its biochemical and physiological consequences, the new group of compounds led to powerful ligands for the high yield affinity chromatography

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purification of this low abundance enzyme (13-15). The pure enzyme, juvenile hormone esterase (JHE) is a very suitable candidate for several biotechnological approaches from the use of the enzyme as a probe for endocrine regulation, to the more detailed research of the molecular biology and molecular genetics of the enzyme, itself. As discussed above the biological role of JHE in lepidopterous larvae was established using highly specific esterase inhibitors. Application of these compounds led to giant larvae which were locked in the feeding stage. The use of trifluoroketone affinity columns led to the purification of large amounts of the enzyme. This availability first led to a dose-response, linear with the quantity of the enzyme applied, observed for the direct injection of the enzyme into several insect species, showing that injected JHE has a clear anti-JH effect. Since such an effect is desirable in agriculture, it becomes important to clone the enzyme. Here again the affinity procedure from chemical approaches provided the protein necessary for development of molecular probes. The injection of purified enzyme in rabbits resulted in JHE specific antibodies needed for screening an expression library as well as for further immunoassay studies. The affinity purified protein also allowed classical aminoacid screening to be done which led to the synthesis of oligonucleotide probes for confirming positives from the expression library. Hopefully the cloned message can be inserted into baculovirus vectors which lead to precocious production of the enzyme. This, in turn, should result in anti-juvenile hormone effects such as cessation of feeding and developmental abnormalities.

These results and future applications of trifluoromethyl ketones show the importance of the "traditional" chemical optimization of compounds in various biotechnological approaches. In this presentation the authors wish to give a summary of the leading research paradigm, Transition State Theory resulting in trifluoromethyl ketones, a group of highly effective inhibitors of JHE. The same concepts can be applied to a variety of polarized carbonyls, carbamates, phosphates and phosphonates as inhibitors of esterases and proteases. Clear targets in the insecticide field will be enzymes involved in insecticide metabolism and neurohormone processing enzymes.

Juvenile Hormone Esterase

Our laboratory is concerned with targeting potential insecticides that disrupt normal development and metamorphosis in insects. Juvenile hormones (JHs), acting in concert with the steroid hormone ecdysone, are believed to control the timing of the larval-larval molts, larval-pupal and pupal-adult transformations of the insects. It has been demonstrated that the events leading to pupation are initiated by reduction of the JH titer in the hemolymph. In addition to a cessation of biosynthesis, this reduction in JH titer is controlled by degradative metabolism (16,17). Hydrolysis of the epoxide and ester functionalities present in active JH are two routes of degradation and subsequent inactivation of JH (18). The primary route of JH metabolism in the hemolymph of last stadium lepidopterous larvae is ester hydrolysis, and it is catalyzed by the enzyme juvenile hormone esterase (JHE). JHE has been shown to

play a crucial role in initiating pupation in lepidopterous insects (19); selective inhibition of this enzyme prevents JH hydrolysis and causes a delay in the onset of pupation (20).

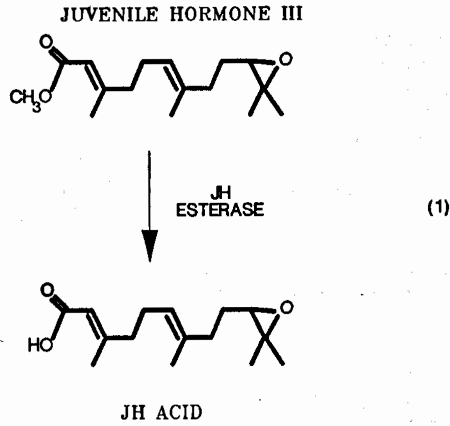
Transition State Theory and JH Ester Hydrolysis

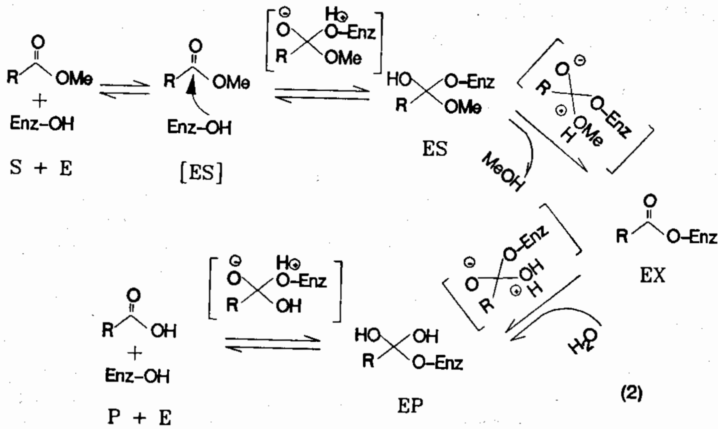
In every chemical reaction the reactants are in equilibrium with an unstable activated complex, the transition state complex (TS), which decomposes to give the product. In his pioneering work, Linus Pauling pointed out that for promoting a reaction without influencing its equilibrium constant, the enzyme should have much higher affinity for the transition state of a reaction than for either the substrate or the product(s), thereby pushing a reaction in the desired direction by continuously removing its transition state (21). Based on this idea, extremely potent inhibitors can be developed for a given enzymatic reaction if one can synthesize "transition state mimics" (TSM): stable chemical compounds resembling the transition state (22).

Because transition states may have lifetimes of only several nanoseconds, in most cases, it is impossible to observe them directly. However, there are numerous lines of evidence for the existence of a tetrahedral-like transition state for non-enzymatic ester hydrolysis: a) substitution at a carbonyl group (as is the case of the hydrolysis of esters) most often proceeds by a tetrahedral mechanism, a second-order addition-elimination (for a review of this mechanism, see (23)); b) the kinetics are pseudo-first order either in the substrate or in the nucleophile, as predicted by the mechanism; c) for the ^{18}O labeled esters, the ^{18}O isotope is detectable in both products (in a "normal" S_N2 reaction all the ^{18}O isotopes should remain in the acid functionality)(24); d) in a few cases tetrahedral intermediates have been isolated or detected spectrally (25).

Ester hydrolysis of the JHs is shown in Equation 1. We have no exact thermochemical data measured for this reaction. In general, the hydrolysis of unsaturated long chain aliphatic acids is thermodynamically neutral under standard conditions (26,27) (i.e. the heat of hydrolysis for *cis*- and *trans*-oleic acid is -1.7 and +0.8 kJ/mol, respectively), which shows that these reactions are generally not favored based solely on thermodynamic considerations. In aqueous solution ester hydrolysis is driven by the high concentration of water. The juvenile hormones will be more resistant to acid or base catalysed hydrolysis because in these compounds conjugation with the α,β-unsaturation greatly stabilizes the carbonyl functionality (19). Therefore, the nonconjugated TS should be difficult to form, and this situation will be reflected by a high activation energy for the uncatalyzed hydrolysis of JH.

The ester hydrolysis catalyzed by hydrolase enzymes (E) proceeds according to Equation 2 and the free energy diagram is shown in Figure 1. After the preliminary noncovalent binding step ([ES]) the enzyme becomes complementary in structure to the substrate (or its TS) and forms a covalent adduct with it (ES). Release of the first product (MeOH) results in the acyl-enzyme (EX), which hydrolyses through a covalent enzyme-product adduct (EP) to the appropriate carboxylic acid and the free enzyme. It has been shown


JUVENILE HORMONE III
CH_3O
O
O
JH
ESTERASE
(1)
HO
O
O
JH ACID


R
OMe
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S + E
[ES]
Enz-OH
O-Enz
OMe
HO
R
ES
MeOH
O-Enz
EX
H₂O
HO
OH
R
O-Enz
EP
OH
P + E
(2)

that the rate limiting step in the reaction is the product release from the acyl-enzyme (28).

Any compound that is recognized by the enzyme as a TS will bind to it competitively with the substrate. It is important to note, however, that the mechanism of enzymatic reaction is not simply the reaction of the enzyme with the transition state of the uncatalyzed reaction; it can be seen by the cartoon in Figure 1, that the enzymatic hydrolysis of esters proceeds through several intermediates and transition states; a putative TSM compound might resemble any or several of these states. Thus, the concept of a "transition state mimic" is somewhat of a misnomer.

Trifluoromethyl Ketones as Transition State Mimics

An exemplary application of Transition State Theory in developing highly active inhibitors for esterase enzymes is the case of trifluoromethyl ketones (29). Replacing the alkoxy group of the carboxylic esters by a trifluoromethyl group results in highly polarized ketones which are sensitive to nucleophilic attack. Subsequently, in the presence of trace amounts of water, the keto-form will be hydrated and be in equilibrium with the corresponding geminal diol. The geminal diol is tetrahedral in geometry and in theory resembles the transition state of the uncatalyzed hydrolysis of esters (TSX). Thus, according to the original Pauling-theory, trifluoromethyl ketones should bind strongly to esterase enzymes. In this reaction they form hemiketals with the serine present at the active site of the enzyme; two possible reaction mechanisms are enzyme addition to the carbonyl (30) or condensation with the geminal diol (31) (enhanced by the hydrophobic aliphatic chain in the molecule) (Equation 3).

The most likely chemical reaction is addition to the carbonyl as shown in the top portion of Equation 3; however, in aqueous solution the majority of the compound exists as the geminal diol. If this situation predominates, either the equilibrium between the diol and carbonyl form must be fast on the time scale of our ability to measure enzyme reactions, or the I_{50}'s are in fact far lower than we have reported. Alternatively, the hydrated carbonyl could react directly with the enzyme. This could happen in two ways. In one case the TSM could be held near the catalytic site due to interaction with the R group. The relative abundance of the carbonyl might then be favored in a nonaqueous microenvironment. This process could even be accelerated catalytically. If there is enzyme involvement in production of the carbonyl from the geminal diol, then these TSMs could be considered "suicide" substrates. Another alternative explanation is a "normal" S_N2 type reaction between a serine anion and a protonated geminal diol with water as the leaving group. These alternative pathways are not mutually exclusive, but additional work on the kinetics and structural biochemistry of the interaction will be needed to indicate the predominant pathway.

The reaction results in an adduct with the enzyme (ETSM) with no ester C-OR bond present in the molecule to be cleaved. It is clear from Equation 3 that the hemiketal may only react by the release of the trifluoromethyl ketone by the enzyme through the transprotonation transition state, thereby acting as a reversible

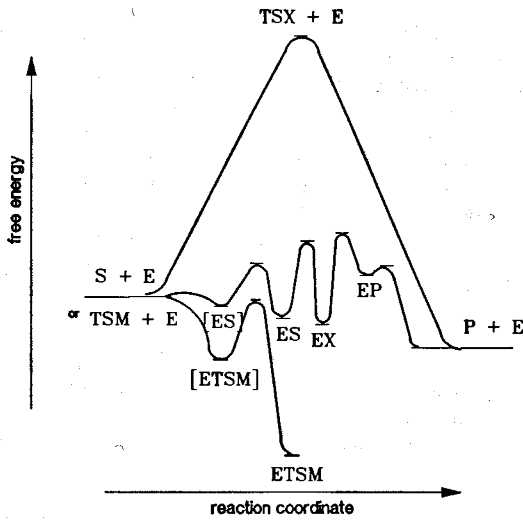
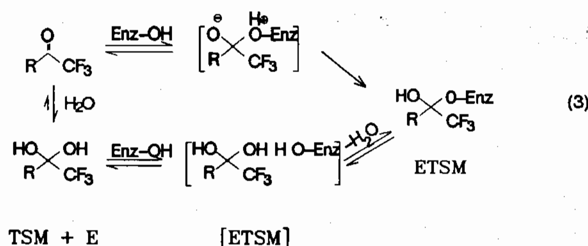
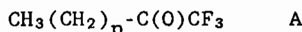


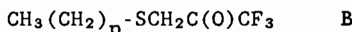
Figure 1. Schematic presentation of the free energy changes in non-enzymatic (ETX) and enzymatic reactions (EX) and in the reaction of a hypothetical transition state mimic (TSM) with the enzyme.



inhibitor. The enzyme-TSM complex (ETSM) is sterically and/or electronically similar to one of the enzyme-substrate Michaelis complexes (possibly to ES), but is favored energetically over the reaction between the enzyme and the substrate. A possible free energy diagram is shown in Figure 1.

Based on the above discussion, trifluoromethyl ketones should inhibit proteases such as chymotrypsin (32), and serine esterases, such as acetylcholinesterase (33,34), carboxylesterases (10), JHE and other esterases with varying selectivity. In a series of some juvenoid-like trifluoromethyl ketones and compounds of the structure A, 1,1,1-trifluoro-2-tetradecanone (TFT) was found to be highly active and selective against JHE (I_{50}: $1x10^{-7}M$) as compared to α-naphthyl acetate esterase (α-NaE) or trypsin (4-6).





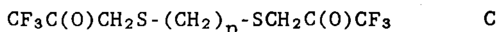
Introducing a sulfur atom β to the carbonyl significantly increased the activity of the resulting compounds (B) on JHE and some but not all other esterases, possibly by bioisosterically mimicking (35) the α,β double bond of the natural JHs. Based on this finding, a series of aliphatic, aromatic (7,8) and terpenoid (9) derivatives were synthesized. The most active compound of these series was 3-octylthio-1,1,1-trifluoro-2-propanone (OTFP). In a recent report, by making a slight modification in the structure of the parent compound, two additional compounds with slightly higher activity have been synthesized (36). TFT and the aliphatic trifluoromethyl ketones appeared to be classical competitive inhibitors, while many trifluoropropanone sulfides were found to be reversible but slow and tight binding inhibitors of JHE (7,11,12).

The outstanding activity of some members of the thiotrifluoropropanones proved useful as ligands and eluting agents for the affinity purification of JHE (13-15). This new method made possible specific purification of JHE with unusually high yields, making possible new research in the areas of immunochemistry and molecular biology for this highly active but low abundant esterase.

In our laboratory, we feel that the scientific and practical use of these inhibitors, by no means, has reached a peak. An obvious need is to try different ligands for affinity elution. Therefore, a new class of transition state analogs was synthesized and tested for their inhibitory potential against JHE, cholinesterase and malathionase.

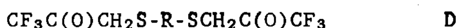
Synthesis

Compounds of the general structure C and D were synthesized and characterized (See Table I)



$CF_3C(O)CH_2S-(CH_2)_n-SCH_2C(O)CF_3$ C

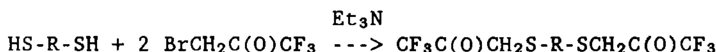
n: 3 - 12 I - X



$CF_3C(O)CH_2S-R-SCH_2C(O)CF_3$ D

R: $-(CH_2)_2-O-(CH_2)_2-$ XI
$-(CH_2)_2-S-(CH_2)_2-$ XII
$-CH(CH_3)(CH_2)_2-$ XIII
$-CH(CH_3)(CH_2)_3-$ XIV

The compounds were synthesized according to a previously published procedure (7) with the modification that triethylamine was used to accelerate the reaction by neutralizing the HBr.



$$HS-R-SH + 2\ BrCH_2C(O)CF_3 \xrightarrow{Et_3N} CF_3C(O)CH_2S-R-SCH_2C(O)CF_3$$

The starting mercaptans were either purchased from Aldrich Chemicals or synthesized from the appropriate α,α'-dihaloalkane *via* the thiourea method (37). The yields were between 59% and 98% for compounds III and V, respectively, and in general higher than 85% depending on the speed of the addition of triethylamine. (Chemical details will be discussed elsewhere: Székács, A.; Hammock, B.D.; Abdel-Aal, Y.A.I.; Philpott, M.; Matolcsy, G.: Pest. Biochem. Physiol., submitted).

Enzyme Assays

The inhibition rate for all inhibitor assays was measured from the initial velocities within the linear time-activity relationships of the control and inhibited samples. The standard deviations were calculated for the I_{50} values of the compounds against the three enzymes used and the maximal values were used to compare the statistical variability within each assay test.

JHE: For the determination of JHE inhibition by the title compounds, the radiometric partition method (38) was used. Hemolymph JHE from Day 2 of the fifth instar larvae of *T. ni* was used (L_5D_2), diluted 1:500 with 0.08M phosphate buffer (pH=7.4 with 0.1% phenylthiourea to inhibit tyrosinases). The main reason for choosing this insect was that a great deal of effort has been put into the characterization of larval carboxylesterases and JHE in *T. ni* (39,40). In L_5D_2 larvae, the JHE titer is near its maximum (19). C10 ^3H labeled JH III (New England Nuclear) and unlabeled JH III (Calbiochem) were used as substrate solubilized in abs.ethanol.

Acetylcholinesterase (AChE): The lyophilized enzyme from electric eel (Sigma) was dissolved in 0.05M phosphate buffer (pH=7.4) at a concentration of 20 μg/mL. Acetylthiocholine iodide was used as substrate at a final concentration of $5x10^{-4}$M in buffer. 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) at a final concentration of $3.8x10^{-2}$M was used to monitor the released thiocholine according to a published procedure (41) with slight modification. Acetone was used as a solvent for the inhibitors.

Malathionase (ME): For measuring the inhibition of malathion esterase activity, general carboxylesterase from porcine liver (Sigma) was used at a final concentration of 16 μg protein/mL in 0.1M Tris HCl buffer (pH=7.5). The procedure involves an indirect determination of the malathionase activity by coupling the hydrolysis of malathion to the reduction of a tetrazolium dye (42). An acetone solution of malathion was used as substrate to a final concentration of $3x10^{-4}$M.

Table I. Inhibition of Esterases by α,α'-alkanebis-thiotrifluoropropanones

compound number	number of carbons	Molar I_{50} Value[a] [M]		
		JHE	AChE	ME
I	3	$9.87x10^{-7}$	$1.14x10^{-5}$	$3.63x10^{-6}$
II	4	$3.06x10^{-9}$	$8.44x10^{-6}$	$3.79x10^{-6}$
III	5	$7.00x10^{-9}$	$4.30x10^{-6}$	$2.38x10^{-6}$
IV	6	$1.50x10^{-8}$	$2.98x10^{-6}$	$9.02x10^{-7}$
V[b]	7	$1.68x10^{-8}$	$2.98x10^{-6}$	$4.89x10^{-6}$
VI[b]	8	$8.17x10^{-10}$	$7.78x10^{-6}$	$4.62x10^{-6}$
VII	9	$2.67x10^{-9}$	$1.34x10^{-5}$	$5.00x10^{-4}$
VIII[b]	10	$4.54x10^{-9}$	$4.35x10^{-6}$	$1.66x10^{-5}$
IX[b]	11	$1.33x10^{-8}$	$5.25x10^{-6}$	$1.03x10^{-3}$
X[b]	12	$8.15x10^{-9}$	$1.03x10^{-5}$	$3.02x10^{-3}$
XI	2-O-2	$2.16x10^{-8}$	$6.01x10^{-6}$	$8.58x10^{-6}$
XII	2-S-2	$4.94x10^{-9}$	$7.12x10^{-6}$	$3.53x10^{-6}$
XIII[b]	3'	$4.68x10^{-7}$	$3.47x10^{-6}$	$4.56x10^{-6}$
XIV[b]	4'	$8.20x10^{-9}$	$8.12x10^{-7}$	$5.22x10^{-5}$

[a] JH III was used as a substrate for JHE from *T. ni*, acetylthiocholine iodide for electric eel AChE and malathion for carboxylesterase from porcine liver.
[b] The dithiol was prepared from alkyl-dibromide.

Summarized in Table I are the I_{50} values for α,α'-alkanebis-thiotrifluoroketones against JHE, AChE and malathionase. All the tested compounds showed much higher inhibitory potency against JHE than against the other two enzymes. The comparative potency in favor of JHE adds to the evidence that these trifluoromethyl ketones are transition state mimics (TSMs) of the enzyme natural substrates (JHs). However, the slopes of the inhibition curves for almost all the compounds were lower with JHE than with the other

two enzymes. Possibly this is due to the fact that JHE from *T. ni* contains multiple JH catalytic sites (43-45), as it has been shown for the tobacco hornworm (*Manduca sexta*)(13). Except for compounds III and IV, the inhibition curves for malathionase inhibition had the highest slopes. The maximal values of the percentage standard error of pI_{50} were 0.77%, 0.18% and 0.66% of the mean for the JHE, AChE and malathionase assays, respectively.

The quantitative structure-activity relationships of the tested compounds are beyond the scope of this paper. However, the empirical structure-activity correlations based on the number of the carbon atoms in the alkyl chain is different for each of the esterases assayed.

It is worth noting that in the JHE assay the trend of the data showed similar structure-activity relationship to the 3-alkylthio-1,1,1-trifluoro-2-propanones (8), which adds some additional evidence for the rational design of these TSMs of JHs. In this assay, however, a definite peak occurs at carbon number eight, for the compound 1,1,1,16,16,16-hexafluoro-4,13-dithia-hexadecane-2,15-dione (I_{50}: 8.2×10^{-10}M), which suggests that after reaching this particular size, the compound might be able to interact with the enzyme(s) in two catalytic sites (or highly hydrophilic parts) at the same time.

In contrast, cholinesterase did not seem to respond in a systematic fashion to the structural variation. Malathionase showed intermediate response with a generally decreasing potency as a function of the number of carbon atoms in the molecule.

In Vivo Assays

Using the irreversible inhibitor EPPAT, Sparks and Hammock (4) demonstrated that pupation could be delayed in *T. ni* presumably by inhibiting JHE and thereby maintaining an abnormally high JH and low prothoracicotropic hormone (PTTH) level. TFT (lacking the thioether moiety) failed to cause this effect while OTFP was effective in delaying pupation when repeated doses of 0.1-0.2 µmol were applied topically on day 1 and 2 of the fifth larval instar (L_5D_1 and L_5D_2)(7).

The two most effective *in vitro* inhibitors of JHE in the new series, VI and II, were screened *in vivo* for their ability to delay pupation in *Trichoplusia ni*, relative to the ability of OTFP, a response shown to be concurrent with the selective inhibition of JHE (7,9).

The compounds (2 µL of 1×10^{-1}M EtOH solution) were topically applied to *T. ni* on L_5D_1 and L_5D_2 at 4, 12 and 17 hrs ALO (after lights on) and the time of pupation was recorded. In the control group, larvae treated with 2 mL EtOH, 90-100% of larvae had pupated by L_5D_5 9 AM. At final doses of 0.2 µmol, VI was as effective as OTFP in delaying pupation relative to the ethanol controls, with only 45-75% of the larvae pupating by L_5D_5. At this concentration, treatment with II did not delay pupation significantly, 75-100% pupated by L_5D_5.

Conclusion

Although trifluoromethyl ketones acting on JHE will probably not result in effective insecticides (delaying pupation is generally not a desired biological effect in agriculture), continued research on them has led to several practical results as well as to new theoretical considerations. Knowledge gleaned by research with trifluoromethyl ketones will lead to a more complete understanding of the role of JHE in insect development. With this understanding of the mechanisms regulating normal development in insects, new avenues may be opened for targeting new and specific ways of insect control. This work with juvenile hormone esterase provides a nice example of chemistry, biochemistry and molecular biology as complementary technologies (19). These potent inhibitors were first used to demonstrate the essential role of JHE in insect development. A knowledge of the chemistry and biochemistry of the interaction of these transition state mimics with JHE led to the development of affinity purification systems for this low abundance enzyme (13). Large amounts of the pure protein allowed more detailed kinetic studies as well as the development of antibodies and nucleic acid probes used to clone the enzyme. It could well be that the resulting clones can be engineered into baculoviruses or other vectors for insect control.

In addition, studies from this and other laboratories illustrate that by mimicking hypothetical transition states one can develop exceptionally potent enzyme inhibitors. These compounds will advance our understanding of catalytic mechanisms, serve as probes for unraveling the roles of particular enzymes in catalytic processes, and allow their rapid purification. They are also promising commercially as pesticides, pesticide synergists, as well as pharmaceuticals. From such work it seems clear that both chemical and biotechnological approaches will be used alone and in combination in the development of biologically active materials.

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Literature Cited

1. Hollingworth, R.M. In Pesticides, Minimizing the Risks; Ragsdale, N.N.; Kuhr, R.J., Eds.; ACS Symposium Series No. 336; American Chemical Society: Washington, DC, 1986; pp. 54-76.
2. Metcalf, R.L. Ann. Rev. Entomol. 1980, 25, 219-56.
3. Hammock, B.D.; Soderlund, D.M. In Pesticide Resistance, Strategies and Tactics for Management; Glass, E., Ed.; (Natl. Acad. Press: Wash.D.C., 1986; pp.111-29.

4. Sparks, T.C.; Hammock, B.D. Pest. Biochem. Physiol. 1980, 14, 290-302.
5. Hammock, B.D.; Wing, K.D.; McLaughlin, J.; Lovell, V.M.; Sparks, T.C. Pest. Biochem. Physiol. 1982, 17, 76-88.
6. Sparks, T.C.; Hammock, B.D.; Riddiford, L.M. Insect Biochem. 1983, 13, 529-41.
7. Hammock, B.D.; Abdel-Aal, Y.A.I.; Mullin, C.A.; Hanzlik, T.N.; Roe, R.M. Pest. Biochem. Physiol. 1984, 22, 209-23.
8. Hammock, B.D.; Mullin, C.A. U.S. Patent 4 562 292, 1985.
9. Prestwich, G.D.; Eng, W-S.; Roe, R.M.; Hammock, B.D. Arch. Biochem. Biophys. 1984, 228, 639-45.
10. Ashour, M-B.A.; Hammock, B.D. Biochem. Pharm. 1987, 36, 1869-79.
11. Abdel-Aal, Y.A.I.; Roe, R.M.; Hammock, B.D. Pest. Biochem. Physiol. 1984, 21, 232-41.
12. Abdel-Aal, Y.A.I.; Hammock, B.D. Insect Biochem. 1985, 15, 111-22.
13. Abdel-Aal, Y.A.I.; Hammock, B.D. Arch. Biochem. Biophys. 1985, 243, 206-19.
14. Abdel-Aal, Y.A.I.; Hammock, B.D. Science 1986, 233, 1073-6.
15. Hammock, B.D.; Abdel-Aal, Y.A.I.; Hanzlik, T.N.; Croston, G.E.; Roe, R.M. In Molecular Entomology; Law, J.H., Ed.; Alan R. Liss, Inc.: New York, 1987 pp. 315-28.
16. Hammock, B.D.; Quistad, G.B. In Progress in Pesticide Biochemistry; Hutson, D.H.; Roberts, T.R., Eds.; Wiley: New York, 1981; Vol.1, pp. 1-83.
17. de Kort, C.A.D.; Granger, N.A. Ann. Rev. Entomol. 1981, 26, 1-28.
18. Slade, M.; Zibitt, C.H. In Insect Juvenile Hormones: Chemistry and Action; Menn, J.J.; Beroza, M., Eds.; Academic Press: New York; 1972; pp. 155-76.
19. Hammock, B,D.; Abdel-Aal, Y.A.I.; Ashour, M.; Buhler, A.; Hanzlik, T.N.; Newitt, R.; Sparks, T.C. In Human Welfare and Pest Control Chemicals-Approaches to Safe and Effective Control of Medical and Agricultural Pests; Sasa, M.; Matsunaka, S.; Yamamoto, I.;Ohsawa, K., Eds.; XVIII. Pesticide Science Symposium and Public Lectures; Pesticide Science Society of Japan: Tokyo, 1986; pp.53-72.
20. Hammock, B.D. In Insect Physiology Biochemistry and Pharmacology; Kerkut, G.A.; Gilbert, L., Eds.; Pergamon Press: New York, 1985; pp. 431-72.
21. Pauling, L. Amer. Sci. 1948, 36, 51-8.
22. Wolfenden, R. Ann. Rev. Biophys. 1976, 45, 271-306.
23. Jencks, W.P. Catalysis in Chemistry and Enzymology; McGraw-Hill: New York, 1969; pp. 463-554.
24. Bender, M.L.; Thomas, R.J. J. Am. Chem. Soc. 1961, 83, 4183-9.
25. Bender, M.L. Chem. Rev. 1960, 60, 53-113.
26. Cox, J.D.: Pilcher, S. Thermochemistry of Organic and Organometallic Compounds; Academic Press: London, New York, 1970; pp. 140-493.
27. Lange's Handbook of Organic Chemistry, 13th Ed.; Dean, J.A., Ed.; McGraw-Hill: New York, 1984; pp. 9-70 - 9-99.
28. Walsh, C. Enzymatic Reaction Mechanisms; Freeman: San Francisco, 1979; pp.53-107.

29. Abdel-Aal, Y.A.I.; Hammock, B.D. In Bioregulators for Pest Control; Hedin, P.A., Ed.; ACS Symp. Ser. No. 276; American Chemical Society: Washington, DC, 1985; pp. 135-60.
30. Stein, R.L.; Strimpler, A.M.; Edwards, P.D.; Lewis, J.J.; Manger, R.C.; Schwartz, J.A.; Stein, M.M.; Trainor, D.A.; Wildonger, R.A.; Zottola, M.A. Biochemistry 1987, 26, 2682-9.
31. Abeles, R.H. Drug Dev. Res. 1987, 10, 221-34.
32. Imperiali, B.; Abeles, R.H. Biochemistry 1986, 25, 3760-7.
33. Brodbeck, U.; Schweikert, K.; Gentinetta, R.; Rottenberg, M. Biochim. Biophys. Acta 1979, 567, 357-69.
34. Gelb, M.H.; Svaren, J.P.; Abeles, R.H. Biochemistry 1985, 24, 1813-7.
35. Thornber, C.W. Chem. Soc. Rev. 1979, 8, 563-80.
36. Linderman, R.J.; Leazer, J.; Venkatesh, K.; Roe, R.M.: Pestic. Biochem. Physiol. 1987, 29, 266-77.
37. Speziale, J.A. In Organic Syntheses; Rabjohn, N., Ed.; Wiley: New York, 1963; Coll. Vol. IV, pp. 401-3.
38. Hammock, B.D.; Roe, R.M. Methods Enzymol. 1985, 111B, 487-94.
39. Wing, K.D.; Sparks, T.C.; Lovell, V.M.; Levinson, S.O.; Hammock, B.D. Insect Biochem. 1981, 11, 473-85.
40. Yuhas, D.A.; Roe, R.M.; Sparks, T.C.; Hammond, A.M.,Jr. Insect Biochem. 1983, 13, 129-36.
41. Ellman, G.L.; Courtney, K.D.; Andres, V., Jr.; Featherstone, R.M. Biochem. Pharmacol. 1961, 7, 88-95.
42. Talcott, R.E. Tox. Appl. Pharmacol. 1979 47, 147-50.
43. Jones, D.; Jones, G.; Rudnicka, M.; Click, A.; Streekrishna, S. Experientia 1986, 42, 45-7.
44. Jones, D.; Jones, G.; Click, A.; Rudnicka, M.; Streekrishna, S. Comp. Biochem. Physiol. 1986, 85B, 773-81.
45. Hanzlik, T.N.; Hammock, B.D. J. Biol. Chem., 1987, 262, 13584-91.

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