Title

Synthesis of halogenated isoprenoids as potential enzyme inhibitors and mechanistic probes

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SYNTHESIS OF HALOGENATED ISOPRENOIDS AS POTENTIAL ENZYME INHIBITORS AND MECHANISTIC PROBES

by

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DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

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ABSTRACT

This dissertation describes work focused on the synthesis of halogenated isoprenoids as potential enzyme inhibitors and mechanistic probes. Specifically, studies have been carried out in three areas.

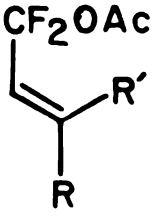
The goal of the first study was to obtain additional information about the active site specificity and topology of squalene synthetase. This enzyme catalyzes the coupling of two farnesyl pyrophosphate molecules to form squalene, one of the intermediates in the biosynthesis of cholesterol. Since high levels of cholesterol are associated with cardiovascular diseases, specific inhibitors of its biosynthesis have been of therapeutic interest. The analog 2-chloro farnesyl pyrophosphate, was synthesized and tested as a substrate of squalene synthetase isolated from yeast. The analog was found to be a competitive inhibitor but the substitution of a chlorine at the 2-position was found to prevent acceptance of the analog as a substrate.

The second study focused on the development of an effective anti-juvenoid, a compound which would interfer with the growth and development processes of insects. Since insect maturation is regulated by levels of juvenile hor-

mone, inhibitors of its biosynthesis should disrupt growth and therefore be advantageous as insecticides. The inhibition of the cytochrome P-450 mediated epoxidation step of juvenile hormone biosynthesis was therefore selected as a target site. Several halogenated and nonhalogenated chemical functionalties shown previously to cause inactivation of rat hepatic cytochrome P-450 isozymes were employed in the design of anti-juvenile hormone analogs. Specifically, the following compounds were prepared: Ethyl 3,7- dimethyl-2(E),6(E),10-undecatrienoate; Ethyl 3,7-dimethyl-2(Z), 6(E),10-undecatrienoate; Ethyl 11-chloro-3,7-dimethyl-2(E),6(E), 10(E and Z)-undecatrienoate; Ethyl 11-chloro-3,7-dimethyl-2(Z),6(E),10(E and Z)-undecatrienoate; Ethyl 3,7-dimethyl-10-yne-2(E),6(E)-decadienoate; Ethyl 3-methylene-7- methyl-10-yne-6(E)-decenoate; and Ethyl 11,11-difluoro-3,7-dimethyl- 2(E),6(E),10-undecatrienoate. All of the analogs caused some destruction (8-19%) of rat hepatic cytochrome P-450 isozymes. The *in vitro* and *in vivo* assays with *Manduca sexta*, however, did not provide evidence for a strong anti-juvenile hormone response but rather for general cytotoxic effects.

The third study involved the development (synthetic and mechanistic studies) and application of new synthetic methods for the preparation of di- and mono-α-fluoro alkyl esters. These novel fluoro substituted compounds may serve as useful probes of squalene synthetase and other enzymes

such as phosphatases and esterases. The synthesis of compounds with the general structures of 95 and 96 was achieved.



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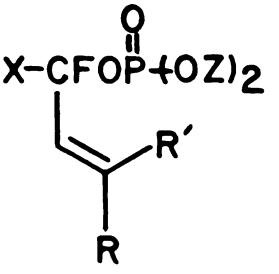

96

X = H, F

Z = Ph, Et

To myself, patience,
and perserverance.

ACKNOWLEDGEMENTS

From my experience of working in the laboratory of Professor Paul Ortiz de Montellano I have gained a new breadth of scientific understanding and a new level of human awareness. I acknowledge these valuable perspectives and the dimensions they add to my future.

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But most of all, I thank Kent Kunze whose support and affection was always there.

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CHAPTER ONE

GENERAL INTRODUCTION

1.1. ENZYMES AS CATALYSTS AND TARGETS FOR INHIBITION

Enzymes are proteins that function as catalysts in biological systems. Each forms a complex with a distinct substrate or group of substrates and subsequently promotes a specific chemical transformation. The active site is that region of the enzyme responsible for both substrate specificity and reaction catalysis (Koshland, 1960). The goal of many studies has been to examine the active site in order to understand the mechanism of enzymes. This understanding can lead to the development of specific inhibitors of an enzyme's catalytic reaction and, in many instances, to the development of clinically important drugs.

A powerful approach to the design of specific enzyme inhibitors is represented by the use of affinity labeling agents. These are substrate-like molecules which resemble the natural substrate but which contain a chemically reactive moiety able to form a covalent bond with a group inside the active site. This may be represented in the simplest case as a two-step process,

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \underset{\text{complex}}{EI} \xrightarrow{k_2} E\text{-}I$$

in which, after formation of the EI complex, the enzyme may undergo: (1) active-site directed irreversible inhibition (a covalent bond forms directly between the enzyme and the inhibitor), (2) suicide substrate inhibition (catalytic turnover of the enzyme generates a reactive intermediate which irreversibly binds to the enzyme), and (3) pseudo-irreversible inhibition[1] (the covalent bond slowly hydrolyzes to regenerate the enzyme) (Santi and Kenyon, 1980). Besides the actual covalent binding event, a number of experimental criteria define the inhibition by affinity labeling agents (Meloche, 1967; Baker, 1967; Wold, 1977). Among these are: (1) the protection against inactivation by substrates or competitive inhibitors, (2) the observation of a time-dependent first-order loss of enzymatic activity, and (3) the stoichiometric incorporation of one irreversible inhibitor molecule per binding site.

The actual chemical design of an affinity labeling agent involves a concerted understanding of the nature and properties of the active site, and of the substrate and its analogs. This understanding evolves from explorations of the kinetic parameters, of the structures of inhibitors and

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(1) This category does not include reversible inhibition, either competitive or noncompetitive, in which noncovalent binding interactions cause the inhibition.

their affinities for the active site, and of the mechanism of action of the target enzyme. Especially important for understanding the intermolecular interactions responsible for binding and specificity of the enzyme are structural modifications of reversible competitive inhibitors, which bind to the active site through noncovalent forces. Investigations of these structural changes include: (1) which portions of the substrate contribute to, detract from, or have no effect on binding, (2) what regions are sensitive to steric effects, and (3) which functional group substitutions augment the interactions with the enzyme. Finally, a basic chemical awareness of the nature of chemical bonds, electrostatic and hydrophobic interactions, nucleophilicity, and leaving group ability, are all necessary for the eventual construction of the inhibitor. The combined knowledge of the chemistry of enzyme-substrate-analog interactions provides the framework for rational design of an affinity labeling agent which takes advantage both of an enzyme's binding selectivity and of the presence of functional groups in the active site suitable for covalent bond formation. The literature contains numerous examples that illustrate the ingenious design of these types of inhibitors. Several review articles are available (Baker, 1967; Shaw, 1970; Maycock and Abeles, 1967; Walsh, 1977; Abeles, 1978; Rando, 1975; 1978).

1.2. THE HALOGENS: USE IN INHIBITOR DESIGN

A variety of chemical modifications of molecules have been used to create a reactive moiety which will inactivate an enzyme by forming a covalent bond within the active site. The emphasis here and in the following chapters will be primarily on the effects of the halogens as substituents and on how substituent effects may alter the chemical reactivity of molecules in the inhibition of enzymes.

The reactivity of any functional group in a molecule is determined, among other things, by electron distribution, polarizability, and steric effects. The halogens, fluorine, chlorine, bromine, and iodine, whose properties are summarized in Tables I and II, readily affect all three of these factors.

With the exception of iodine, the halogens are more electronegative than carbon or hydrogen. Thus, covalent bonds formed between a carbon and a fluorine, chlorine, or bromine atom will have an unevenly distributed electron cloud. The electron density will reside over the halogen and leave the carbon atom with a partial positive charge. In turn, this carbon will compensate for its loss of electron density by withdrawing electrons from its adjacent carbon. This is known as an inductive effect in which the carbon-carbon bond is polarized by the presence of the electronegative halogen substituent (Figure 1).

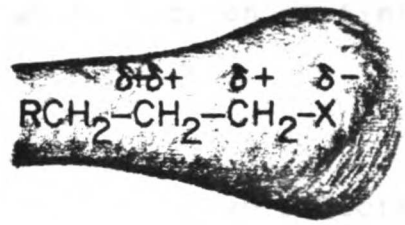
Table I[a]

	Electro-negativity	Electron Affinity (ev)	Ionization Potential (V)	Van der Waals radii (Å)
F	4.10	3.45	17.426	1.35
Cl	2.83	3.61	13.02	1.80
Br	2.74	3.36	11.85	1.95
I	2.21	3.06	10.457	2.15
H	2.20	0.747	13.598	1.2
C	2.50	1.2	11.267	---
CH_3	---	---	---	2.0

Table II[a]

	Bond Lengths (Å)	Bond Energies (kcal/mole)
$F-CH_3$	1.379	108
$Cl-CH_3$	1.767	83.5
$Br-CH_3$	1.938	70
$I-CH_3$	2.207	56
$H-CH_3$	1.096	104
CH_3-CH_3	1.537	88

(a) Values taken from Gordon, A.J. and Ford, R.A., 1972.



X = F, Cl, Br

FIGURE 1: The inductive effect of halogens: F, Cl and Br.

The polarizability of bonds, the ability to undergo distortion and orientation of charge distribution, is also a property of individual atoms. Of the halogens, iodine is the most polarizable atom. It is not sufficiently electronegative to polarize the carbon-iodine bond. However, its other properties, namely a low ionization potential, an electron affinity greater than carbon, and a long carbon-iodine bond length, allow its own electron cloud to be easily polarized and to accommodate a formal negative charge in its outer low energy d orbitals. Together these properties allow iodine to be easily displaced in nucleophilic reactions. The leaving group ability of the other halogens is in the order I>Br>Cl>>F. These two factors, electronegativity and polarizability, have three effects on molecular chemical reactivity: (1) a saturated carbon bonded to a halogen is susceptible to nucleophilic attack, (2) the δ+ charge on the halogen-substituted carbon atom tends to pull electrons away from adjacent β-carbon atoms making their protons more

acidic, and (3) the electrophilicity of a group, other than a saturated carbon atom (e.g. an olefin), is increased by an attached halogen.

Chemical reactivity is also affected by steric effects. For enzyme-substrate interactions these are the conformational and electrostatic interactions that occur in the substrate and to the formation of the EI complex. When a new group is attached to the natural substrate these interactions affect the binding and "fit" of the substrate analog in the active site, or more specifically, the proper alignment of substrate groups with the appropriately oriented enzyme functionalities. Tables I and II show that the size of halogens and the length of their bonds to carbon increase in the order F>Cl>Br>I. As a comparison of molecular size, chlorine is about the same size as a methyl group and fluorine about the same as a hydrogen (Table I). These differences in size may or may not be critical when a halogen is substituted for another group. The halogens, as already mentioned, are centers of high electron density and are consequently capable of electrostatic interactions. For example, halogen replacement of a hydrogen which is normally located in a negatively charged region of the enzyme would result in a repulsive, and hence unfavorable, interaction. Considering both size and electronic effects, if a halogen is to be substituted for another atom or group on a substrate molecule it must be ascertained to what extent the

substitution detrimentally affects the ability of the enzyme to actively recognize and bind the analog.

The following examples illustrate the application of halogens in affinity labeling agents. Figure 2 shows an α-haloketone substituent used for active-site-directed irreversible inhibition of serine proteases (Baker, 1967; Shaw, 1970), enzymes which normally catalyze the hydrolysis of amino acid amides or esters.

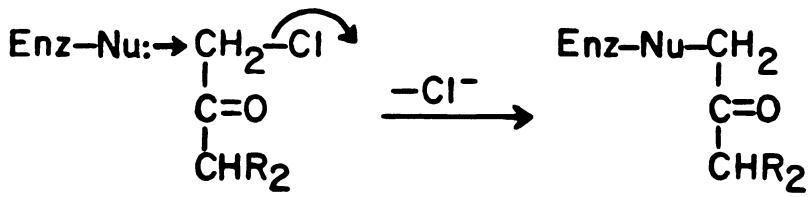


FIGURE 2: Enzyme alkylation by an α-haloketone group, active-site-directed irreversible inhibition.

Through the R-groups, the enzyme recognizes and binds the analog such that the halomethyl group is juxtaposed to a nucleophilic group within the active site. This nucleophile subsequently displaces the halide to form a direct covalent attachment of the enzyme to the inhibitor. A second example, shown in Figure 3, portrays how enzymatic loss of HCl generates a reactive species which alkylates the enzyme. This type of affinity labeling agent is termed a suicide substrate and is an irreversible inhibitor.

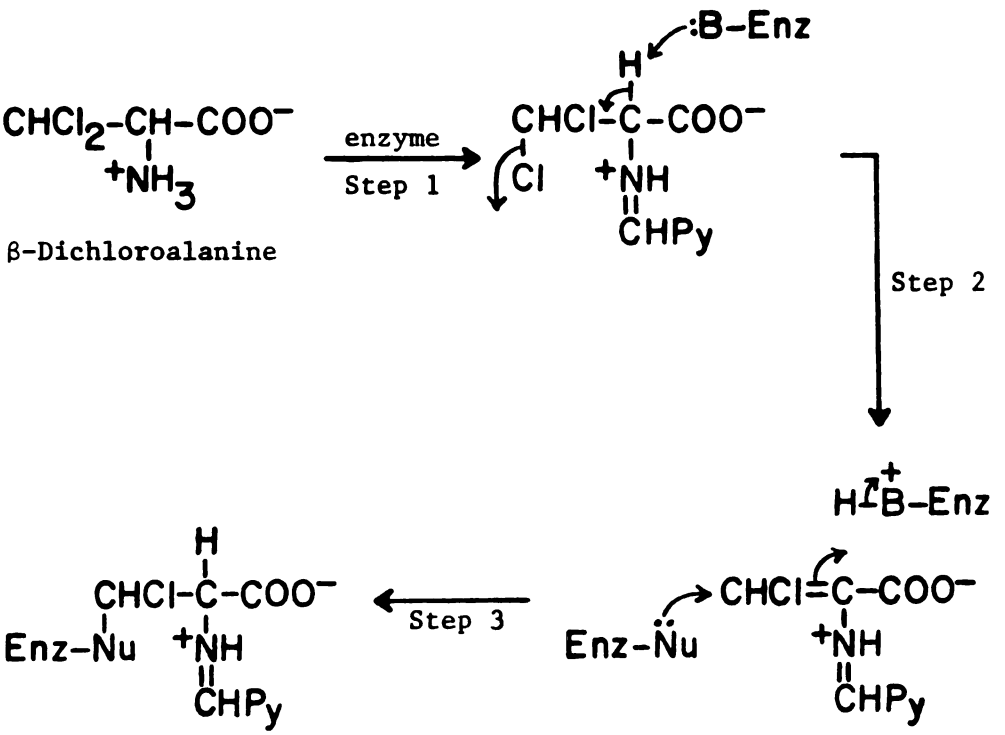


FIGURE 3: Enzyme alkylation by β -dichloroalanine, suicide substrate irreversible inhibition.

β-Dichloroalanine inactivates the enzyme, α-cystathionase, through a series of proposed steps (Maycock and Abeles, 1976). Normal schiff base formation with the enzyme (Step 1) and α-proton abstraction by an enzymic base are followed by a loss of halide (Step 2). The resulting unsaturated imine, an enzymatically created moiety, undergoes a Michael-type addition of an active-site nucleophile and thereby inactivates the enzyme. The role of the halogens in this inhibitor is two-fold. First, one halogen is required as a good leaving group, and second, the other halogen facilitates the Michael addition by making the double bond more electrophilic. The latter effect is evident in that,

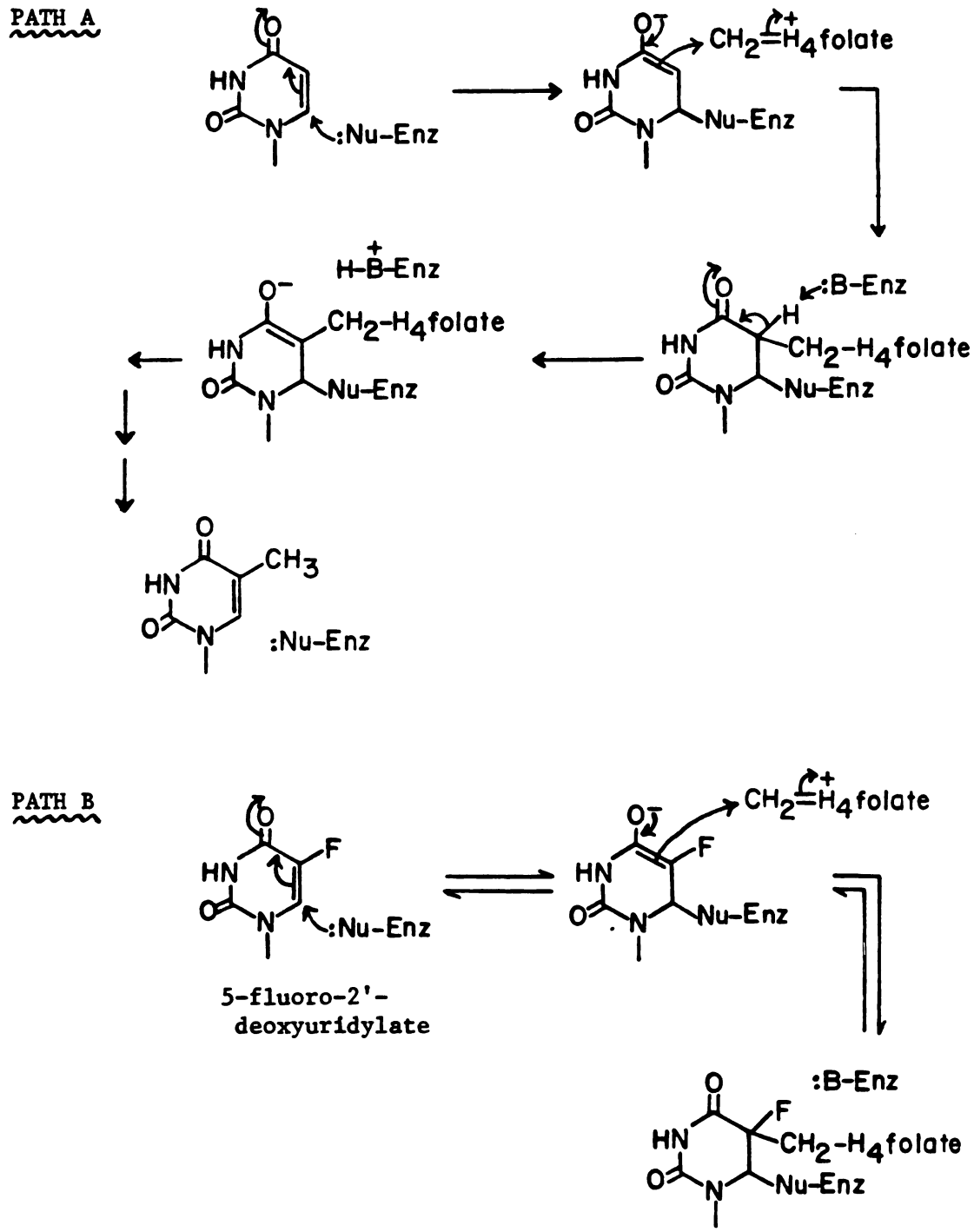


FIGURE 4: Normal catalysis of thymidylate synthetase (PATH A) and enzyme alkylation by 5-fluoro-2'-deoxyuridylate, pseudoirreversible inhibition (PATH B).

without the second halogen, inactivation does not occur. A third example, shown in Figure 4, illustrates one type of pseudoirreversible inhibitor (Pogolotti and Santi, 1977). The enzyme, thymidylate synthetase, commonly catalyzes the conversion of 2'-deoxyuridylate and 5,10-methylene tetrahydrofolate to thymidylate and dihydrofolate. Part of this enzymatic sequence is shown in Path A. The inhibitor, 5-fluoro-2' deoxyuridylate, contains a fluorine in place of the hydrogen which is normally abstracted during the enzymatic process. Fluorine thus prevents the enzyme from completing the catalytic reaction since it cannot be removed. The covalent bond, formed as part of the normal sequence, is slowly broken to regenerate the unchanged inhibitor and enzyme (Path B).

In summary, if a halogen is to be used to prepare an affinity labeling agent it is necessary to determine: (1) the nature of the chemical catalytic event and how it may be utilized for the design of an affinity label, and (2) the constraints on substrate "fit" in the enzyme and the nature of the steric restrictions. Together, these provide a framework for the analysis of an enzymatic reaction and for the rational design of irreversible inhibitors.

CHAPTER TWO

2-CHLORO FARNESYL PYROPHOSPHATE: A MECHANISTIC PROBE OF SQUALENE SYNTHETASE

2.1. INTRODUCTION

The synthesis of squalene (3) from two units of farnesyl pyrophosphate (1) is an enzymatic reaction common to biological systems that synthesize steroids from acetate. Squalene synthetase, the membrane-bound enzyme involved, catalyzes an obligatory step in the production of cholesterol. Since high plasma cholesterol levels appear to be related to atherosclerosis and associated cardiovascular disorders (Friedman et al., 1968; NIH Task Force Report, 1971), a specific inhibitor of squalene synthetase is of potential therapeutic value.

The overall enzymatic reaction (Figure 5) is a reductive, head-to-head coupling of the pyrophosphate esters of two allylic alcohols (For a comprehensive review see Popjak and Agnew, 1979). The condensation is a stereospecific, asymmetric process (Popjak and Cornforth, 1966) which

entails the formation of a distinct isolable intermediate, presqualene pyrophosphate (2) (Rilling, 1966; Epstein and Rilling, 1970; Edmond et al., 1971; Coates and Robinson, 1971; Popjak et al., 1975; Corey and Volante, 1976).

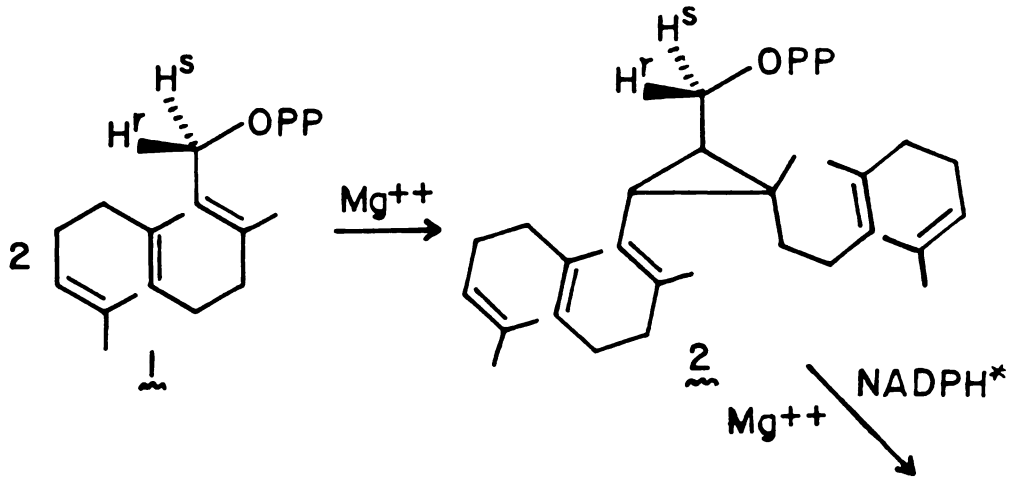
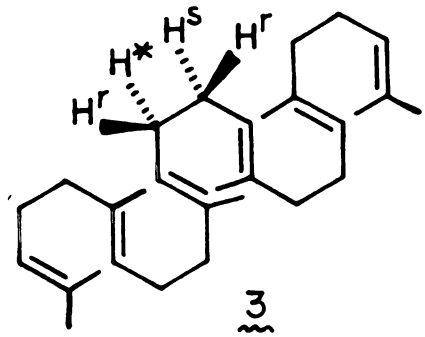


FIGURE 5: The condensation of two farnesyl pyrophosphates to form squalene.



In the first step the pro S hydrogen is removed from one farnesyl pyrophosphate and in the second step it is replaced by a hydride from the β-face of the pyridine ring in NADPH in such a way that no inversion of configuration occurs. The C-1 of the second farnesyl pyrophosphate undergoes inversion of configuration (Popjak and Cornforth, 1966). The reaction requires the presence of a divalent cation, Mg^{++} or Mn^{++} (Beytia et al., 1973).

A kinetic analysis (Dugan and Porter, 1972; Beytia et al., 1973; Popjak and Agnew, 1979) of the reaction mechanisms has led to the postulate of two discrete steps. The first step, the condensation of two farnesyl pyrophosphates to form presqualene pyrophosphate, has been assigned a ping-pong, bi-bi mechanism (Figure 6).

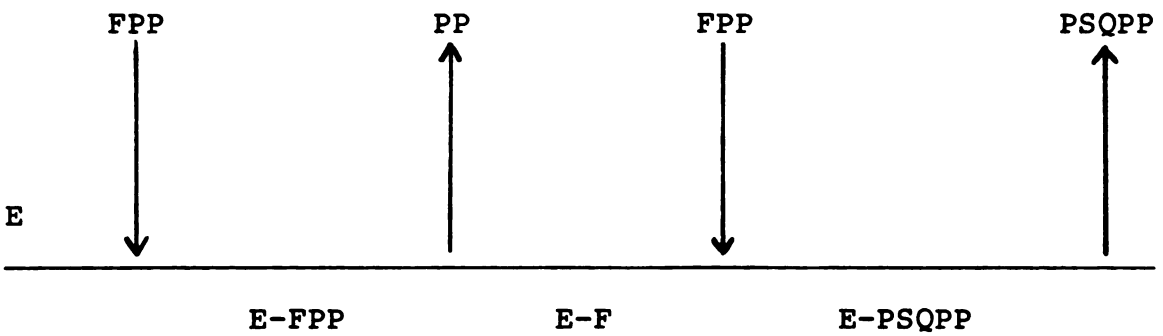


FIGURE 6: Ping-Pong, bi-bi mechanism of presqualene pyrophosphate formation.

In this scheme, the first molecule of farnesylpyrophosphate (FPP) binds to the enzyme (E) to form a complex (E-FPP) that releases inorganic pyrophosphate (PP), followed by binding of a second molecule of farnesyl pyrophosphate to give the enzyme-presqualene pyrophosphate complex (E-PSQPP). In the absence of NADPH, presqualene pyrophosphate subsequently dissociates from the enzyme. Additional evidence to corroborate two binding sites has been provided by experiments in which several farnesyl pyrophosphate analogs clearly revealed differences in their affinities for the two far-

nesyl pyrophosphate binding sites (Ortiz de Montellano, 1976a; 1976b). The second step, the conversion of presqualene pyrophosphate to squalene, was suggested to be of an ordered sequential bi-ter type (Figure 7).

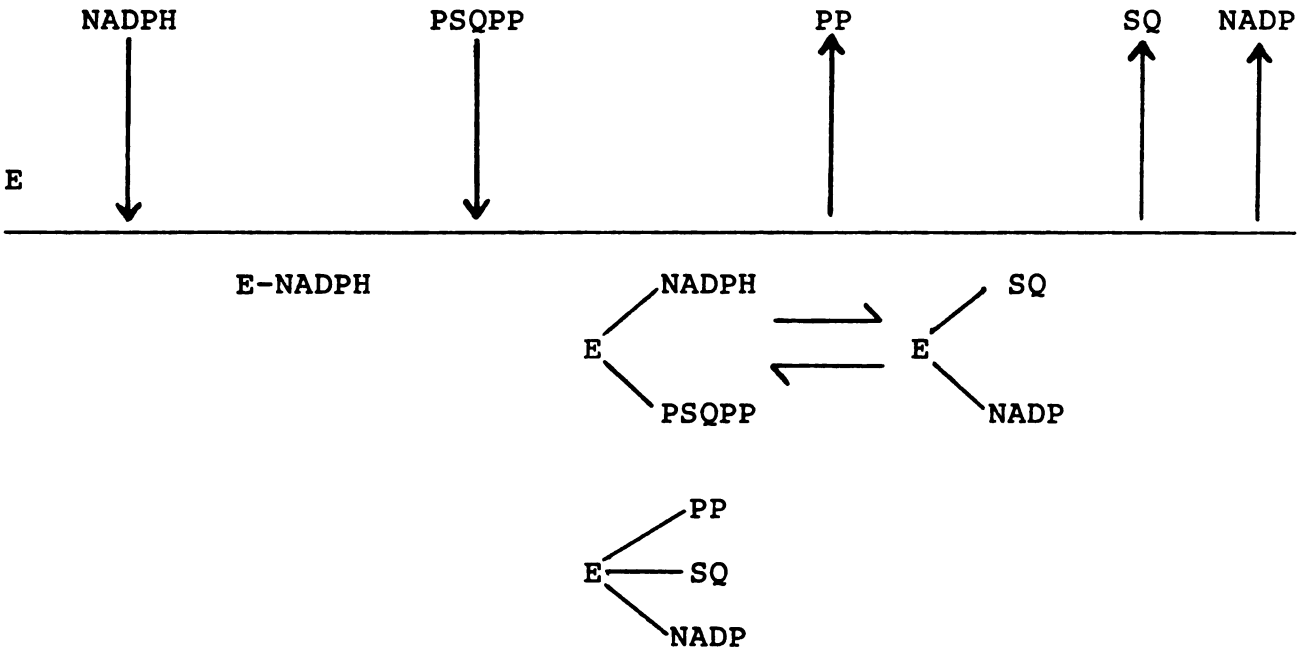


FIGURE 7: Ordered sequential bi-ter mechnism of squalene formation.

In this sequence, NADPH binds first to the enzyme and then presqualene pyrophosphate. The order of product release was thought to be inorganic pyrophosphate, squalene, and finally NADP. The determination of the enzyme kinetics of squalene synthetase has been difficult. In each of the two proposed steps, the kinetics were based upon studies of the uncoupled reactions of solubilized enzyme. It is unclear if the intact enzyme will display the same kinetic parameters.

Several enzymatic mechanisms have been suggested for the formation of the intermediate presqualene pyrophosphate (2) and, subsequently, of squalene itself (3) (Epstein and Rilling, 1970; Edmond et al., 1971; Van Tamelen and Schwartz, 1971; Beytia et al., 1973; Poulter et al., 1974). Any mechanism which purports to explain the formation of presqualene pyrophosphate must account for nucleophilic displacement of a pyrophosphate group, nucleophilic or electrophilic addition to a double bond, proton removal from the C-1 position of one farnesyl pyrophosphate molecule, and cyclopropane formation. Likewise, the following conversion of presqualene pyrophosphate to squalene must account for loss of pyrophosphate, ring opening, and hydride transfer from NADPH, to give the enzymatic (not chemical, 5) product. In Figures 8 and 9 are presented some of the more attractive mechanistic possibilities (Poulter et al., 1974; Castillo, 1977).

2.2. THE RATIONALE FOR THE PREPARATION OF THE 2-CHLORO FARNESYL PYROPHOSPHATE ANALOG

Previous work in our laboratory (Ortiz de Montellano et al., 1977a; Ortiz de Montellano et al., 1977b; Castillo, 1977) involved the synthesis of various farnesyl pyrophosphate analogs and their use to study the structure-activity-relationship of the enzyme. Of special interest is the available evidence of the biological activity of the

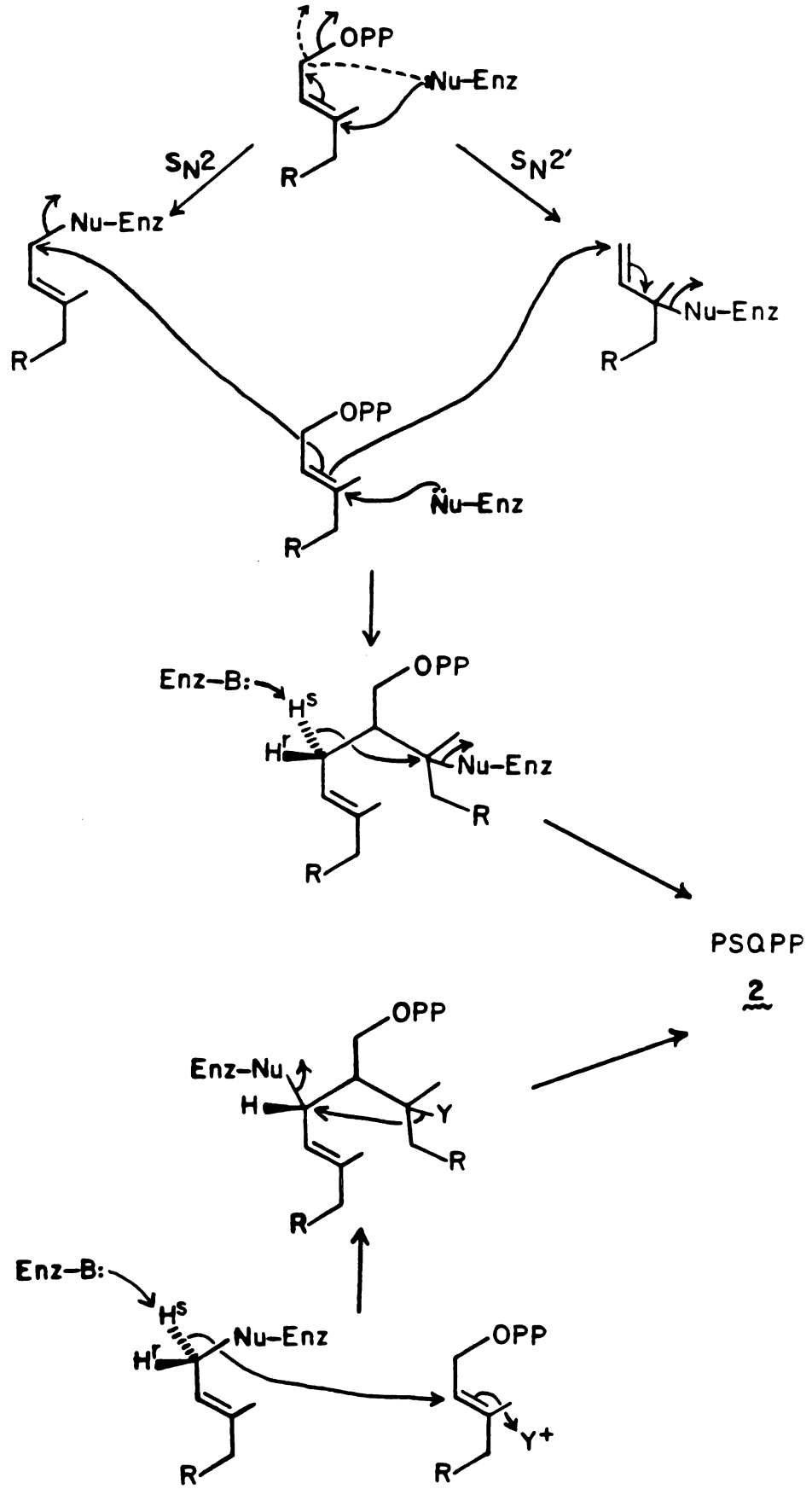


FIGURE 8: Postulated enzymatic mechanisms for the formation of presqualene pyrophosphate. R= 

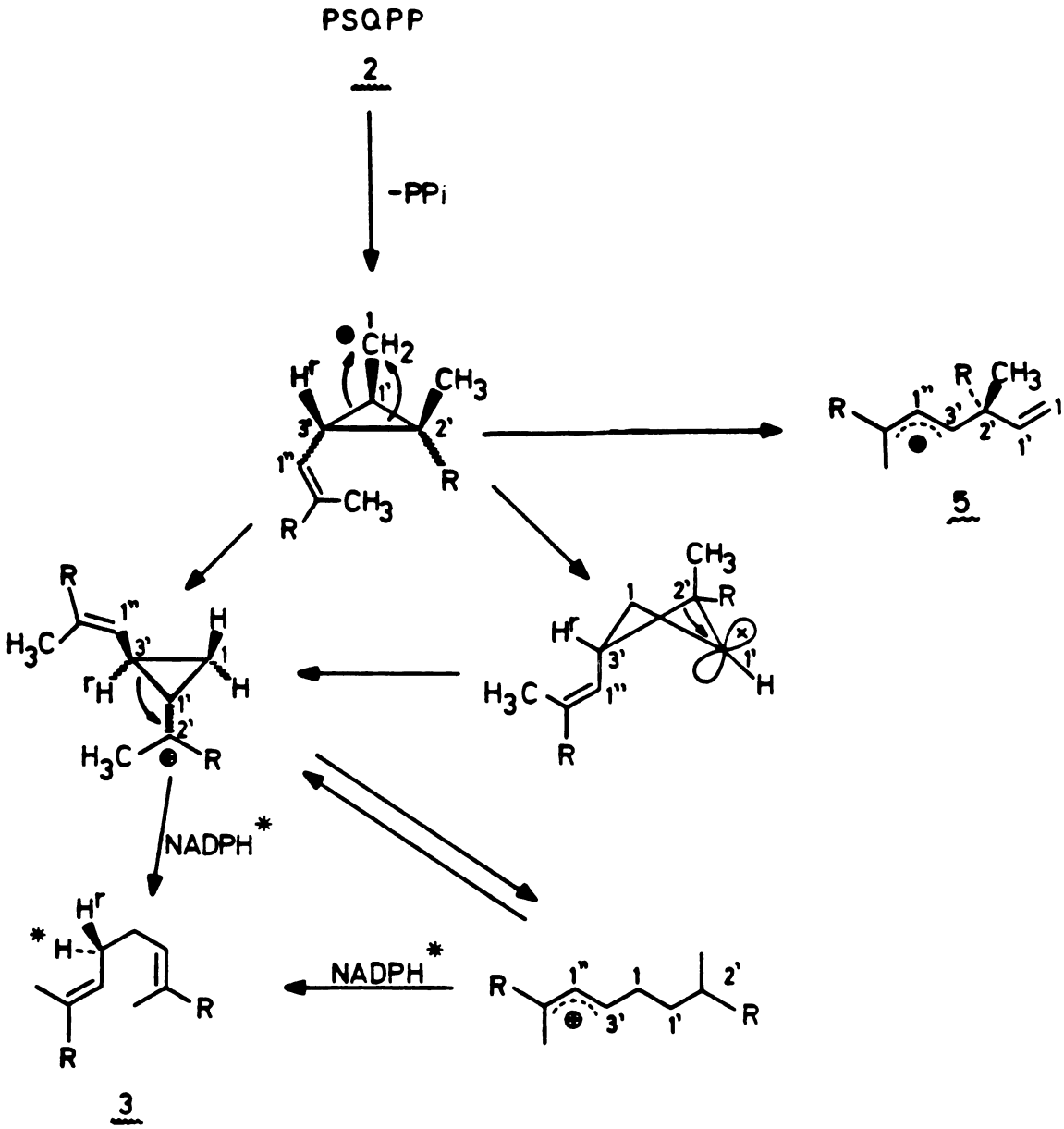
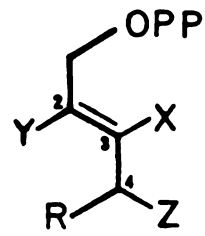


FIGURE 9: Postulated enzymatic mechanism for the formation of squalene from presqualene pyrophosphate.

analogs as a result of the electronic and steric modifications about the 2,3 double bond, the molecular region directly involved in the catalytic process.

The studies carried out so far tenatively suggest a correlation between the electron density of the 2,3-double bond and the amount of inhibition of squalene product formation. The inhibition seems to increase with substituents of the double bond that increase the electron density and decrease with electron withdrawing substituents.

Table III[(a)]



Analog	X	Y	Z	%Relative Inhibition
6	-Me	-Me	-H	87
7	-Et	-H	-H	82
8	-H	-H	-H	72
9	-Me	-H	-SMe	66
10	-I	-H	-H	64
11	-H	-I	-H	63
12	-Me	-H	-F	see Table IV

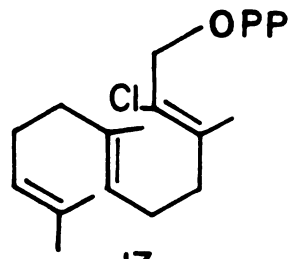
(a) Taken from Castillo, 1977.

Table III lists some values which were determined by measuring the decrease in enzymatic incorporation of (1-^{3}H)-farnesyl pyrophosphate into squalene relative to control incubations with no inhibitor (for details of procedure

see Ortiz de Montellano et al., 1977a). The type of inhibition was subsequently shown to be competitive with the substrate, farnesyl pyrophosphate (Ortiz de Montellano et al., 1977a). These results could be explained by two interdependent effects: (1) the analog binds more tightly to the active site through specific 2,3 double bond π interactions, that is, the analog has a better binding affinity than farnesyl pyrophosphate ($K_I < K_M$), and (2) the analog binds sufficiently to the active site but the ability of the double bond to react as an electrophile is thwarted by electron releasing substituents. If only steric effects are considered, it is difficult to explain why the 3-ethyl FPP analog (7) is more active than the 3-desmethyl FPP analog (8). However, there may be a hydrophobic region in the active site that associates with the C-3 substituent and results in stronger binding of an ethyl group than a hydrogen. The other analogs 9, 10, 11 and 12 may also be less active as inhibitors due to conformational and electrostatic interactions which reduce binding.

Further studies have shown that the catalytic requirements of the enzyme include the necessity for: (1) the 2,3 double bond at both farnesyl pyrophosphate binding sites, (2) for a C-3 methyl group at the second site to possibly anchor the substrate (Ortiz de Montellano et al., 1976b), and (3) for the absence of C-2 steric congestion at the first site (demonstrated by the inability of the 2-methyl FPP analog to function as a first site substrate, Ortiz de

Montellano et al., 1976a).



13

The 2-chloro FPP analog (13) was prepared to extend our study of the substrate specificity and because rational analysis suggested it might irreversibly inhibit the enzyme. Figure 10, in conjunction with Figure 8, presents some of the possible mechanisms which, after elimination of chloride ion, would result in affinity labeling of the enzyme. The mechanisms shown in Path A and B (X=H) are the more likely candidates for enzyme inactivation since a chlorine atom and a methyl group are of approximately the same size (Table I) and a methyl group has been found to be acceptable at the second site (Ortiz de Montellano et al., 1976a). The alkylation mechanisms shown in Path C or D or Path A or B (X=Cl) are less likely to take place because they would require acceptance of the analogue at the first site, an unlikely occurence since the 2-methyl FPP analog is not accepted as a substrate at this site.

2.3. SYNTHESIS OF 2-CHLORO FARNESYL PYROPHOSPHATE

The initial step in the preparation of the 2-chloro FPP analog (13) was to react monochloro phosphonate 14 with

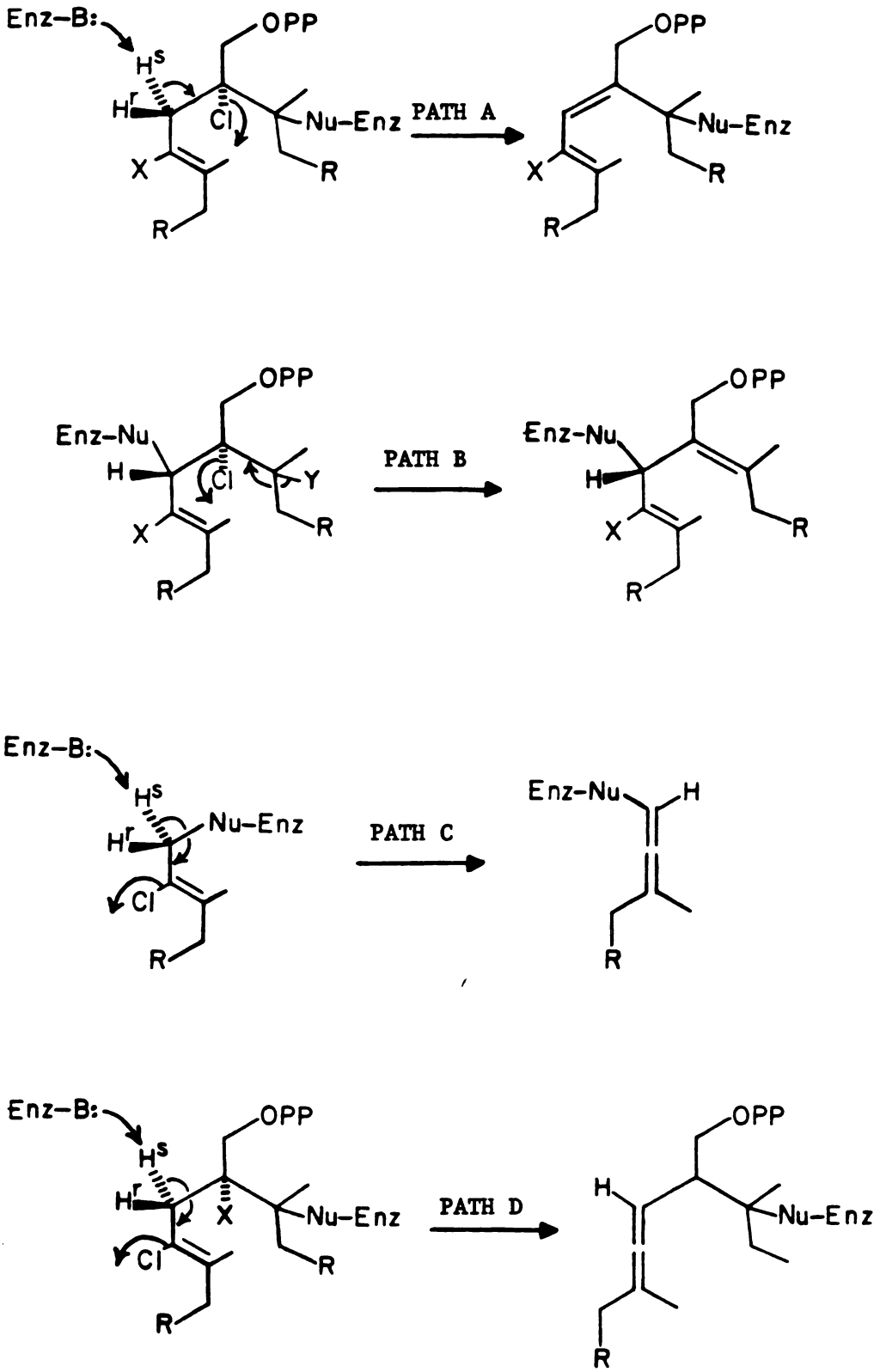
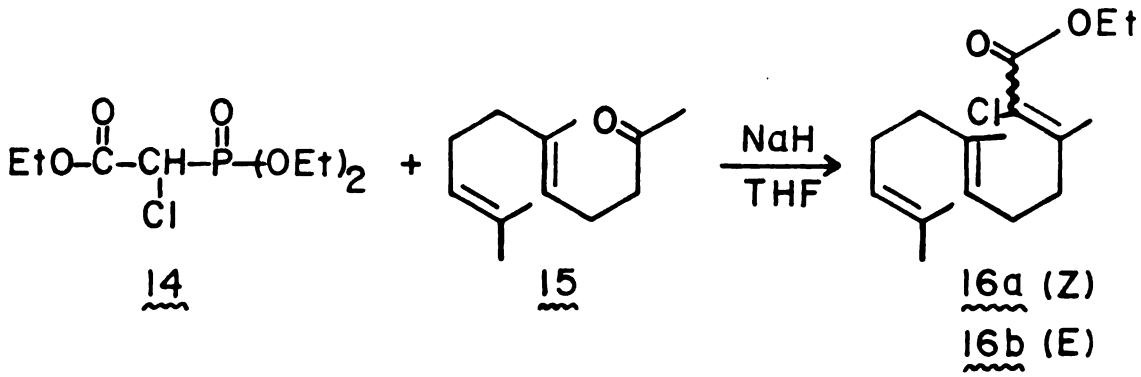


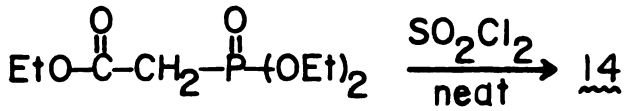
FIGURE 10: Postulated mechanisms for enzyme alkylation using 2-chloro farnesyl pyrophosphate. (X = H,Cl)

E-geranyl acetone (15) under the conditions described by Wadsworth and Emmons (1961) to give a mixture of isomers of ethyl 2-chloro farnesoate (16a and 16b).



14 15 16a (Z) 16b (E)

The starting reagent 14 is not commercially available and was therefore synthesized. A survey of the literature revealed a Russian article (Shevchenko et al., 1962) in which the synthesis of 14 in 47% distilled yield by reaction of diethylethoxycarbonylmethyl phosphonate (17, Aldrich) with one equivalent of sulfuryl chloride was claimed.



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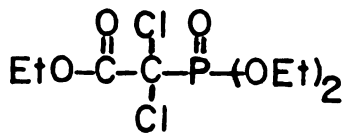
However, the reaction invariably produced in our hands a mixture of the monochlorophosphonate 14, the dichlorophosphonate 18, and starting material 17.



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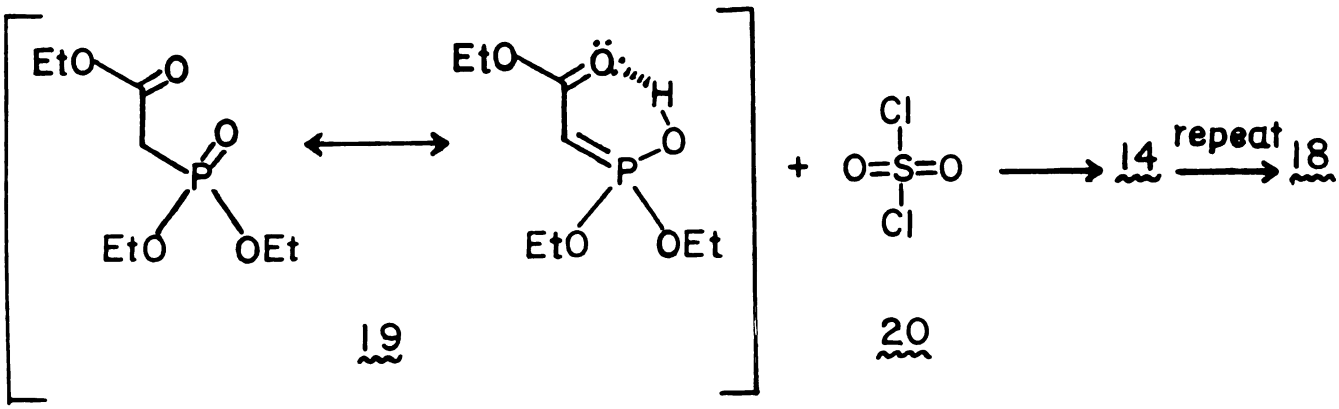
This observation is consistent with a rate-determining step in which enolization (19) is followed by addition of a chloronium ion from sulfuryl chloride (20).

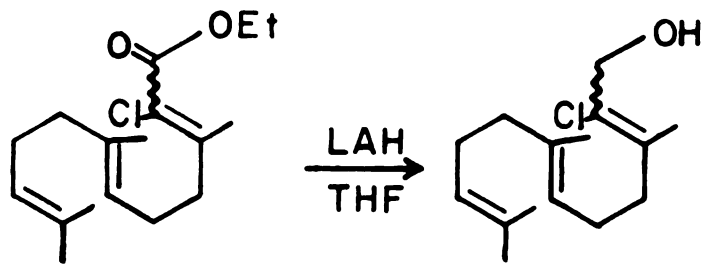


19 20

The reaction would then be expected to occur even more readily with 14 than 17 because the inductive effect of the chlorine atom in 14 would increase the acidity of the remaining central hydrogen atom and favor the enolization. A second approach to the synthesis of 14 was based on the report that the dichlorophosphonate 18 could be reduced to the monochlorophosphonate 14 in 98% yield using sodium bisulfite/methanol (Nicholson et al., 1971). The dichlorophosphonate was prepared in this earlier study using the hypohalite halogenation procedure (Quimby et al., 1968). With the latter procedure, we were unable to observe any of the desired 18 but found instead another unidentified product. However, since some 18 was obtained by the sulfuryl chloride procedure, the desired material was prepared by

forcing the reaction toward complete production of 18 by employing an excess of sulfuryl chloride. Selective reduction of dichlorophosphonate 18 provided the necessary reagent for condensation with E-geranylacetone (15) without appreciable formation of 17, [2]. The yield in the condensation reaction was approximately 75% of a mixture of 2E, 2Z isomers, of 16a and 16b.

Lithium aluminum hydride (LAH) reduction[3] of the ester functionality of 16a and 16b provided the alcohols 21a and 21b in nearly quantitative yield.



16a (Z)
16b (E)

21a (Z)
21b (E)

LOBAR chromatography separated the two isomers and the 2Z and 2E configurations were assigned using NMR spectroscopy and the shift reagent, $Eu(fod)_3$ (Rondeau et al., 1971;

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(2) Upon completion of this work, a publication, Synthesis 31(1978), described a new method for preparation of 16.

(3) For some of the enzyme studies it was necessary to prepare a labeled compound. This was done by using tritiated LAH.

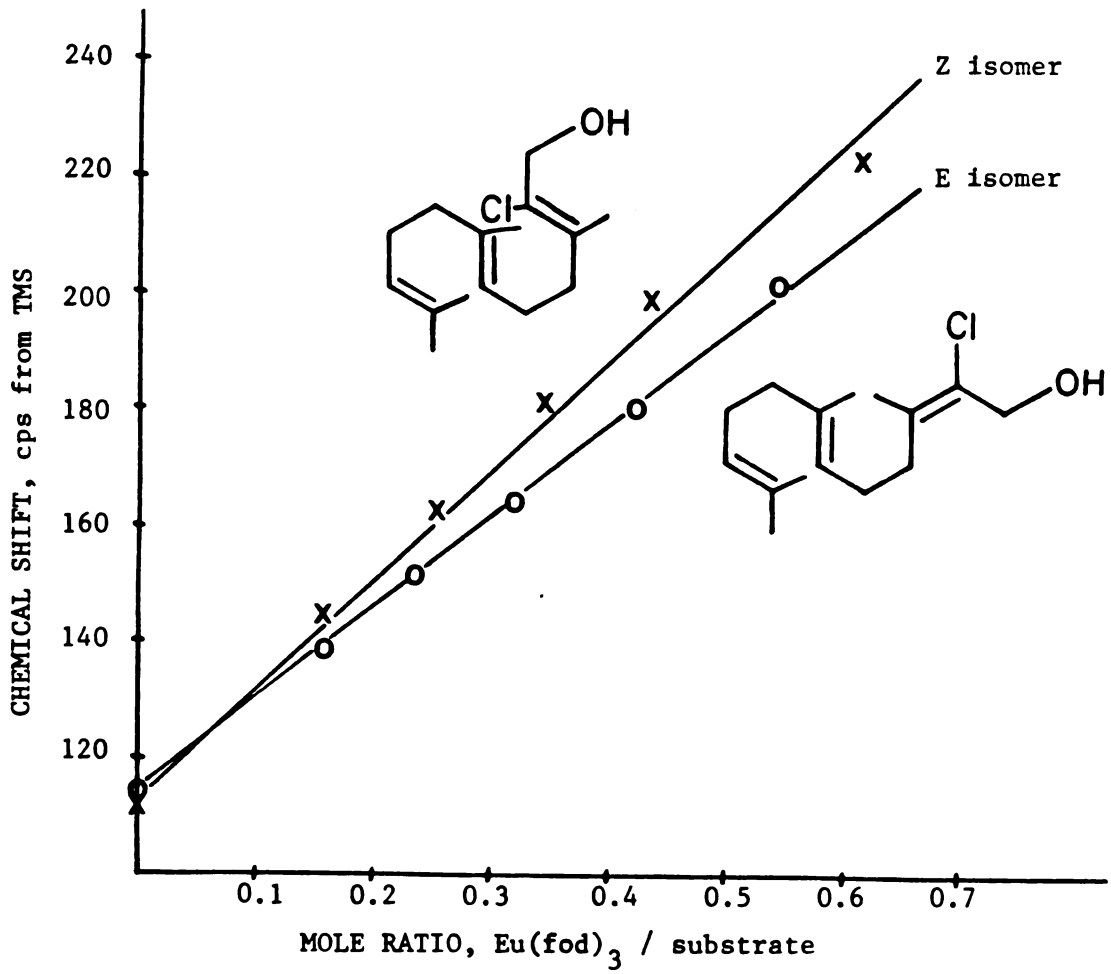
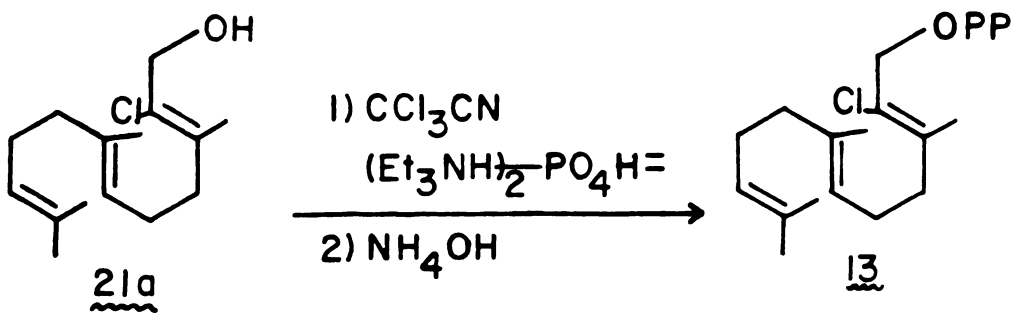


FIGURE 11: Variation in chemical shift of the 3-methyl protons of each isomer upon addition of $Eu(fod)_3$ in $CDCl_3$.

Cockerill et al., 1973). A series of NMR spectra were taken for each isomer with increasing amounts of shift reagent, and the effect on the chemical shift of the 3-methyl protons was measured. The graph in Figure 11 shows the shift differences as a function of the $Eu(fod)_3$ concentration. The isomer in which the 3-methyl group was more responsive to the shift reagent was assigned the 2Z stereochemistry.

Pyrophosphorylation of 21a, the 2Z isomer, by a standard procedure (Ortiz de Montellano et al., 1977a) afforded the 2-chloro FPP analog (13) in about 20% yield.



2.4. BIOLOGICAL RESULTS

The 2-chloro FPP analog (13) was tested as an irreversible inhibitor of yeast squalene synthetase (the procedure is described in Section 5.21). Irreversible binding would have been characterized by a time-dependent increase in observed inhibition (Aldridge and Reiner, 1972). However,

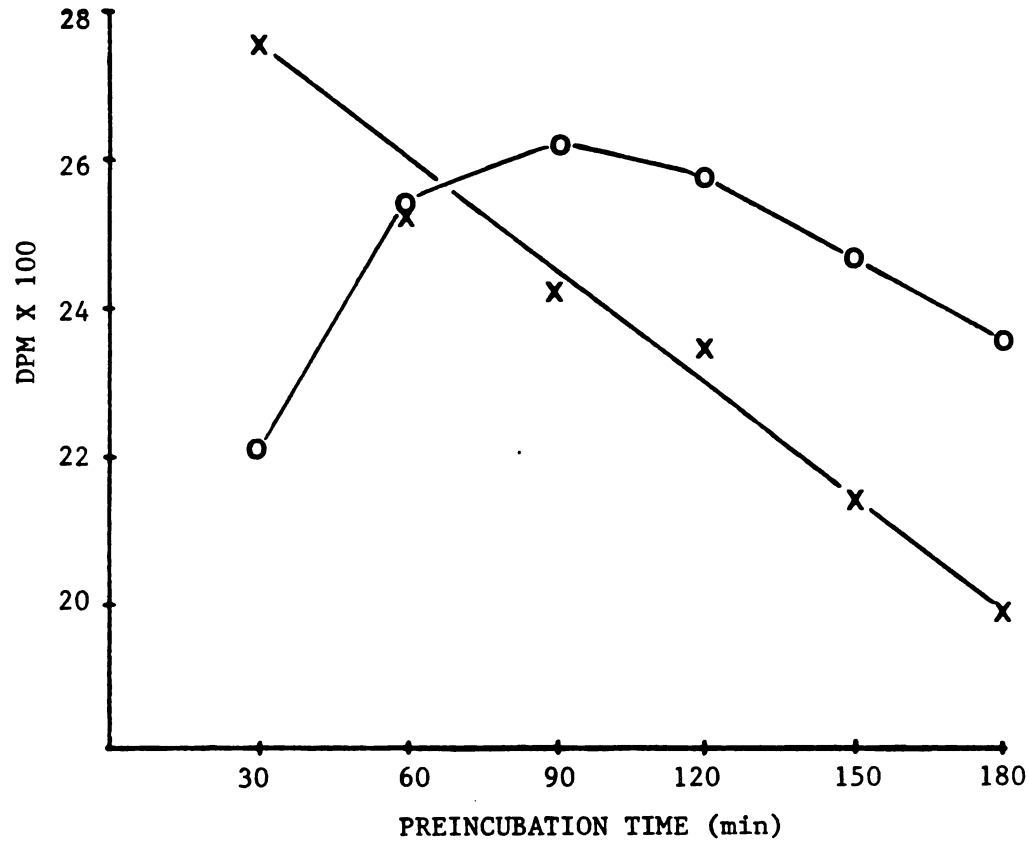


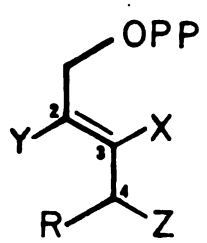
FIGURE 12: Effect of preincubation of analog 13 (o) and control (x) on squalene synthetase at 37°.

our results, shown in Figure 12, are rather complex. It is possible to conclude that 13 does not inactivate the enzyme since a time-dependent decrease in inhibition is observed. This decrease may be due to slow enzymic and chemical degradation of the analog.

(1-^{3}H)-13 was prepared (specific activity of ~ 7.2mci/mmole) and analyzed for acceptance as a substrate by the enzyme. Using procedures previously developed in this laboratory (see Section 5.21 and references therein), 13 was found to be unacceptable to the enzyme as a substrate at either the first or second catalytic site. This conclusion was reached after silica gel tlc and liquid scintillation counting of extracted plate sections from a large scale incubation revealed that nearly all the radioactivity (98.6%) corresponded to a polar non-squalene component which remained at the origin. Similarly, using the standard bioassay, no radioactivity was detected for a squalene product after column separation. Substrate activity of 0.1% that of 1 would have been detectable using 13.

Finally, in collaboration with G.S. Yost (1977) in our laboratory, the 2-chloro FPP analog was tested as a competitive inhibitor of squalene product formation.

Table IV[(a)]



Analog	X	Y	Z	%Relative Inhibition
6	-Me	-Me	-H	64
13	-Me	-Cl	-H	41
7	-Et	-H	-H	31
12	-Me	-H	-F	27

(a) Yost, 1977.

Table IV shows the observed values, which were calculated in the same manner as reported in Table III. Since the values reported in the two Tables were not obtained with the same controls or enzyme preparation, they are not directly comparable to each other. However, the inhibition of 41% noted for the 2-chloro FPP analog appears to be significant when compared to 64% for the 2-methyl FPP analog (6), which in Table III gave 87% inhibition.

2.5. DISCUSSION

Formation of a covalent enzyme-substrate complex (Figure 8) presumably does not occur since 2-chloro FPP (13) is neither a substrate nor an irreversible inhibitor. At first glance this is a surprising result. Since the 2-methyl FPP

analog (6) is not accepted at the first site, it is to be expected that the 2-chloro FPP analog would not be either ($-C\!\!\perp\!\!CH_3$, Table I). On the other hand, 6 is a substrate at the second site and so the similar size of a methyl group and chlorine atom suggests that 13 should also be acceptable. The negative results therefore strongly suggest that steric effects do not alone govern substrate acceptance at the active site but that the electronic effect of the chlorine substituent must also influence the course of the enzymatic process.

The inability of the 2-chloro FPP analog to act as a first site substrate reinforces the previous observation that this site tolerates nothing larger than a hydrogen at the 2-position (Ortiz de Montellano et al., 1976b). A reasonable explanation for its inability to function at the second site may be rationalized in terms of chlorine's electronic effects. The properties of this halogen on the double bond allow it to exert two effects (Section 1.2). First, its greater electronegativity defines its area as δ^- and second, its inductive effect draws electrons away from the double bond. These effects, in turn, may cause the 2-chloro FPP analog to lose its ability to be accepted as a substrate by: (1) decreasing the ability of the analog to bind to the active site if the double bond associates with a cationic region and/or if the 2-position cannot tolerate electron density, or (2) decreasing the nucleophilicity of the double bond thereby preventing its reaction with an

electrophilic species (see Figure 8). Since 2-chloro FPP was found to inhibit squalene product formation, it presumably is bound to the active site, but it is apparently not catalytically acceptable because none was incorporated into a squalene product. One possible explanation is the aforementioned electronic effect upon the nucleophilicity of the double bond. Each of the enzyme mechanisms proposed in Figure 8 require a nucleophilic 2,3 double bond in the second site substrate. This reactivity would be compromised by the chloro substituent.

Apparently the substitution of a chlorine for a hydrogen at the 2-position of the natural substrate, farnesyl pyrophosphate, is not tolerated by yeast squalene synthetase.

CHAPTER THREE

ANTI-JUVENILE HORMONE ANALOGS

3.1. INTRODUCTION

Considerable attention has been directed toward the possibility of controlling insects by interfering with growth and development processes that are unique to the class, Insecta. This would offer a more selective and environmentally safe means of insect pest control.

The typical life cycle of an insect involves the transformation of an immature larvae or nymph into a reproducing adult of different form and structure (metamorphosis). The two types of metamorphosis, shown in Figure 13, are hemimetabolous development (the juvenile forms resemble the adult forms except for minor differences), and holometabolous development (the juvenile forms differ markedly from the adult forms).

Early research (Kopec, 1922; Wigglesworth, 1934, 1940) showed that insect moulting and metamorphosis are regulated

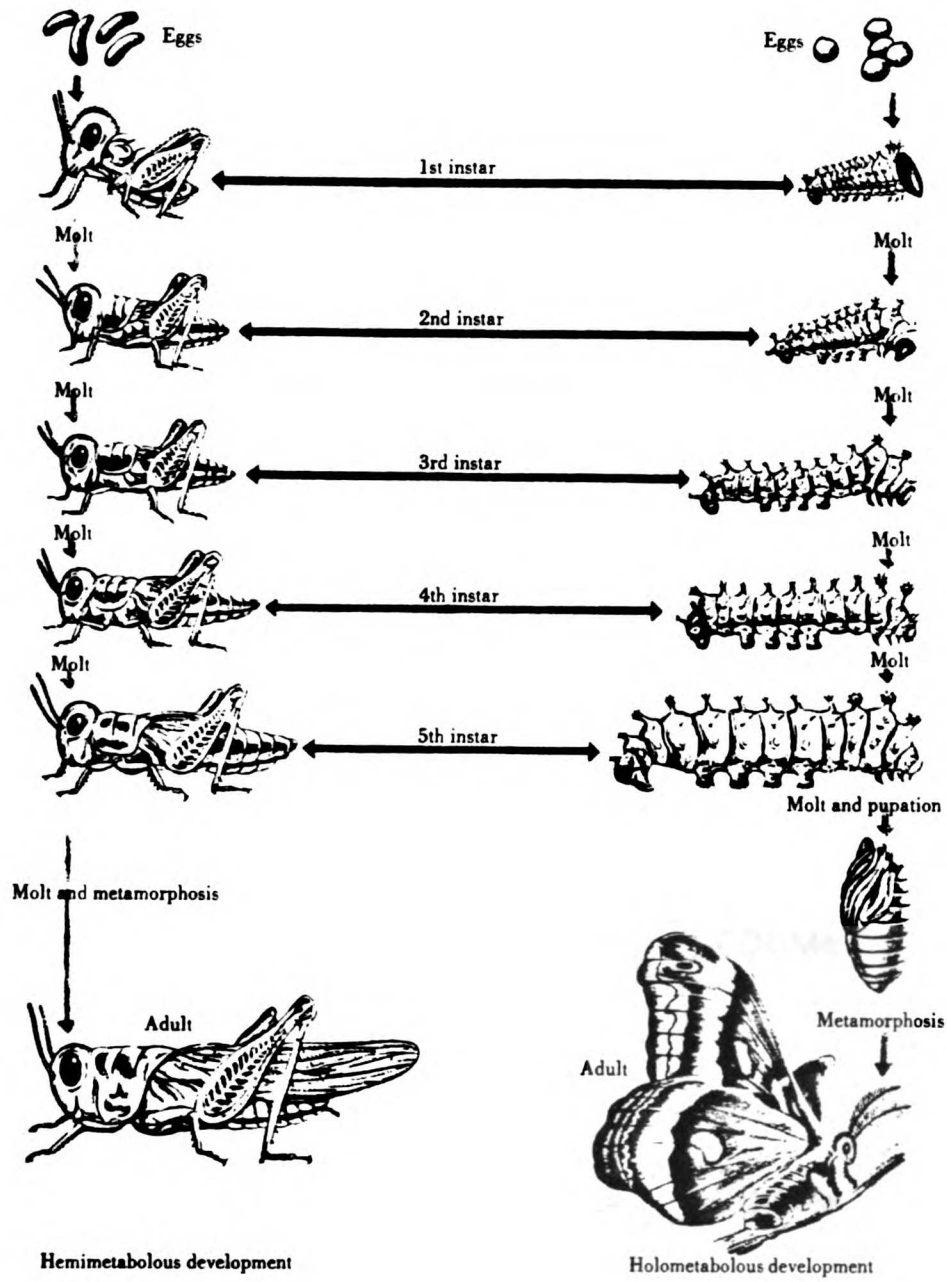
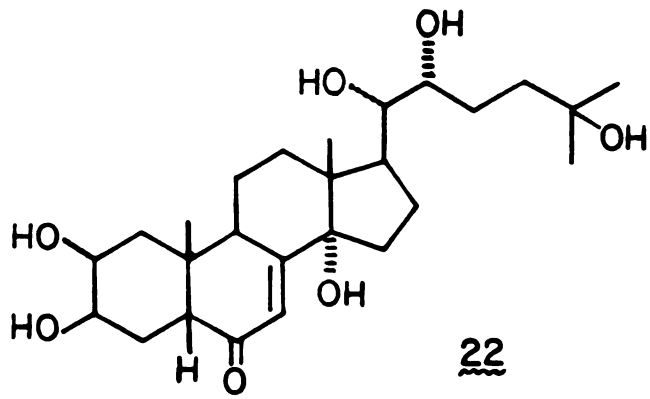


FIGURE 13: The hemimetabolous development of a grasshopper and the holometabolous development of a moth (Fingerman, 1969).

by hormones. Later, these hormones were isolated and shown to consist of brain hormones, which are probably polypeptides (Ishizaki and Ichikawa, 1967; Yamazaki and Kobayashi, 1969; Ishizaki et al.; 1978), β-ecdysone 22 (Butenandt and Karlson, 1954; Huber and Hoppe, 1965; Karlson et al., 1965), and juvenile hormones 23, 24 and 25 (Roller et al., 1967; Meyer et al., 1968; Judy et al., 1973).



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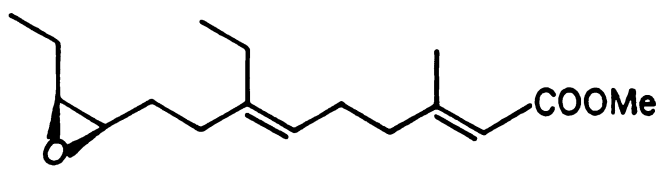
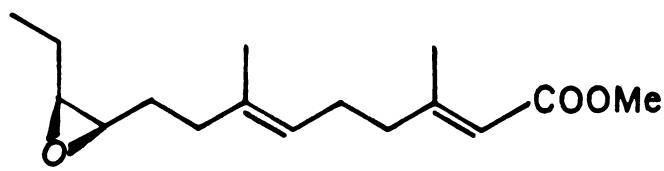
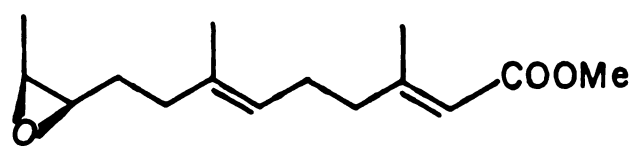


JH I (23)



JH II (24)



JH III (25)

This endocrine system works in concert to orchestrate the growth and development of an insect (see Figures 14 and 15). The brain hormones, secreted by neurosecretory cells, stimulate the prothoracic glands (Williams, 1947, 1952; Schaller and Charlet, 1980; Ishizaki and Suzaki, 1980). These glands, in turn, secrete α-ecdysone which is metabolically converted to β-edysone by hydroxylation at the C-20 position (King et al., 1974; Chino et al., 1974; King and Mark, 1974; Romer et al., 1974). Ecdysone is responsible for initiating metamorphosis and moulting (for review see Burdette, 1974; Hoffmann, 1980). The juvenile hormones are secreted by a pair of glands called the corpora allata (Akamatsu et al., 1975 and references therein) which are controlled by nerve signals carried via connecting neurons from the brain and by brain hormones carried via the hemolymph (Williams, 1976; Gilbert et al., 1977; Ishizaki and Szaki, 1980). The role of the juvenile hormones, which varies with species, was found to be fourfold (for review see Gilbert, 1976; Kramer and Law, 1980a). First, high levels of juvenile hormone are necessary to maintain the insect juvenile form. These high levels in the immature insect exert an inhibitory effect on the formation of typical adult features. Second, the presence or absence of juvenile hormone is necessary for larval and adult diapause, the period of dormancy which allows an insect to survive periods of climatic stress. Third, in the adult female, levels of juvenile hormone rise once again and stimulate the reproductive system. And fourth, in some

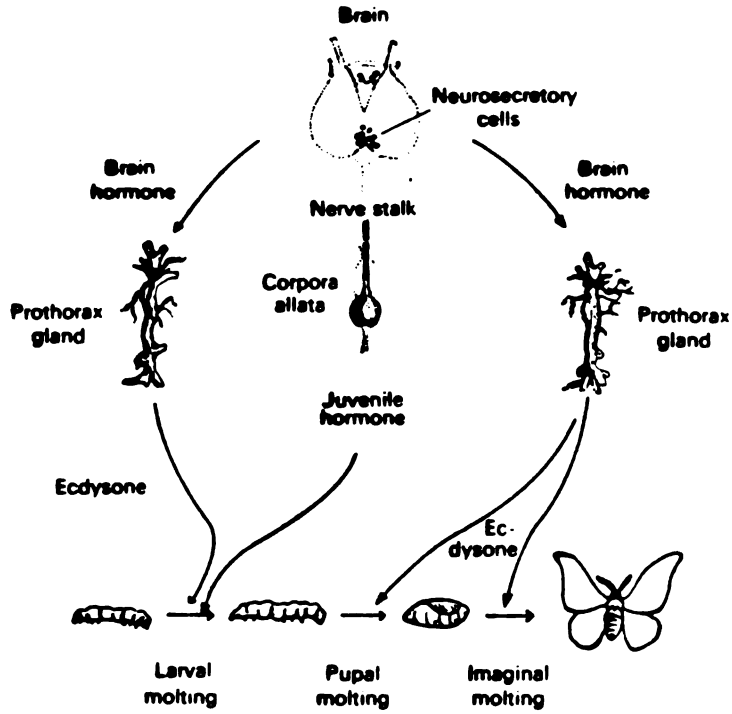


FIGURE 14: Origin of insect hormones and their action on development in a holometabolous insect (Karlson, 1975).

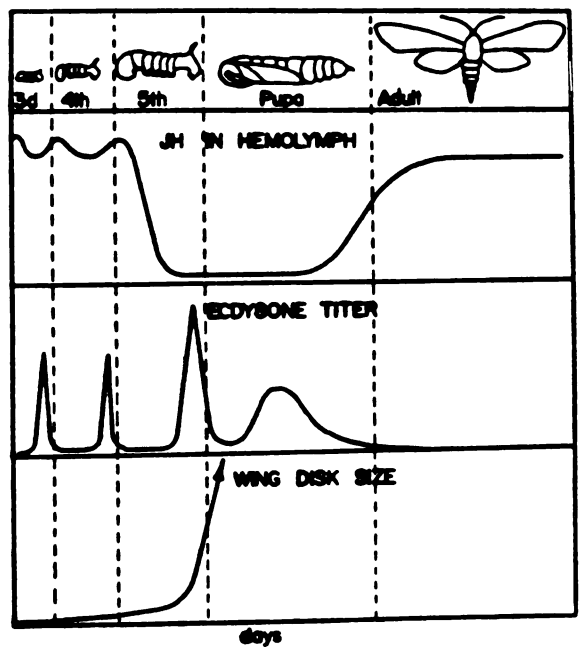


FIGURE 15: Idealized hormone levels during development in a holometabolous insect (Akamatsu et al., 1975).

species, juvenile hormone is necessary for the production of characteristic sex pheromones.

Since insect maturation is, in part, controlled by levels of juvenile hormone, then agonists of the biological action of juvenile hormone (JH analogs) or antagonists of its biosynthesis, release, or uptake (anti-JH analogs) would be expected to be disruptive to growth and possibly advantageous as insecticides. A JH analog enhances the natural juvenile hormones' biological action either by directly mimicking the juvenile hormones or by synergistically protecting them from metabolic degradation. Juvenile hormone is present throughout most stages of an insect's life cycle except during the last instars when differentiation to the adult stage occurs. If a JH analog is applied at this time, when low titers of endogenous juvenile hormone are usually present, then adult morphogenesis is upset and insects appear with a mosaic of adult and juvenile characteristics. These precocious adults are unable to feed, mate, or reproduce and eventually die (Williams, 1956; Bowers, 1971a, 1971b). The major drawback of the JH analogs as insecticides is the brief periods during which insects are sensitive to them. Immature and adult insects are unaffected by an excess of juvenile hormone and therefore pests in these stages, like the caterpillar and mosquito, are able to inflict their damage. In contrast, anti-JH analogs are intended to decrease the titers of juvenile hormone. A decrease in juvenile hormone affects an insect throughout

its developmental and adult stages by provoking precocious metamorphosis during the immature stages (early instars), by interrupting larval and adult diapause, and by causing female sterility. Thus, an anti-JH analog is potentially more useful as an insecticide because there is less need for critical timing of application. In addition, since the larval stages are responsible for producing most of the crop damages, an anti-JH analog would be of economic importance for controlling these insect pests.

Our search for an anti-JH analog was sparked by the work of several investigators on the epoxidation step of juvenile hormone biosynthesis (see Section 3.2) and by work in our own laboratory on the suicide destruction of rat hepatic cytochrome P-450 (see Section 3.3).

3.2. THE EPOXIDATION STEP OF JUVENILE HORMONE BIOSYNTHESIS

The biosynthesis of juvenile hormones (Figure 16) seems to occur wholly in the corpora allata (Akamatsu et al., 1975 and references therein). Our focus on the epoxidation step of the pathway, path A or B, stems from the fact that it may be a cytochrome P-450 mediated reaction.

W.S. Bowers began a search for an anti-JH analog in the 1960's. Because plants were known to contain compounds which affected insect activity, he began to challenge immature insects throughout their development with the organic

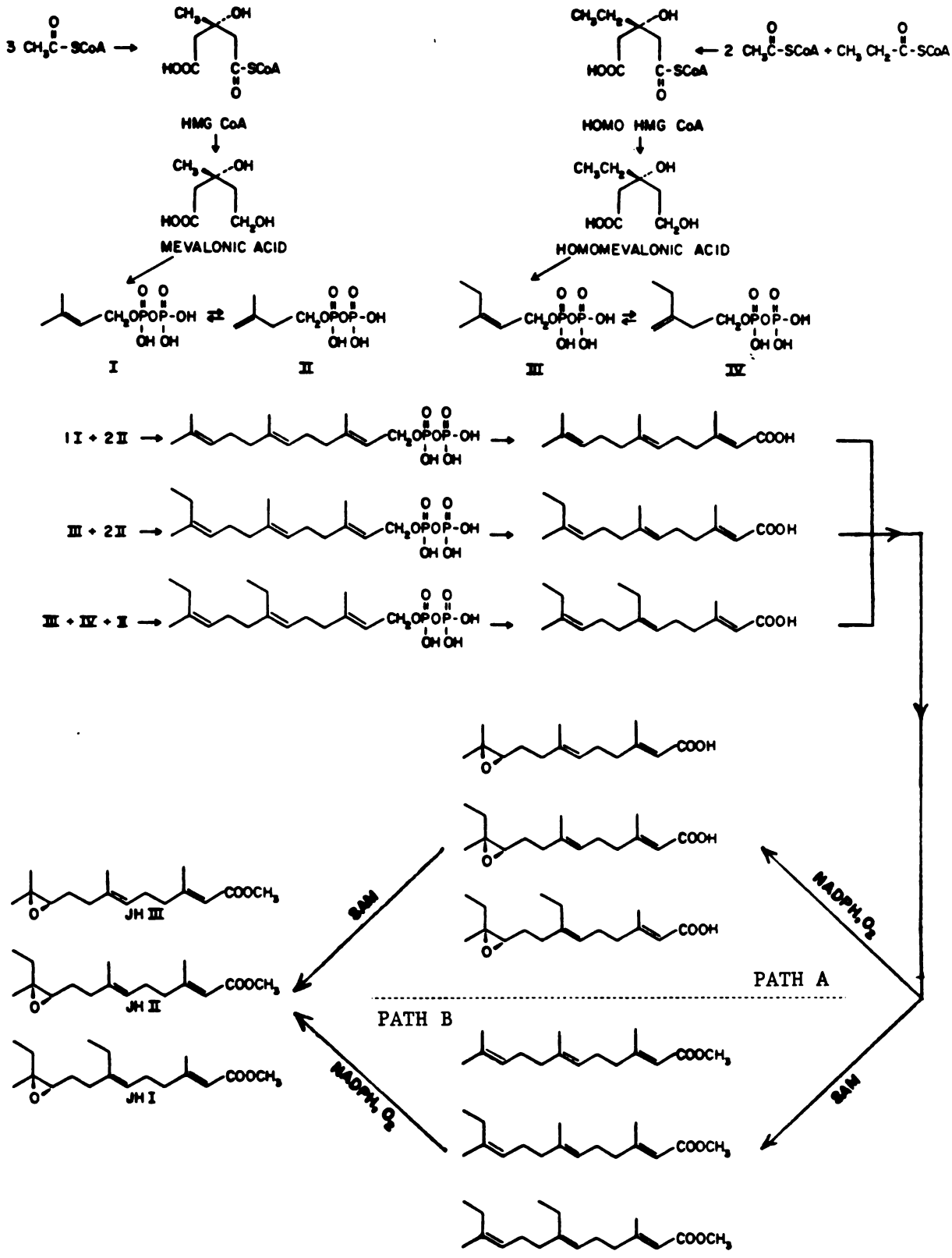
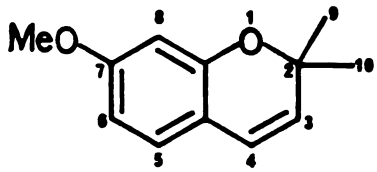


FIGURE 16: Postulated pathways for the biosynthesis of the juvenile hormones I, II, and III.

extracts of plants. In this way he isolated from the plant, Ageratum houstonianum, two chromenes with anti-JH activity which he named Precocene I (26) and Precocene II (27) (Bowers, 1976a; Bowers et al., 1976b).



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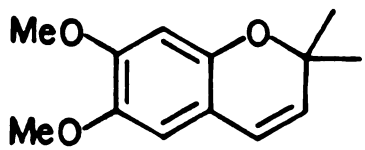

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He established that these two compounds caused precocious metamorphosis, prevented ovarian development, and induced diapause in several hemipteran species of insects. Other insect species were shown to exhibit all or some of these effects (Bowers et al., 1976b; Onta et al., 1977; Pener et al., 1978; Nemec et al., 1978).

Although Bowers observed that some precocene-induced effects could be reversed by application of juvenile hormone (Bowers et al., 1976b), the exact mechanism of action of the precocenes was undetermined. However, since his initial discovery, experimental evidence has accumulated which shows that Precocenes I and II, and their analogs (Bowers, 1977; Matolesy et al., 1980; Chenevert et al., 1980), cause selective necrosis of the secretory cells of the corpora allata (Unnithan et al., 1977; Schoonveld, 1979). The resulting inactivation of the corpora allata prevents its growth and

biosynthesis of juvenile hormone (Pratt and Bowers, 1977; Unnithan et al., 1977; Pener et al., 1978; Unnithan and Nair, 1979). The mechanism of inactivation suggested to involve an oxidative biotransformation (Brooks et al., 1979), is catalyzed by a juvenile hormone biosynthetic enzyme (Unnithan and Nair, 1979; Masner et al., 1979) associated with the microsomal fraction from corpora allata homogenates. The enzyme appears to be a mixed function oxidase because it requires NADPH and molecular oxygen (Ohata et al., 1977) and is inhibited by carbon monoxide and mixed function oxidase inhibitors (Ohta et al., 1977; Brooks et al.,1979; Pratt et al., 1980). From metabolism studies (Ohta et al., 1977; Burt et al., 1978; Soderlund et al., 1980; Pratt et al., 1980), it has been deduced that oxidation of the 3,4-double bond of the precocenes by an enzyme in the corpora allata gives a reactive epoxide (28) (Jennings and Ottridge, 1979) which alkylates cellular nucleophiles (29) (Brooks et al., 1979; Pratt et al., 1980) (see Figure 17). This reactive epoxide was postulated because a mixture of cis and trans 3,4-dihydrodiol (30) metabolites was observed. These diols probably are formed by chemical hydration because epoxide hydrolases give exclusively trans dihydrodiols (Brooks et al., 1970) and none of these enzymes have been detected in the corpora allata (Hammock, 1975; Pratt et al., 1980). The more stable juvenile hormone epoxide is apparently metabolized outside the corpora allata to give 97% trans dihydrodiol (Nakanishi et al., 1971). The

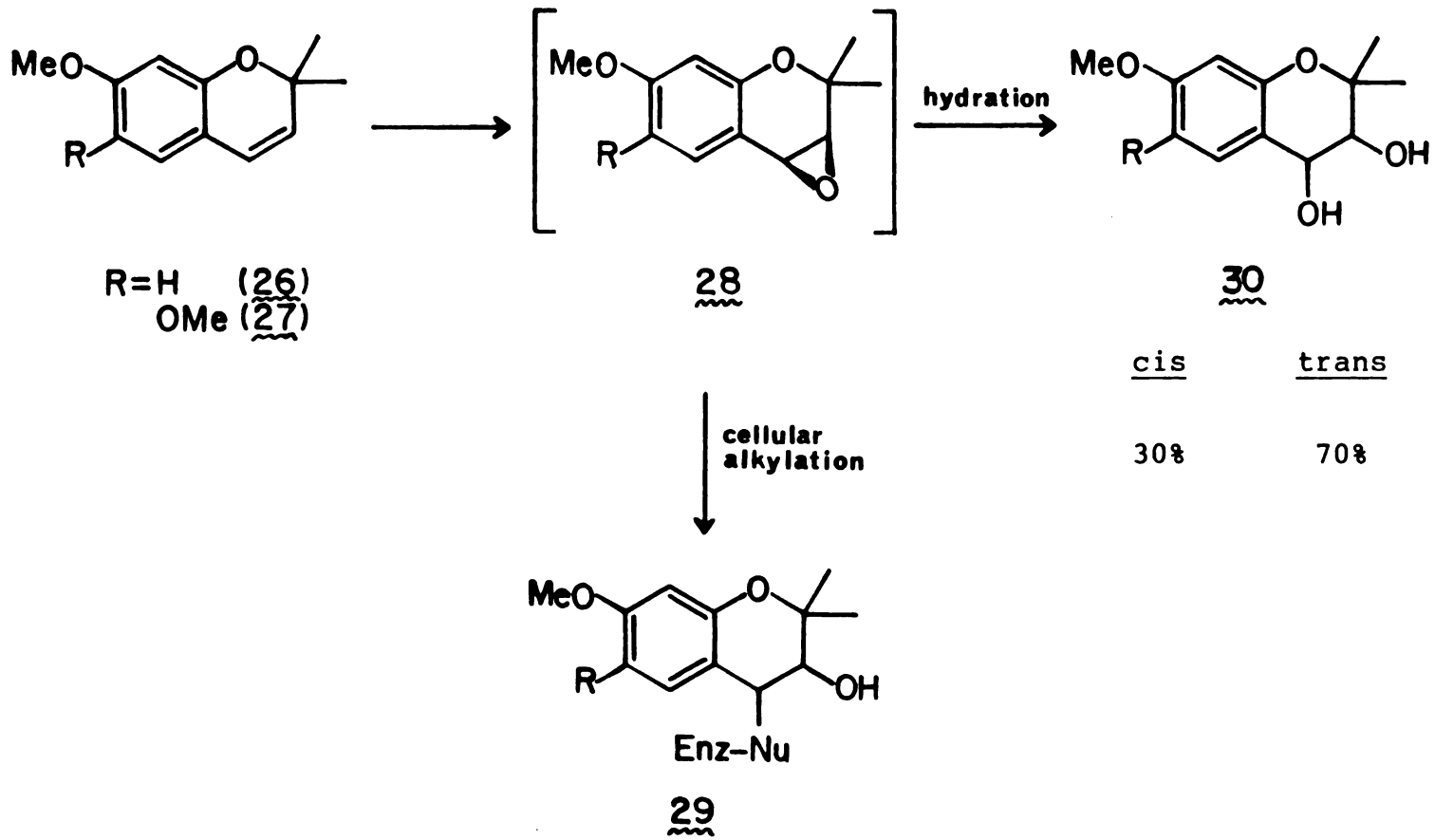


FIGURE 17: Precocene metabolism to the intermediary epoxide which subsequently undergoes chemical hydration or cellular alkylation.

information available on the metabolic activation of the precocenes, indicates the involvement of a step similar to the epoxidation step of juvenile hormone biosynthesis. Epoxidation of juvenile hormone also occurs in the microsomal fraction (100,000g) of corpora allata homogenates (Hammock, 1975; Tobe and Pratt, 1976; Reibstein et al., 1976), requires NADPH (Akamatsu et al., 1975; Hammock, 1975; Feyereisen etal., 1981) and molecular oxygen (Tobe and Pratt, 1974; Hammock, 1975; Feyereisen et al., 1981), and is inhibited by carbon monoxide and mixed function oxidase inhibitors (Hammock; 1975; Hammock and Mumby, 1978; Feyereisen et al., 1981). Furthermore, the NADPH supported epoxidation is inhibited by oxidized cytochrome C (Feyereisen et al., 1981). All of these criteria are consistent with a cytochrome P-450 mixed function oxidase enzyme (Wislocki et al., 1980; White and Coon, 1980).

Although the evidence is not complete, the enzyme responsible for the epoxidation of the juvenile hormones now appears to be the same enzyme responsible for the bioactivation and subsequent cytotoxicity of the precocenes.

3.3. FORMATION OF REACTIVE EPOXIDES AND INACTIVATION OF MAMMALIAN HEPATIC CYTOCHROME P-450

The cytochrome P-450 enzymes (for reviews see Sato and Omura, 1978; Wislocki et al., 1980; White and Coon, 1980) are membrane bound hemoproteins that, in conjunction with an

electron transfer protein (NADPH cytochrome P-450 reductase), are capable of donating atomic oxygen to a broad variety of substrates. These enzymes, widely distributed in nature, participate in monooxygenase reactions involved in the synthesis and degradation of endogenous substrates and in the oxidative metabolism of exogenous lipophilic compounds. The latter metabolism generally enhances the elimination of intrusive xenobiotics by converting them to polar derivatives which are directly excreted or are functionalized for further metabolism. However, this protective process occasionally converts compounds to chemically reactive species which produce toxic effects (Miller, 1970).

Among other reactions, the cytochrome P-450 enzymes oxidize olefinic π-bonds to epoxides which, because of their highly strained ring system, readily react with nucleophiles. Thus, in biological systems epoxides may undergo chemical or enzymatic transformation to the corresponding dihydrodiols or glutathione conjugates, or they may alkylate cellular nucleophiles (Garner, 1976). Examples of carbon-carbon double bonds known to be bioactivated to toxic epoxides are provided by those in polycyclic aromatic hydrocarbons (Hulbert, 1975; Gelboin, 1980), aflatoxins (Swenson et al., 1977), styrene (Marniemi et al., 1977), ethylene (Ehrenberg et al., 1981), and vinyl halides (Guengerich et al., 1981). In addition to toxic epoxide formation, however, several investigators have established that irreversible inactivation of cytochrome P-450 can occur with the

π-bonds of olefins (Levin et al., 1972; DeMatteis, 1978; Ortiz de Montellano and Mico, 1980a), vinyl halides (Guengerich and Strickland, 1977; Pessayre et al., 1979; Mico, 1980; Ortiz de Montellano et al., 1981a), acetylenes (White and Muller-Eberhard, 1977; White, 1978; Ortiz de Montellano and Kunze 1980b), and allenes (Ortiz de Montellano and Kunze, 1980c). In the presence of NADPH, oxygen, and a catalytically competent enzyme, these functionalities are oxidatively activated to chemically reactive species which destroy the cytochrome P-450 enzyme. This enzyme inactivation is usually accompanied by loss of heme and, in some instances, by the formation of abnormal porphyrin pigments. The isolation and characterization of some of these pigments have demonstrated that enzyme inactivation is due to covalent attachment of the substrate to a nitrogen of the prosthetic heme moiety. The structure of the ethylene adduct has been identified as the iron complex of N-(2-hydroxyethyl) protoporphyrin IX (Ortiz de Montellano et al., 1980d; 1981c) and that of the propyne alkylated heme as N-(2-oxopropyl) protoporphyrin IX (Ortiz de Montellano and Kunze, 1981) (see Figure 18). Evidence to clearly support a suicidal destructive process has been obtained only with 2-isopropyl-4-pentenamide (Ortiz de Montellano and Mico, 1981b), the prototype for this type of cytochrome P-450 inhibitor (DeMatteis, 1978).

The evidence so far available thus suggests that substrates with certain chemical structural patterns are

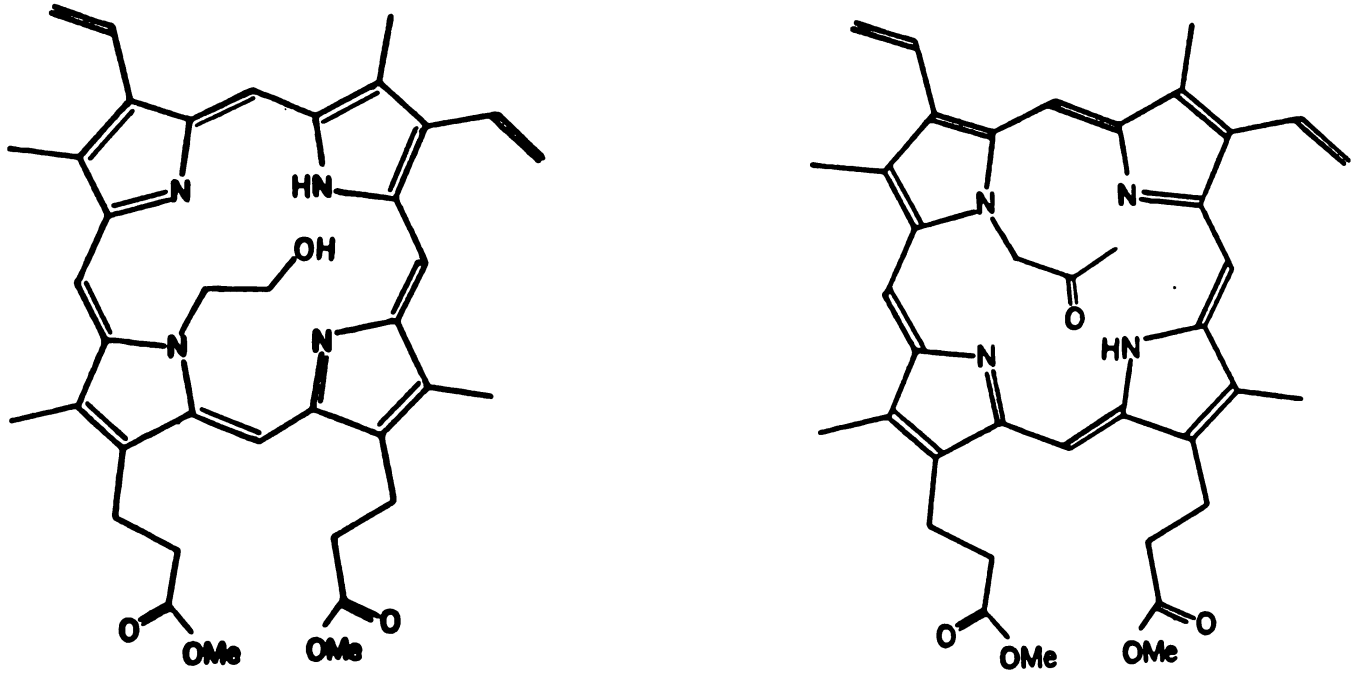


FIGURE 18: Structure of the ethylene adduct (left) and of the propyne adduct (right).

oxidatively activated to either a toxic epoxide, which covalently binds to macromolecules, or to a species which covalently binds to the prosthetic heme of the cytochrome P-450 enzyme.

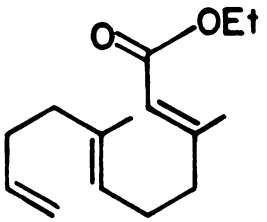
3.4. THE RATIONALE FOR THE DESIGN OF THE ANTI-JUVENILE HORMONE ANALOGS

The design of anti-juvenile hormone analogs was predicated on two assumptions:

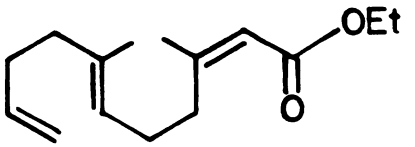
(1) The biosynthetic epoxidation of juvenile hormone is mediated by a cytochrome P-450 monooxygenase (Section 3.2).

(2) The chemical functionalities that cause inactivation of hepatic cytochrome P-450 isozymes (Section 3.3) can be employed for the inactivation of the juvenile hormone cytochrome P-450 enzyme.

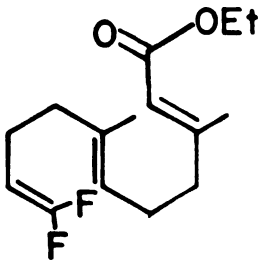
Those cytochrome P-450 enzymes responsible for xenobiotic metabolism exhibit broad and overlapping substrate specificities, while those which are part of biosynthetic pathways display relatively high regioselectivity and stereoselectivity. Juvenile hormone epoxidase is part of a biosynthetic pathway and therefore probably has a high substrate specificity. For this reason, our approach to the design of an anti-juvenile hormone analog was to preserve the structural integrity of juvenile hormone III while



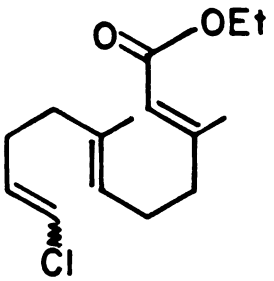
31a



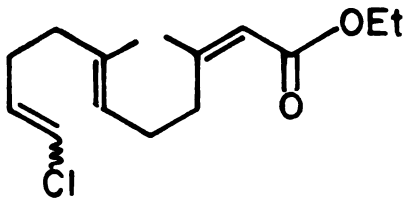
31b



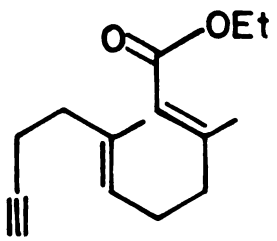
35



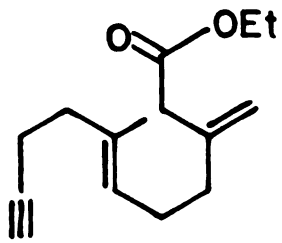
32a



32b



33



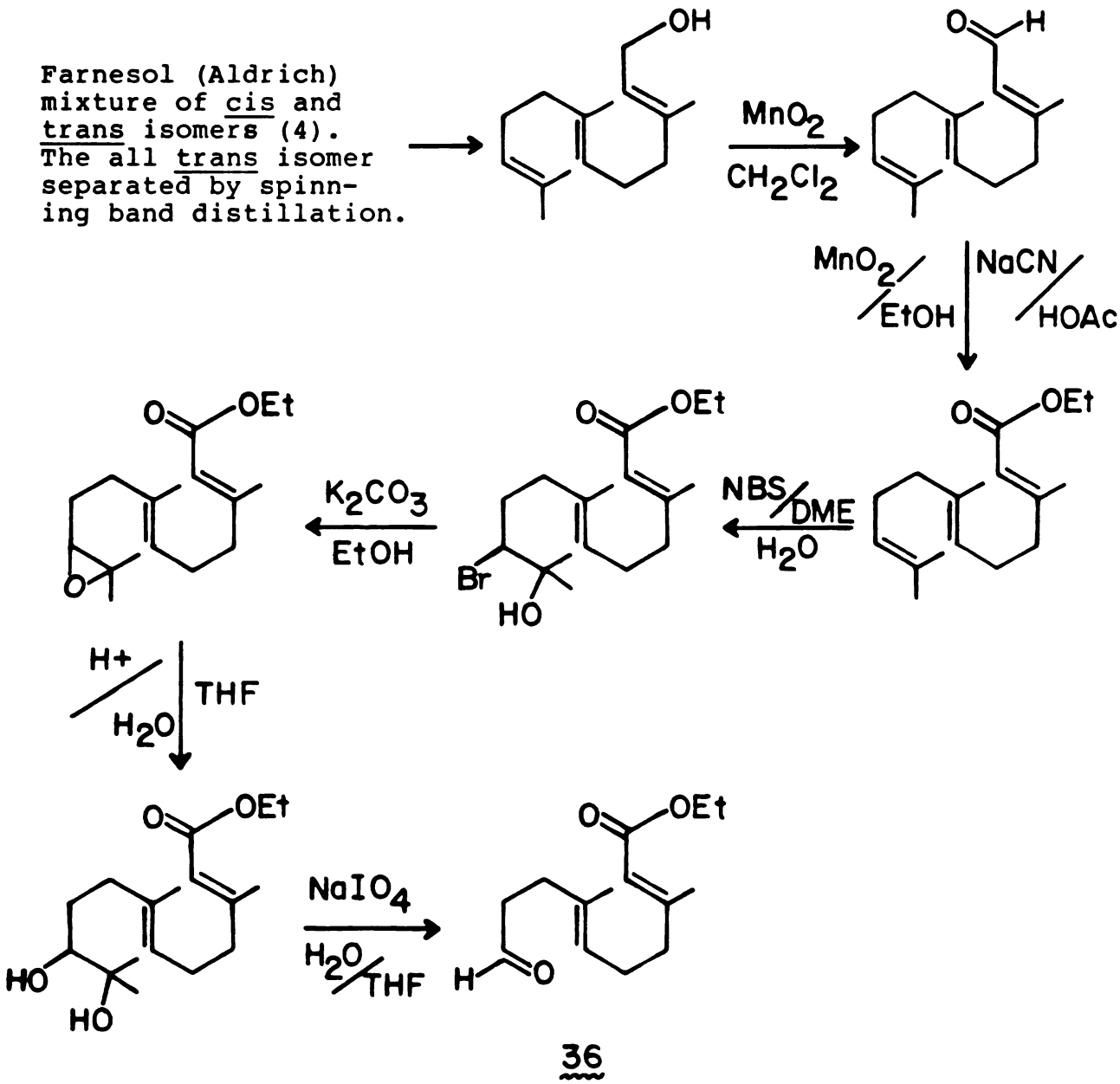
34

FIGURE 19: The synthetic anti-JH analogs.

introducing chemical modifications about the 10,11 double bond, the area of epoxidation. The analogs we prepared are shown in Figure 19. In all of the analogs, the ethyl ester instead of the methyl ester of juvenile hormone III was used because it has been shown to be less readily hydrolyzed by esterases (Weinrich and Wren, 1973). Similarly, analogs 31b and 32b, which contain the Z configuration about the 2,3 double bond, are also less susceptible to hydrolysis by esterases (Weirich and Wren, 1973; Hammock and Quistad, 1976). Analog 34 containing the 3-methylene group was obtained as a major side product in the preparation of 33. Each of the new functionalities at the terminal position, $-CH=CH_2$, -CH=CHCl, $-CH=CF_2$, and $-C\equiv CH$, had previously been shown to destroy rat hepatic cytochrome P-450 isozymes. In the case of all but the vinyl chloride funtionality, the enzyme inactivation was accompanied by covalent attachment of the substrate to the prosthetic heme moiety (see Section 3.3).

3.5. SYNTHESIS

The synthetic strategy for the preparation of analogs 31a and 31b, 32a and 32b, and 35 was to form the different appropriate ylides and react each with ethyl3,7-dimethyl -10-oxo-2-(E),6(E)-decadienoate (36). Analog 33 was then to be prepared from 32a by dehydrochlorination. However, it was first necessary to prepare aldehyde 36. This was done

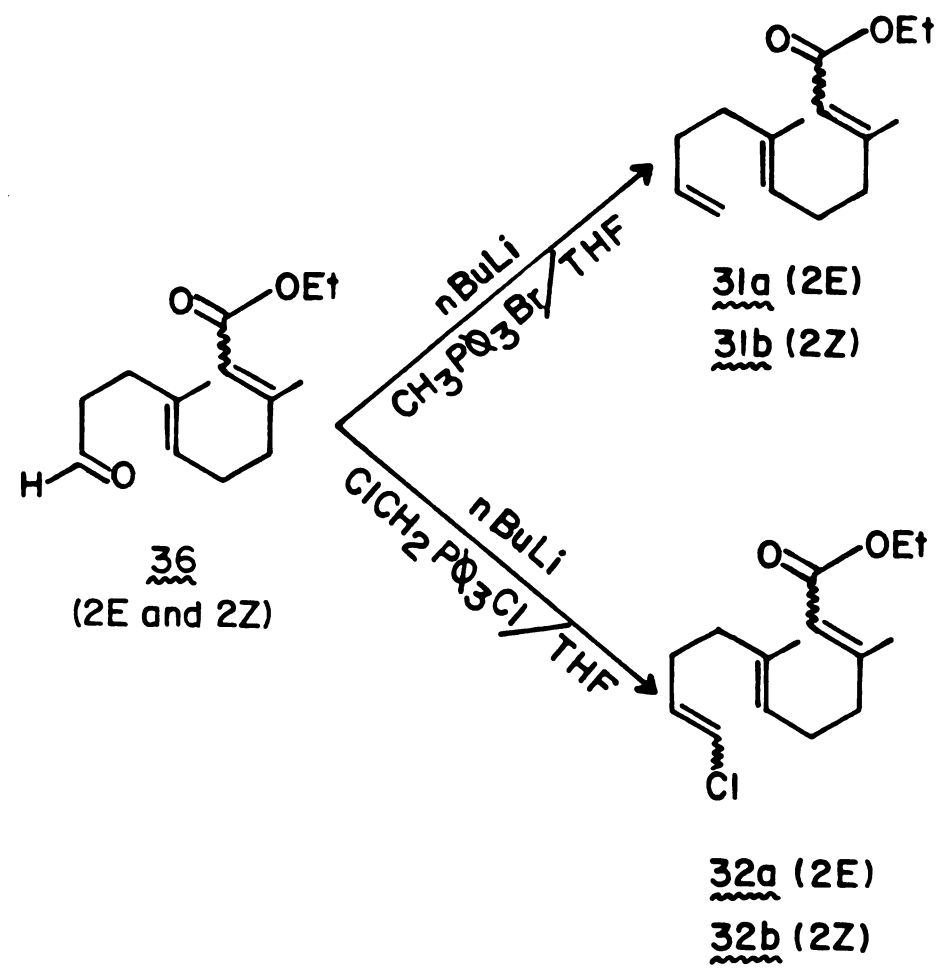


36

FIGURE 20: Synthesis of ethyl 3,7-dimethyl-10-oxo-2(E),6(E)-decadienoate (36).

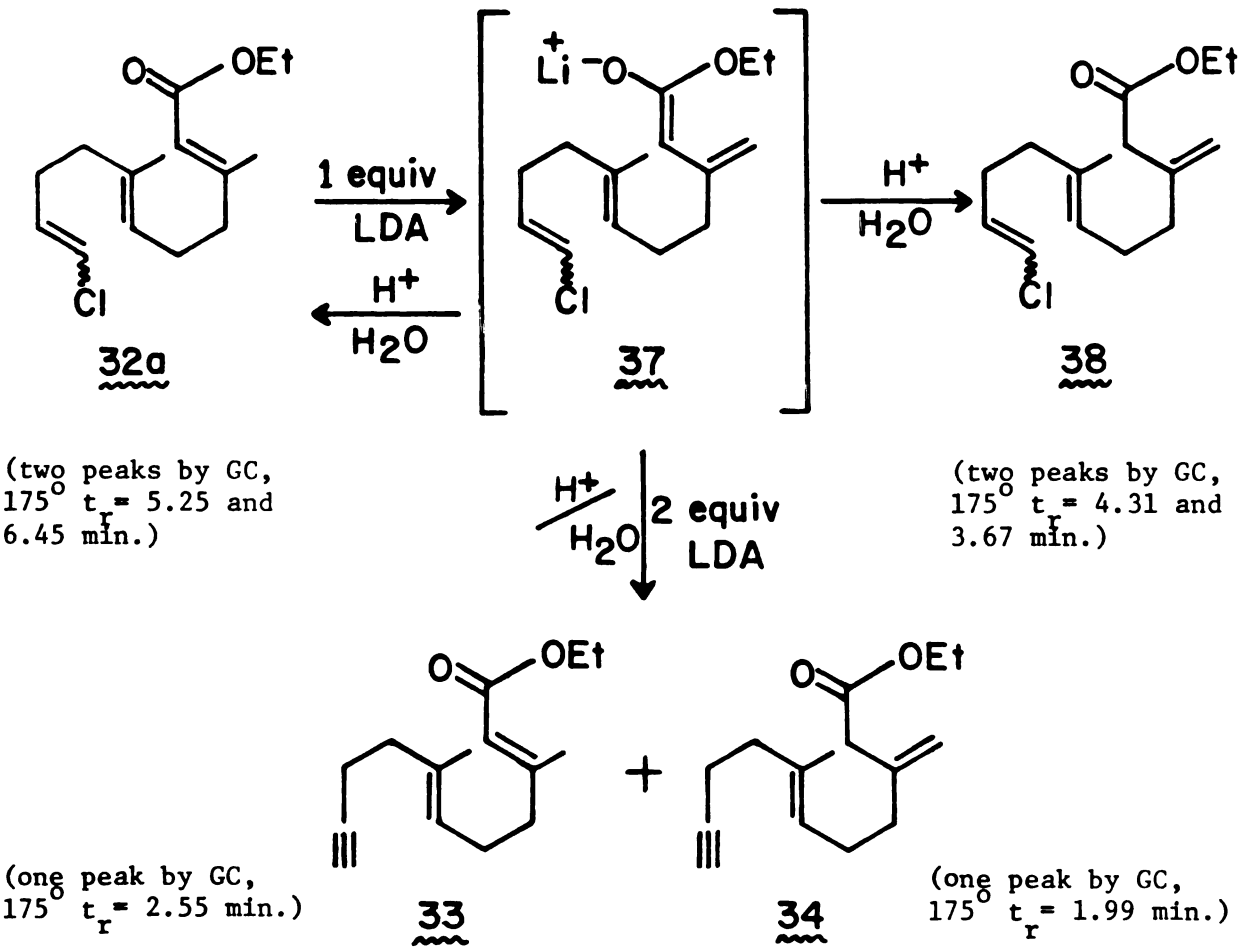
according to the literature procedure outlined in Figure 20 (Yamamoto, 1971; Anderson et al., 1972; Boparai, 1977). The 2Z isomers, 31b and 32b, were prepared beginning with an isomeric mixture (2E, 2Z) of farnesol.

Wittig reaction of aldehyde 36 with the orange-yellow ylide generated from treatment of methyltriphenylphosphonium bromide with n-butyllithium, as described by Boparai (1977), provided compounds 31a and 31b in 60% combined yield. Similarly, the corresponding ylide from (chloromethyl) triphenylphosphonium chloride also reacted with aldehyde 36 to give 32a and 32b in 56% combined yield.



The 2E and 2Z isomers of each pair, 31a and 31b, and 32a and 32b, were separated by LOBAR chromatography and their stereochemistry was confirmed by NMR (Bates et al., 1963; Burrell et al., 1966). The cis and trans chloro isomers of the 10,11 double bond were prepared as a mixture but were not separated.

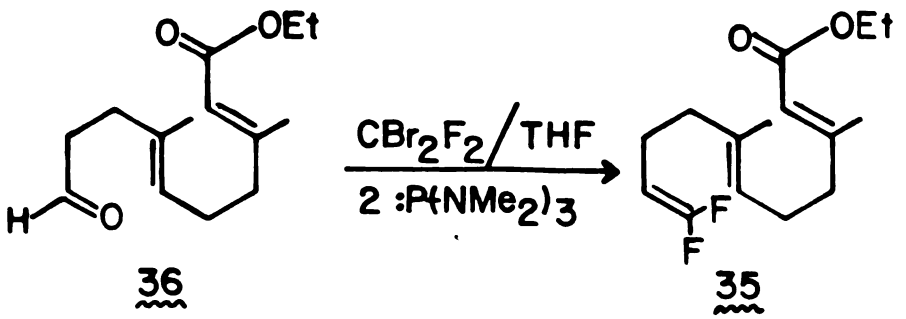
The preparation of ethyl 3,7-dimethyl-10-yne-2(E), 6(E)-undecadienoate (33) and ethyl-3-methylene-7-methyl-10-yne-6(E)-undecaenoate (34) was accomplished by reacting three equivalents of the base, lithium diisopropylamide (LDA), with chloro olefin 32a at -50° (Villieras et al., 1975; Metcalf et al., 1981).



To conserve the ester functionality it was necessary to use the sterically congested base LDA rather than the conventionally used but nucleophilic n-butyllithium. All attempts to exclusively prepare the desired product, 33, failed. The C-3 methyl protons are apparently acidic enough to be abstracted first by the base, resulting in intermediate 37 which gives 38 and starting material 32a on quenching, or 33 and 34 if reacted with two additional equivalents of LDA before quenching. Careful monitoring of the reaction by GC after the addition of one equivalent of LDA showed a partial loss of the two peaks corresponding to 32a and the formation of two new peaks with shorter retention time, presumably the two isomers of 38. Upon the addition of another two equivalents of LDA the two pairs of GC peaks of 38 and 32a disappear and the peaks of 33 and 34 (even shorter retention time) appear simultaneously. Both products are formed in nearly a 1:1 ratio when the reaction is rapidly quenched in cold 2N sulfuric acid.

Ethyl 11,11-difluoro-3,7-dimethyl-2(E),6(E),10-undecatrienoate (35) was prepared by the modified Wittig reaction of Naae and Burton (1973). The ylide was generated by reacting one equivalent of dibromodifluoromethane (39) with two equivalents of tris-dimethylaminophosphine (40). The first equivalent of 40 reacts with 39 to form the phosphonium salt and the second equivalent removes a Br^+ to create the reactive phosphorus ylide which subsequently

reacts with aldehyde 36 to give 35.



36 35

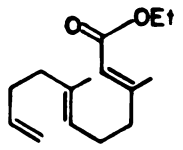
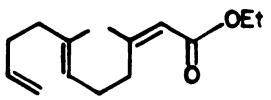
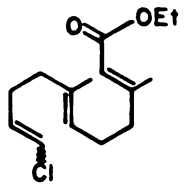
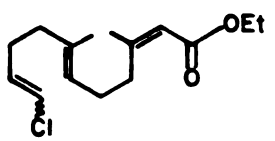
All of the prepared analogs (31-35) were characterized by normal spectroscopic and analytical techniques.

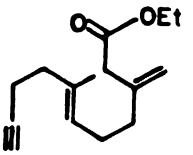
3.6. BIOLOGICAL RESULTS

The Zoecon corporation performed *in vitro* and *in vivo* assays with each of the synthetic analogs (31-35) to determine if they had anti-juvenile hormone activity (the procedures are described in Section 5.2.3.). The first *in vitro* assay involves removing the intact corpora allatum and testing each of the analogs for its ability to inhibit the biosynthesis of juvenile hormone in the gland. A good anti-juvenile hormone response in this assay would be 100% inhibition at a 10^{-7}M concentration of the inhibitor. Table V shows that all of the analogs inhibit juvenile hormone synthesis, to varying degrees, at a 10^{-4}M concentration. The most potent analog was 33, which gave 100% inhibition at a concentration of 10^{-4}M and 40% inhibition at 10^{-5}M. In an

Table V

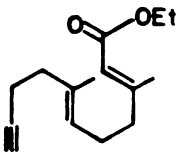
Effects of different compounds on Manduca sexta corpora allata activity *in vitro*, both in the absence and presence of farnesoic acid.

COMPOUND (10^{-4}M)	%INHIBITION OF JH BIOSYNTHESIS	
	w/o farnesoic acid	w/$2X10^{-5}$M farnesoic acid
 31a	94	53
 31b	91	6
 32a	68	68
 32b	74	34



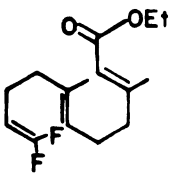
34

100 --



33

100(*) 100



35

100 --

(*) This analog also gave 40% inhibition at 10^{-5}M concentration.

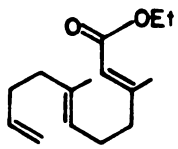
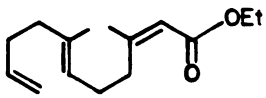
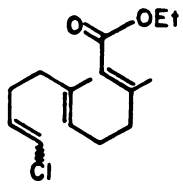
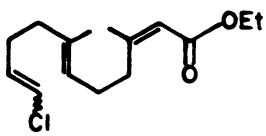
attempt to more precisely determine if inhibition was occurring at the epoxidation or methylation steps, a second *in vitro* assay was run. In this assay, the analog was coincubated with the intermediate, farnesoic acid, and the amount of inhibition of juvenile hormone synthesis again measured. A decrease of inhibition can result if the analog and farnesoic acid compete for the same site, either the methylation or epoxidation steps. Table V reveals that partial or no decrease of the inhibition occurred. Analog 31b, whose inhibition decreased from 91% to 6%, appears to be the only significant competitive inhibitor of either of the two steps.

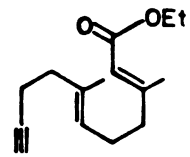
Anti-juvenile hormone activity in the *in vivo* assay is observed as two types of effects. First, intense black pigmentation, and second, precocious metamorphosis marked by nonviable miniature pupae or intermediates between larvae and pupae with a varying mosaic of larval and pupal cuticle. As revealed by Table VI, only analog 32a had detectable anti-juvenile hormone activity, observed as a very slight black pigmentation. Some of the analogs ellicited a toxic response resulting in larval mortality, but otherwise the analogs produced no response at the concentrations tested.

The analogs were also tested as *in vitro* destructive agents of rat hepatic cytochrome P-450 isozymes from phenobarbital pretreated rats (the procedures are described in Section 5.2.2). The functionalities incorporated into

Table VI

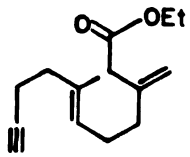
Effects of different compounds on Manduca sexta *in vivo* activity. Unless noted, no response was observed at the given dosage.

COMPOUND	LD_{50}		
	Assay 41(ug/III)	Assay 108(ppm)	Injection(*)
 31a	>250	>100	--
 31b	250(toxic)	>100	--
 32a	>250(**)	>100(**)	--
 32b	>250	>100	--



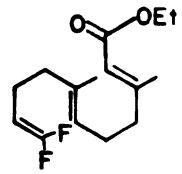
33

250(toxic) 24(toxic) 50%(toxic)



34

>250 >100 14%(toxic)



35

190(toxic) 12(toxic) 21%(toxic)

(*) Injections were made in DMSO solutions at the rate of 10ug/insect in the dorsal neck region directly posterior to the head capsule of 24hr post-molt III instar larvae. Insects were then reared and scored as per Assay 41.

(**) Very slight black -- indication of very minor degree of anti-JH activity.

our analogs had previously been shown to cause inactivation of cytochrome P-450 (20%-50% enzyme loss). It was important, however, to test whether these functionalities retained their activity when attached to the molecules in question. In addition, this assay provides one initial estimate of mammalian toxicity. The results of these studies are presented in Table VII. The analogs were found to destroy the enzyme, the loss ranging from 8-19%, with the most effective being the vinyl chloride 32b. The precocenes, which are known to be toxic to several species of insects, exhibited no destruction of cytochrome P-450 in either phenobarbital or 3-methylcholanthrene induced microsomes at a nominal 10mM concentration. Allylisopropylacetamide (AIA) was run as a standard and gave its usual 31% loss of the enzyme.

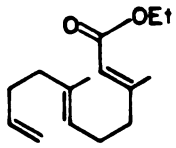
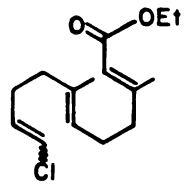
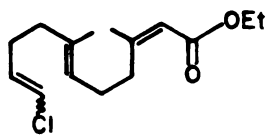
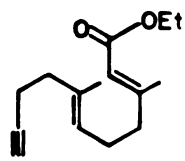
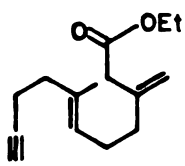
3.7. DISCUSSION

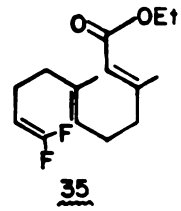
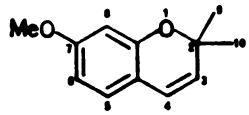
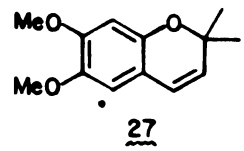
Our aim in this study was to determine if juvenile hormone epoxidase could be inactivated by analogs designed to inhibit a cytochrome P-450 mediated oxidation. This would provide a potential route for the design of specific anti-juvenile hormone analogs.

The in vitro assay results indicate that all of the compounds inhibited juvenile hormone biosynthesis by the corpora allata. Since for some analogs, and especially 31b, the percent of inhibition decreased upon coincubation with

Table VII

Destruction of cytochrome P-450 on incubation of substrates with hepatic microsomes from phenobarbital-pretreated rats.

SUBSTRATES	NOMINAL CONCENTRATION	PERCENT LOSS OF CYTOCHROME P-450		
		10 min	20 min	30 min
 31a	2.0mM	(*) 5.7	7.5	9.4
 32a	2.0mM	4.3±2.4	12.5±3.1	14.0±2.9
 32b	2.0mM	10.8±3.4	15.4±4.4	19.0±5.5
 33	2.0mM	6.5±2.4	13.0±5.0	10.2±1.7
 34	2.0mM	2.8±1.1	7.5±1.6	8.0±1.1

 35	2.0mM	10.3±1.0	17.4±3.7	12.9±4.6
 26	10.0mM	--	--	0(**)
 27	10.0mM	--	--	0(**)
Allylisopropyl acetamide	10.0mM	--	--	31.3±2.1

* tested only once.

** same results from 3-methylcholanthrene induced microsomes.

franesoic acid, it appears as if they are at least in part competitive inhibitors of the epoxidation (or methylation) step and therefore possibly anti-juvenoids. Two of the analogs, 32a and 33, gave the same amount of inhibition with or without coincubation with farmesoic acid. Possible explanations for this last result are: (1) inhibition of the juvenile hormone biosynthetic machinery is occurring at some other biosynthetic or regulatory point along the pathway such that farnesoic acid is not able to circumvent the blocked step, or (2) the binding of these analogs is so much better than that of farnesoic acid that the 5 fold lower concentration of farnesoic acid does not allow conpetitive inhibition to be detectable, or (3) the analogs produce a general cytotoxic effect.

In the *in vivo* studies only analog 32a gave an anti-juvenile hormone response. Its *in vitro* inhibition was not affected by coincubation with farnesoic acid, however, suggesting that the anti-juvenile hormone activity is due to a noncompetitive or irreversible mechanism or due to mechanisms anatagonistic to the release or uptake of juvenile hormone. The *in vivo* results for the other analogs indicated that they were either ineffective (no response) at the tested doses or that they illicited a toxic response (mortality). The interpretation of these results requires consideration of the fact that in the *in vivo* assay, where the intact insect is under observation, the analogs are subject to alternative metabolic degradative pathways. Hydrolysis

of the ester functionality is a major pathway for these types of compounds. It is signigicant that many of the most potent juvenile hormone analogs have either ester functions which are difficult to hydrolyze or a non-hydrolyzable group in place of the ester (Jacobsen et al., 1971). Thus hydrolysis of the ester to a polar excretable metabolite could decrease the potency of the analogs. In addition to the juvenile hormone epoxidase, cytochrome P-450 enzymes associated with xenobiotic metabolism are known to be found in the fat body, Malpighian tubules, and midgut of insects (Agosin, 1976; Hodgson and Tate, 1976). These enzymes may be responsible for not only metabolism to polar, excretable metabolites but also for the formation of a reactive intermediate responsible for the observed toxic in vivo responses. Thus, overall, the analogs may not be sufficiently resistant to other metabolic pathways to be effective as in vivo anti-juvenoids.

In both the in vitro and the in vivo assays general cytotoxic responses to the analogs have been implicated. One likely possibility is that the prescribed functionalities of these analogs, which previously have been shown to be bioactivated to toxic intermediates by cytochrome P-450 enzymes (see Section 3.3), are, in fact, undergoing metabolic transformation to the epoxide. As with vinyl chloride (Pessayre, 1979; Guengerich et al., 1981), the toxic epoxide would then be sufficiently stable to migrate and alkylate cellular tissues.

The assays for destruction of rat hepatic cytochrome P-450 show that each of the analogs causes some destruction even though the precocenes gave no destruction in our assay. However, Precocene II has recently been shown to be hepatotoxic in rats (Hsia et al., 1981). Their results strongly imply the involvement of a highly reactive 3,4 epoxide as a result of metabolic activation by cytochrome P-450 enzymes. This observation parallels the insect studies with the precocenes (Section 3.2). Thus, in our assay, the precocenes do not inactivate the enzyme but nevertheless are probably converted to the epoxide.

In conclusion, the results do not permit a definitive statement about inhibition of the juvenile hormone epoxidase. Rather they suggest that more sensitive and directed *in vitro* assay procedures need to be developed.

CHAPTER FOUR

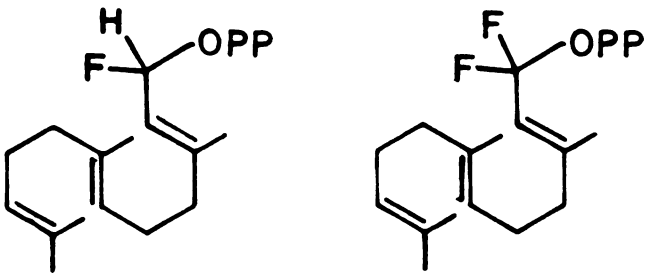
THE SYNTHESIS OF α-FLUOROALKYL ESTERS: POTENTIAL MECHANISTIC PROBES OF SQUALENE SYNTHETASE, ESTERASES, AND PHOSPHATASES

4.1. INTRODUCTION

The general interest in fluorinated organic compounds for medicinal and biochemical applications has grown immensely. In recent years many important advances have been made by substituting the fluorine atom on intermediates of various metabolic pathways. The studies of these fluoro substituted analogs have focused upon their potential as metabolic inhibitors, as substrates in enzymatic reactions, and as probes of protein structure and conformation. For example, fluorinated compounds have been succcessfully employed as antibacterials (Kollonitsch and Barash, 1976), as antiinflammatory agents (Toscano et al., 1977; Vitali et al., 1977), as antiarrythmics (Christy et al., 1977), as anesthetics (Larsen, 1969; Denson et al., 1976), as insecticides (Brown et al., 1975), as prochiral and chiral probes in enzymatic reactions (Goldstein et al., 1978), and as probes for nuclear magnetic resonance studies of macromolecules (Dwek, 1972).

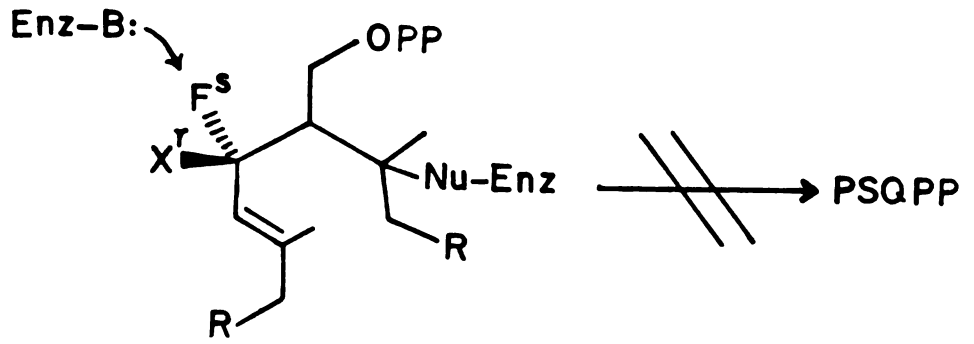
Our initial interest in fluorinated molecules stems

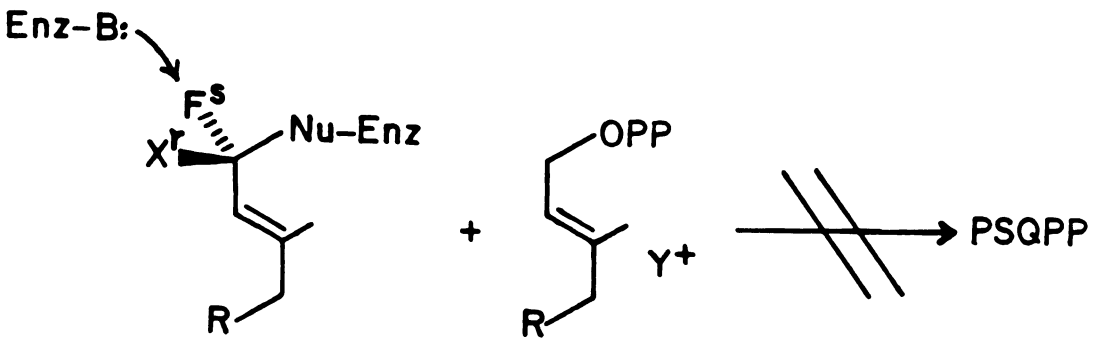
from the possibility that analogs 41 and 42 could serve as irreversible inhibitors and/or as mechanistic probes of the enzyme, squalene synthetase.



41 42

The rationale for suggesting these two analogs as potential inhibitors and probes is based on the proposed mechanisms of the enzyme discussed in Chapter Two. First, since the pro-S hydrogen at the C-1 carbon of the farnesyl pyrophosphate which binds at the first catalytic site is stereoselectively removed subsequent to formation of an enzyme-substrate complex, substitution of this hydrogen with a fluorine could be expected to foil the enzyme because the fluorine could not be removed in the same manner. The result would be a covalently bound intermediate and irreversible inhibition. Figure 8 (see Chapter Two) illustrates the postulated enzyme mechanisms for presqualene pyrophosphate formation and Figure 21 shows the proposed intermediates of these mechanisms which would be thwarted from further reaction by the fluorine substituent. Theoretically, a racemic mixture of analog 41 would be 50% as effective as 42 since the racemate with the pro-R fluorine could still be accepted as a substrate. However, although the replacement of hydrogens with





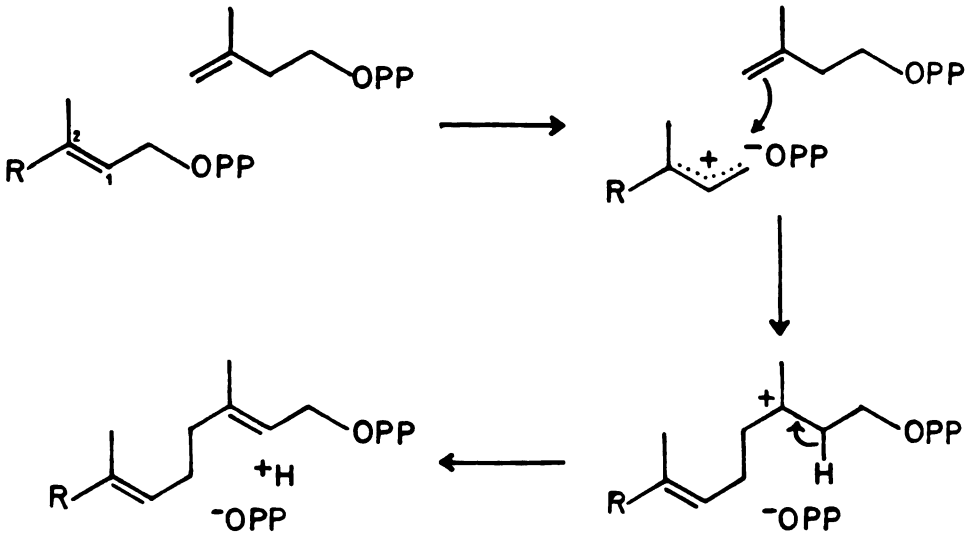
X = H 41

F 42

FIGURE 21: Inhibition of squalene biosynthesis by fluoro-substituted intermediates (also, see Figure 8).

fluorine should not cause large steric perturbations due to the small size of fluorine (1.35A vs. 1.2A for hydrogen), the electronegative nature of fluorine could result in an unfavorable electrostatic binding interaction. This interaction, which might be enhanced by two fluorines, could reduce the binding of 42 prior to formation of the covalent intermediate and make it less effective than 41 as an inhibitor. A second stage in the mechanism which could be altered by a fluorine substituent is the loss of inorganic pyrophosphate that occurs during each half of the reaction. The first pyrophosphate is lost as the two molecules of farnesyl pyrophosphate condense (head-to-head) to form presqualene pyrophosphate. How the enzyme manipulates these substrates to promote eventual loss of pyrophosphate is unknown. Poulter and Rilling (1978) have performed mechanistic studies on a similar enzyme, prenyl transferase, which catalyzes the head-to-tail coupling of isopentenyl pyrophosphate. Two mechanisms had been proposed for this enzymatic reaction: (1) ionization-condensation-elimination and (2) condensation-elimination (see Figure 22). To distinguish between the two mechanisms these workers compared the chemical and enzymatic behavior of the normal allylic substrate to that of analogs in which the hydrogen atoms about the 2,3-double bond had been selectively replaced with fluorine. Their hypothesis was that if carbon-oxygen bond cleavage was rate-determining, the inductive electron withdrawing properties of fluorine would retard an ionization

IONIZATION-CONDENSATION-ELIMINATION



CONDENSATION-ELIMINATION

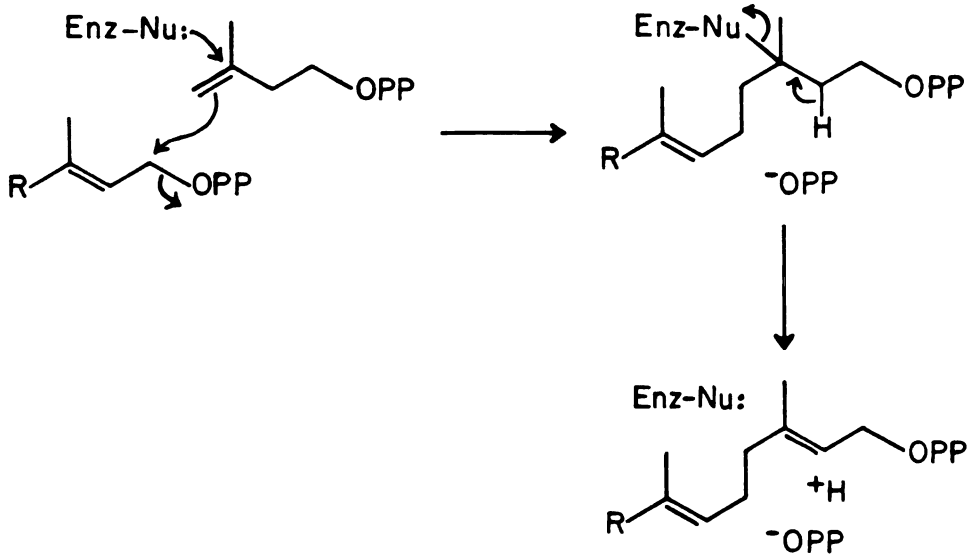
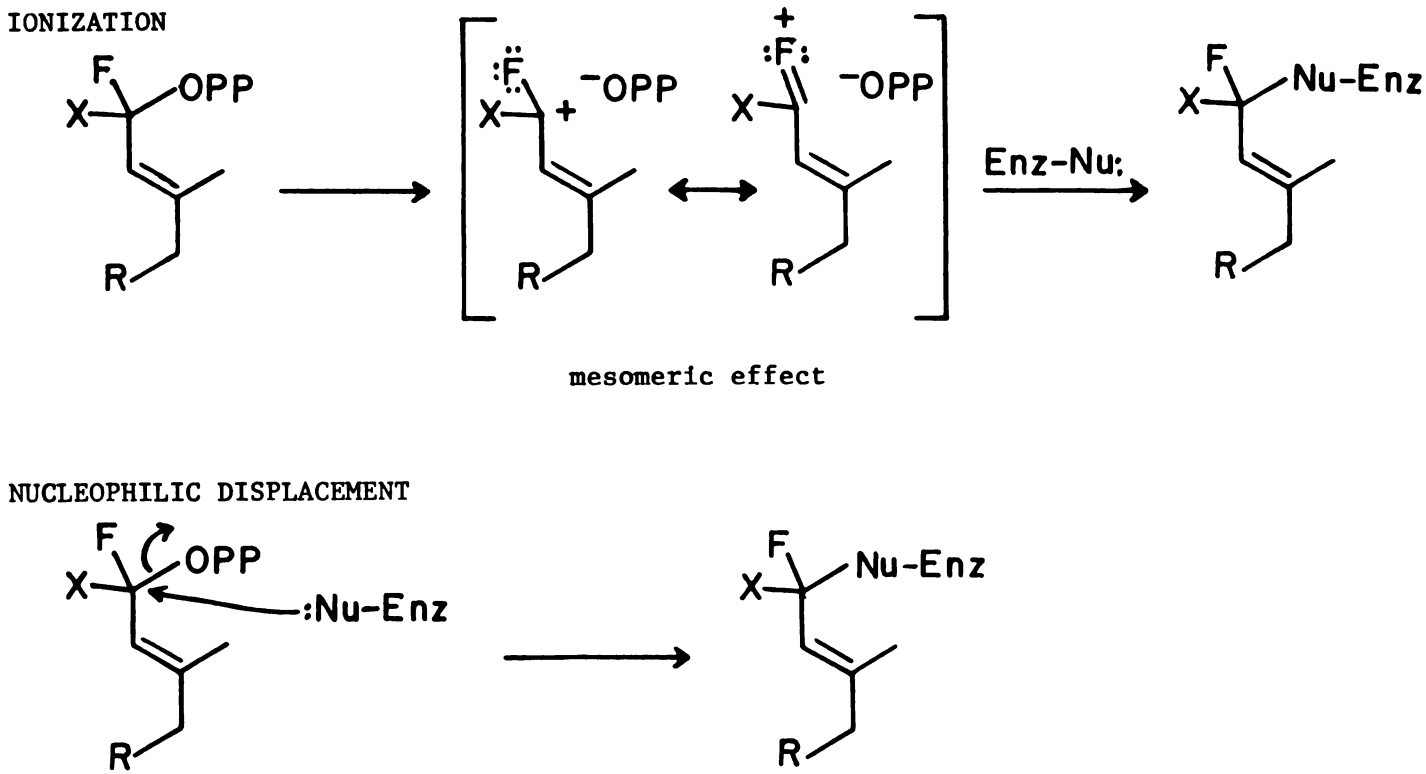


FIGURE 22: Postulated mechanisms of the enzyme, prenyl transferase.

step (destabilize the carbonium ion) but would not greatly affect a nucleophilic displacement of the pyrophosphate. Their results showed that the reaction was greatly retarded by fluorine substitution and thus supported an ionization-condensation-elimination mechanism. Since prenyl transferase and squalene synthetase utilize structurally similar substrates, it is conceivable that there are also common mechanistic features. This is an intriguing possibility which could be partially explored with analogs 41 and 42. If the pyrophosphate is lost by ionization (SN1), as in prenyl transferase, then the mesomeric (electron releasing, p-π interaction) effect of fluorine at the C-1 position could stabilize the carbonium ion (Martin et al., 1966; Baird and Datta, 1971; Olah et al., 1972; Modena and Scorrano, 1973) and might increase the rate of reaction (see Figure 23), assuming carbon-oxygen bond cleavage is rate-determining (Hine and Rosscup, 1960). On the other hand, if the pyrophosphate is displaced (SN2) by an enzymic nucleophile, then the low polarizability and high electron density of the C-1 fluorine(s) could retard the approach of the nucleophile (see Figure 23). Fluorine could also affect the loss of the second pyrophosphate from presqualene pyrophosphate through similar interactions. However, since this step is considered to be SN1, it would be expected that the fluorine(s) would have a stabilizing effect.

Our second interest in fluorinated molecules was the applicability of α-fluoroalkyl esters as potential



X = H (41)

F (42)

FIGURE 23: Postulated mechanisms for the loss of inorganic pyrophosphate in the first half reaction of squalene biosynthesis.

inhibitors of phosphatases and esterases, enzymes which hydrolyze phosphorus and carbon esters respectively. Figure 24 shows how normal enzymatic hydrolysis of an α-fluoroalkyl ester 43

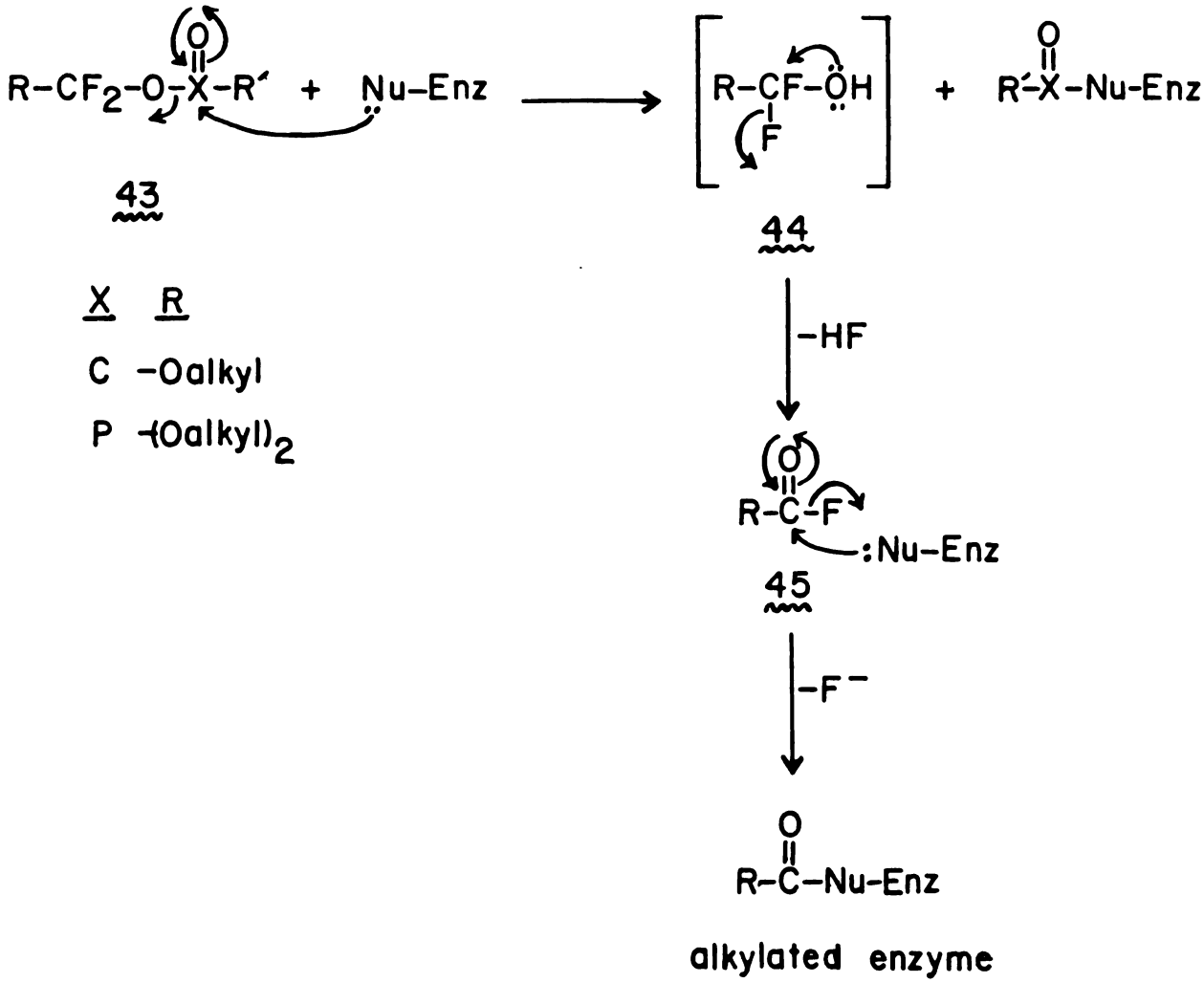


FIGURE 24: Proposed mechanism of enzyme alkylation by alpha-fluoroalkyl esters.

produces an α-fluorocarbinol 44 which would rapidly lose hydrofluoric acid (HF) (see Section 4.2.1) to form the reactive acyl fluoride 45, which in turn could react with an enzymic nucleophile to alkylate and irreversibly inhibit the

enzyme. The chemical and biological reactivity of acyl fluorides as reactive acylating agents has been established (Patai, 1972).

The lack of synthetic methodology for the preparation of α-fluoroalkyl esters has precluded their application to specific biomedical problems such as those just described. The remainder of this chapter will discuss the development in our laboratory of new preparative methods for these types of compounds. The actual utilization of the methodology in biological reasearch, however, is left for future investigations.

4.2. SYNTHETIC STUDIES

4.2.1. Synthetic strategy

α-Monofluorinated or difluorinated alcohols are highly unstable compounds which rapidly lose hydrofluoric acid to give the corresponding carbonyl products (Haszeldine, 1951; Lovelace et al., 1958; Seppelt, 1977; Kloter and Seppelt, 1979) (Figure 25).

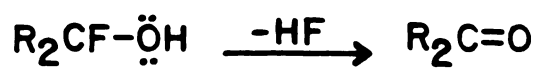
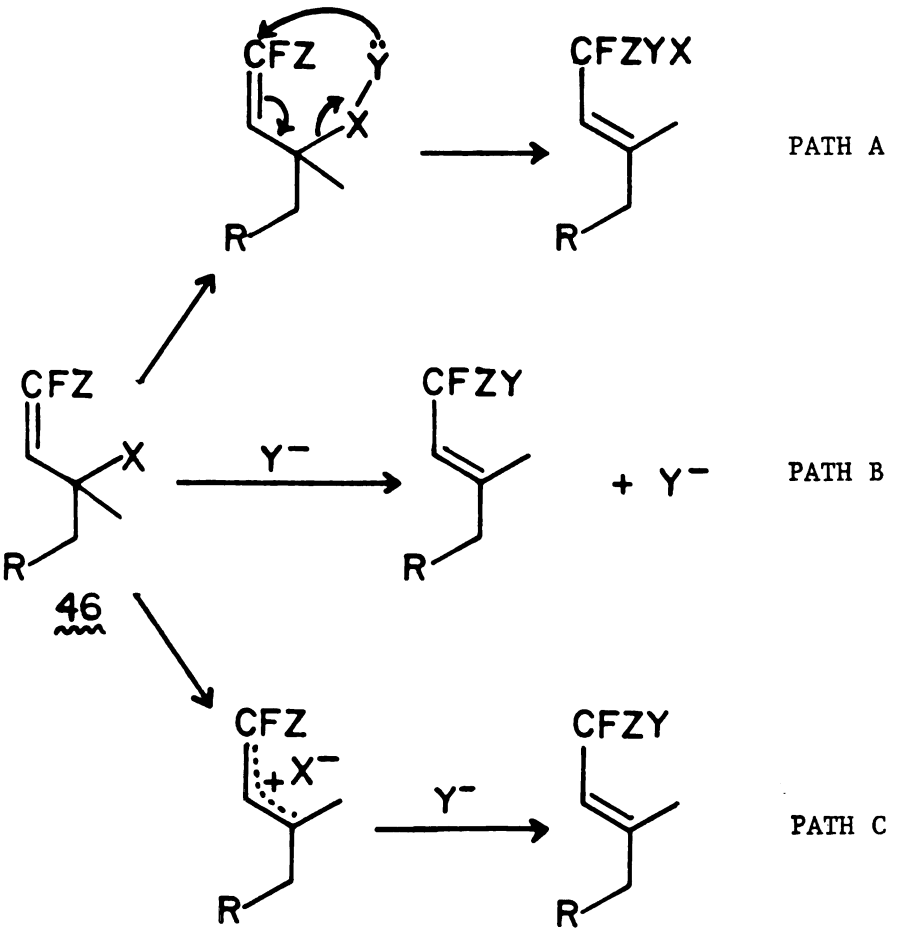


FIGURE 25: Hydrofluoric acid elimination from from α-fluoroalcohols

This instability has prevented the synthesis of

α-fluoroalkyl esters by conventional approaches such as acylation and phosphorylation. Therefore, our approach, as diagrammed in Figure 26, has been to prepare an intermediate (46) that could undergo either: (1) an allylic transposition of functionality (Path A), or (2) a nucleophilic displacement reaction under SN2 or SN2' conditions (Path B), or (3) a nuclephilic displacement reaction with ionic intermediates (Path C).



Z = H, F

X = OR′, halogen

FIGURE 26: Strategies for the synthesis of α-fluoroesters

Examples of these general transformations in non-fluorinated systems are described in the literature (Braude, 1950; Babler and Olsen, 1974; Babler, 1976; Oelberg and Schiavelli, 1977; Overman et al., 1978). Since no synthetic methodology was available for the preparation of the desired pivotal fluoro intermediates (46), our initial interest was to investigate synthetic techniques which could be successfully utilized for their preparation.

4.2.2. Synthesis of gamma difluorovinyl alcohols

The methodology for the synthesis of the gamma difluorovinyl alcohol 48, the precursor for a key intermediate, was worked out in our laboratory by W. A. Vinson and has been previously communicated (Ortiz de Montellano and Vinson, 1979). Figure 27 reviews the synthetic procedures beginning with ketone 47. To further extend the applicability of our newly developed synthetic methodology we examined other compounds for their ability to undergo these reactions. Using the described procedures, ketones 49, 50, and 51 were successfully employed to give their corresponding difluoro products, 52[4], 53[4], and 54.

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(4) Prepared by B. Spahic, 1981.

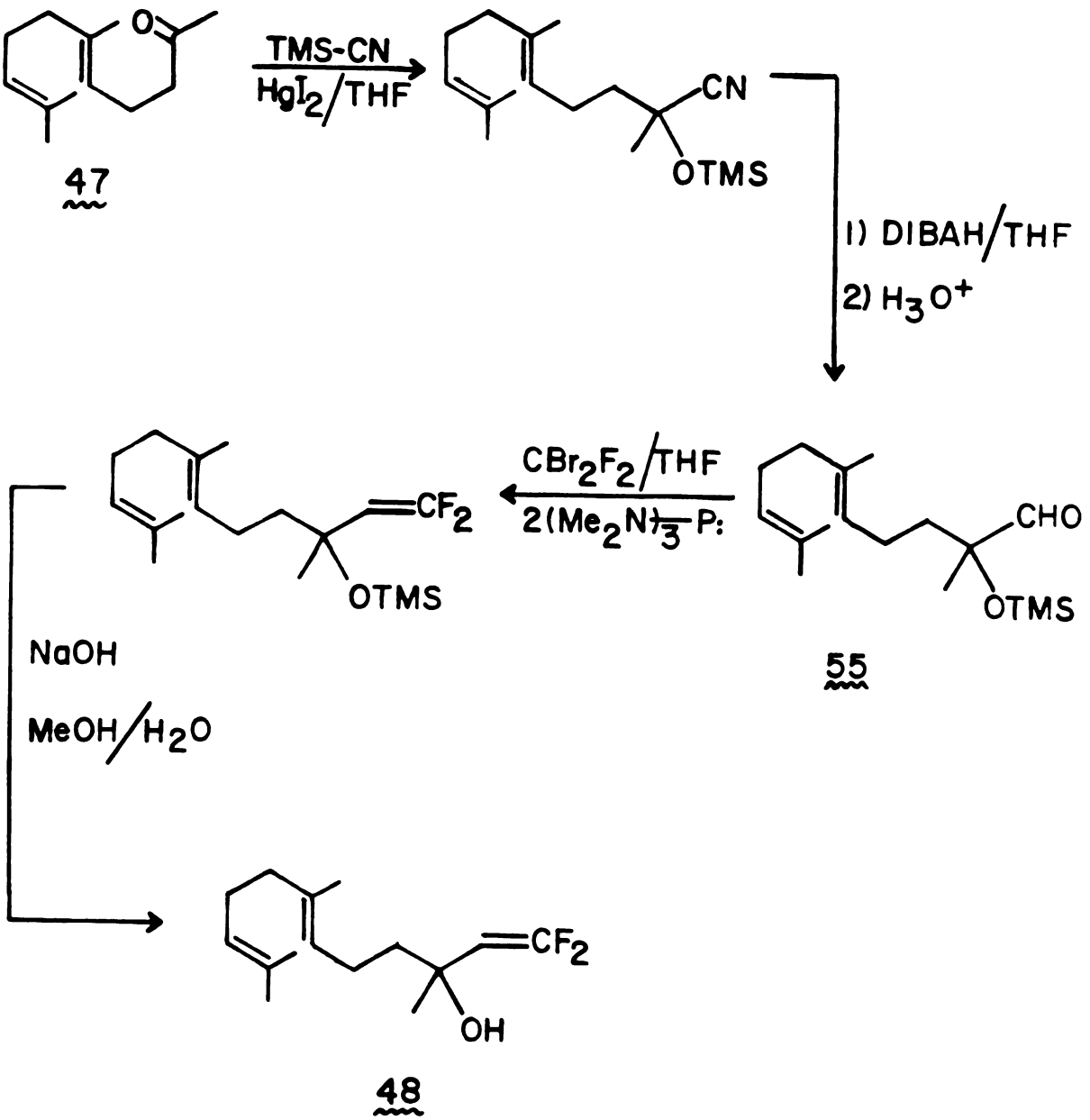
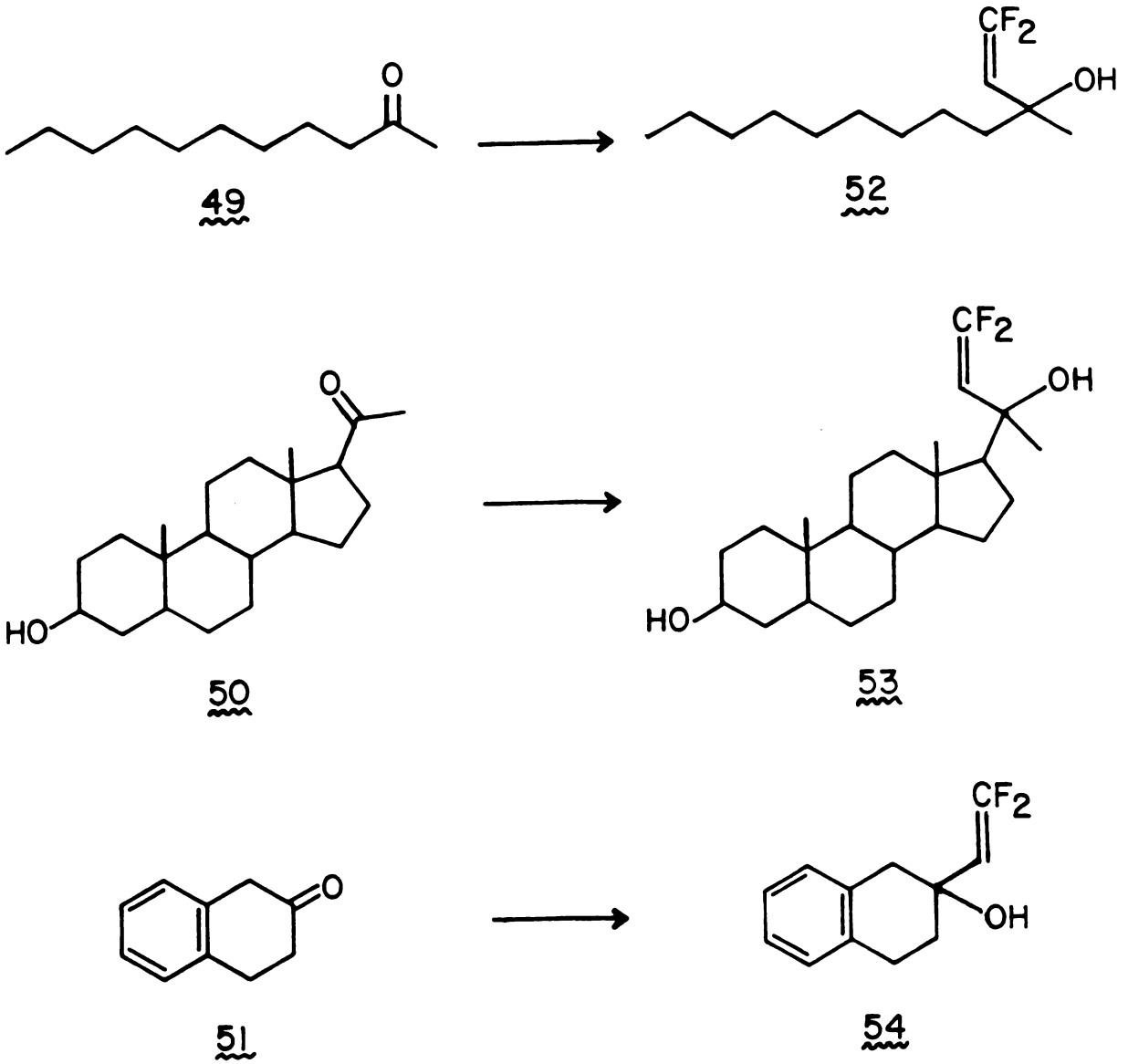
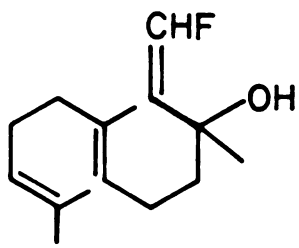


FIGURE 27: Synthesis of γ-difluorovinyl alcohols.



4.2.3. Synthesis of gamma monofluorovinyl alcohols

The synthesis of our second precursor, the gamma monofluorovinyl alcohol 56, was attempted first by the



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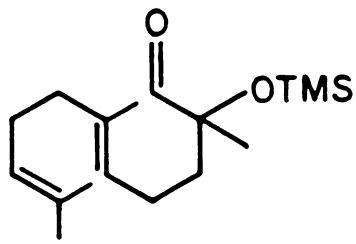
method of Burton and Greenlimb (1975) in which an aldehyde is combined with fluoroiodomethyltriphenylphosphonium iodide and zinc-copper couple in DMF to yield the fluoroolefin (Figure 28).

FIGURE 28: Method of Burton and Greenlimb (1975).

However, this approach failed to produce satisfactory yields with aldehyde 55,



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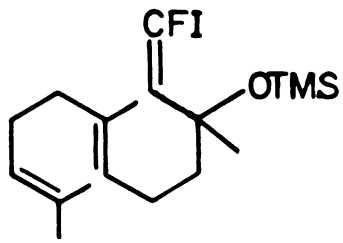
even after the TMS group was removed to alleviate steric hindrance. An attempt to improve the reaction by changing

the solvent from DMF to THF resulted in the formation of 57 in 64% yield as a mixture of cis and trans isomers (Vinson, 1978).



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An alternate approach became available at this time, however, with the publication by Normant and coworkers (Sauvetre et al., 1978; Masure et al., 1978) of a paper which demonstrated that the lithium salts of trifluorovinyl carbinols could be conveniently reduced to their difluoro analogs using lithium aluminum hydride (LAH) (Figure 29).

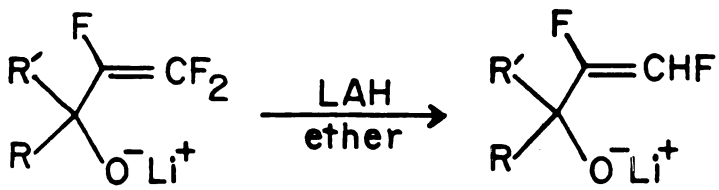
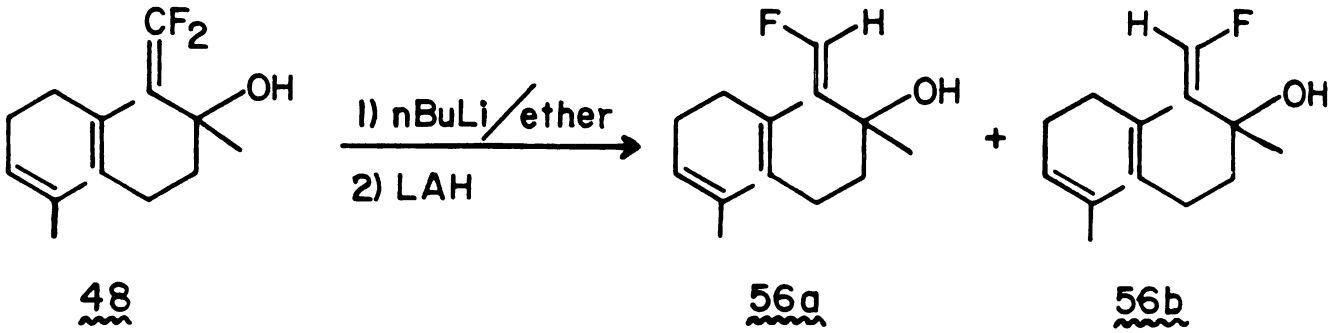


FIGURE 29: Method of Normant and coworkers (Sauvetre et al., 1978).

Application of the procedure to our difluorovinyl alcohol 48 did, in fact, provide the cis and trans monofluorovinyl alcohols 56a and 56b in 90% combined yield and in a 9:1

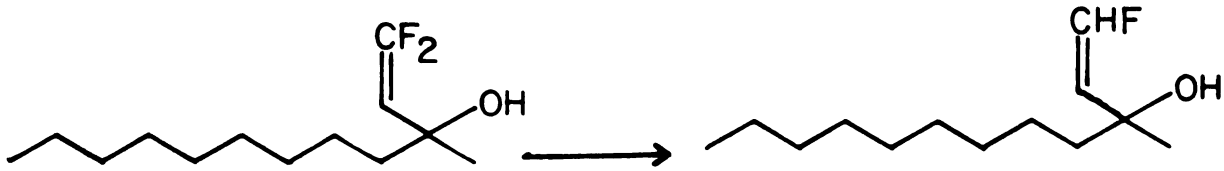
ratio, repectively. The proton NMR spectrum of 56a showed a doublet of doublet (J=21 and 10Hz) for the C-1' vinyl proton due to cis coupling to fluorine and trans coupling to the C-2' vinyl proton. The C-2' vinyl proton of 56a also showed a doublet of doublets (J=85 and 10Hz) due to geminal coupling to the fluorine and trans coupling to the C-1' vinyl proton. The fluorine NMR spectrum of 56a, in agreement with the proton NMR data, showed a doublet of doublets with J=85 and 21Hz. The proton NMR spectrum of 56b showed a doublet of doublets (J = 47 and 5Hz) for the C-1' vinyl proton due to trans coupling to fluorine and cis coupling to the C-2' vinyl proton. The C-2' vinyl proton of 56b showed a doublet of doublets (J=85 and 5Hz) due to geminal coupling to the fluorine and cis coupling to the C-1' vinyl proton. The fluorine NMR spectrum of 56b showed a doublet of doublets with J=85 and 47Hz. In addition, infared analysis of both, 56a and 56b, showed a strong band at 1670 cm^{-1} characteristic of the fluorovinyl group.



48 56a 56b

Again, in an effort to apply this methodology to other

compounds, the difluorovinyl alcohols 52, 53, and 54 were similarly reduced to give their corresponding monofluoro products 58[5], 59[5], and 60 in the same cis and trans ratios.



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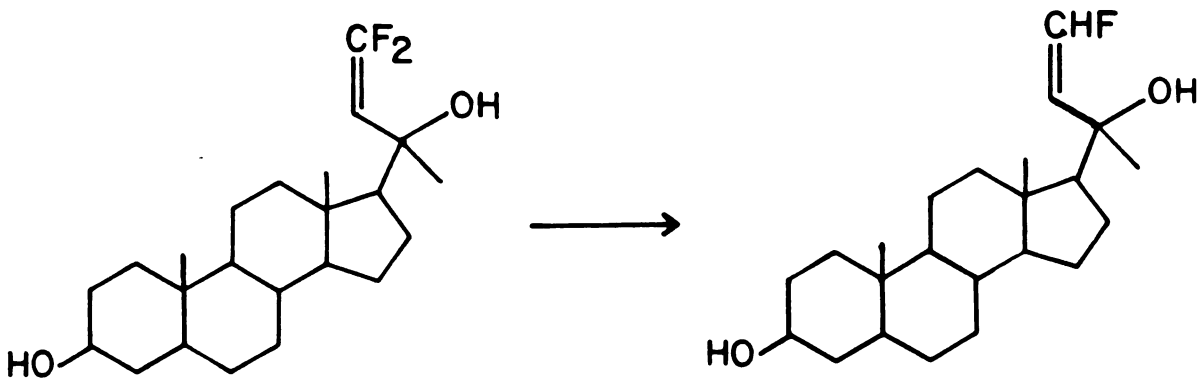

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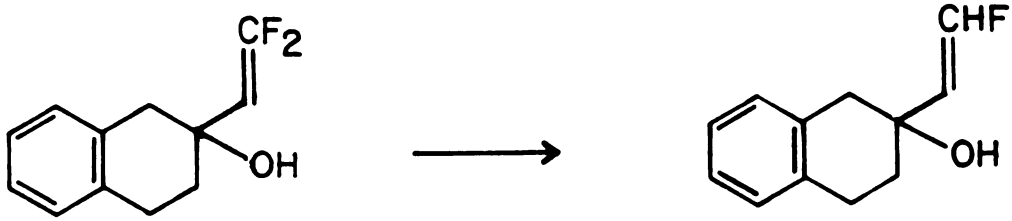


54 60

Since the mechanism of the reductions of the difluorovinyl alcohol 48 to the monofluorovinyl alcohols 56a and 56b was unknown, it was explored. Lithium aluminum hydride

(5) Prepared by B. Spahic, 1981.

(LAH) normally does not react with isolated carbon-carbon double bonds. However, studies with activated [6] allylic alcohols have shown that LAH is able to completely reduce the double bond in these substrates. Labeling experiments (Borden, 1970; Wong and Gray, 1978; Gammill et al., 1980) using combinations of a $LiAlH_4$ or $LiAlD_4$ reduction with a H_2O or D_2O workup, have demonstrated that hydride transfer occurs stereoselectively at C-2 (with an activating group at C-3), via intramolecular delivery from an alkoxyaluminum hydride salt (Gammill et al., 1980). The proton from water is then introduced during workup at the C-3 position. These results are illustrated in Figure 30.

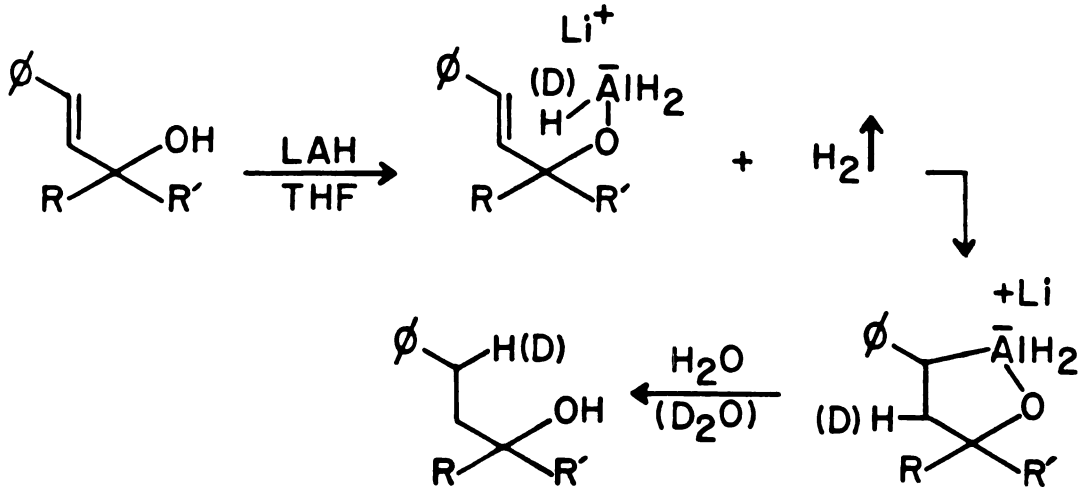


FIGURE 30: Lithium aluminum hydride (LAH) reduction of an allylic alcohol.

(6) Allyl alcohol itself is not readily reduced by LAH (Hochstin and Brown, 1948.)

However, with our difluorovinyl alcohol 48, we did not observe a saturation of the double bond but rather a nucleophilic displacement of fluorine by hydride. To explore this difference, we also carried out a series of reductions with $LiAlH_4$ or $LiAlD_4$ followed by workup in H_2O or D_2O. Our results, presented in Figure 31,

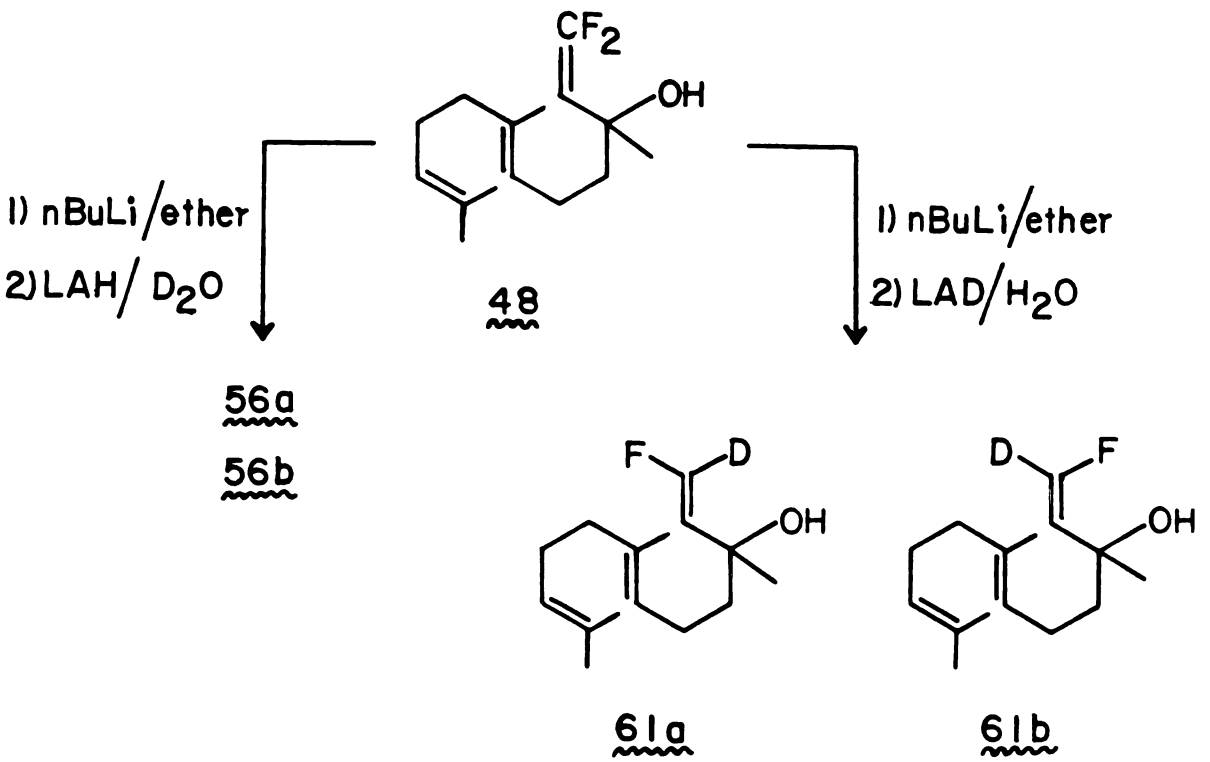


FIGURE 31: Reduction studies with difluoro vinyl alcohol 48.

show that the hydride adds to the C-3 (not C-2) position and that either no stable carbon-aluminum bond forms, since no proton from the aqueous workup is incorporated, or if formed, that it undergoes elimination rather than displace-

ment during the workup. The proton and fluorine NMR chemical shifts and coupling constants of 61a clearly establish its identity. In addition, we found that without n-butyllithium the reaction proceeded much more slowly and side products appeared. Therefore, consistent with all of the experimental evidence, we proposed the mechanism as seen in Figure 32.

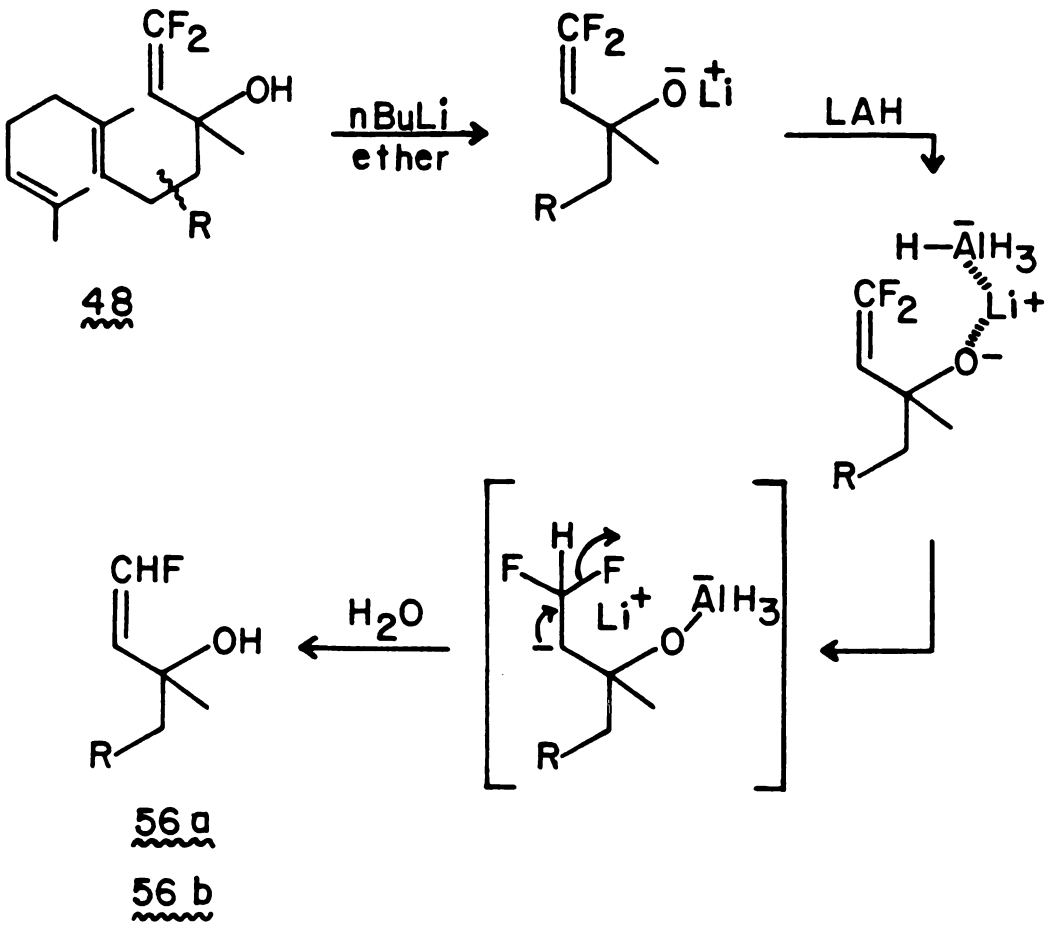


FIGURE 32: Proposed mechanism for LAH reduction of difluorovinyl alcohols.

This mechanism is also compatible with reports from the literature which reveal that (1) strong electron withdrawal

by vinyl fluorine activates the double bond to nucleophilic attack (Chambers and Mobbs, 1965), (2) the largest induced positive charge on the fluorinated double bond is on the carbon bearing the two fluorines (Chambers and Mobbs, 1965), and (3) a β-fluorine stabilizes a carbanion more than an α-fluorine (Andreades, 1964) or α-hydrogen (Feast et al., 1966; Modena and Scorrano, 1973).

The stereochemistry of nucleophilic vinylic substitution depends on the configuration of the substrates and the route involved (Rappoport, 1969). Therefore, to explain the observed 9:1 ratio of trans and cis isomers, it is necessary to examine the various conformers of each of the steps. Figure 33 presents the possibilities which are based on three assumptions: (1) the hydride attacks perpendicularly to the plane of the molecule giving a tetrahedral arrangement around the α and 3 carbons, (2) inversion of the carbanion is rapid, and (3) elimination of the leaving group occurs trans to the carbanion. Since two fluorines exist at the terminal vinyl position, it is difficult to differentiate between retention and inversion of configuration. However, by looking at the effects of the steric and electronic interactions it may be possible to predict the dominant trans isomer. For the trans isomer, both of the rotations (60^{o} and 120^{o}) which occur subsequent to elimination, arrange the groups such that a hydrogen rather than a fluorine is between the carbanion and the negatively charged aluminum complex. In addition, upon elimination of fluoride

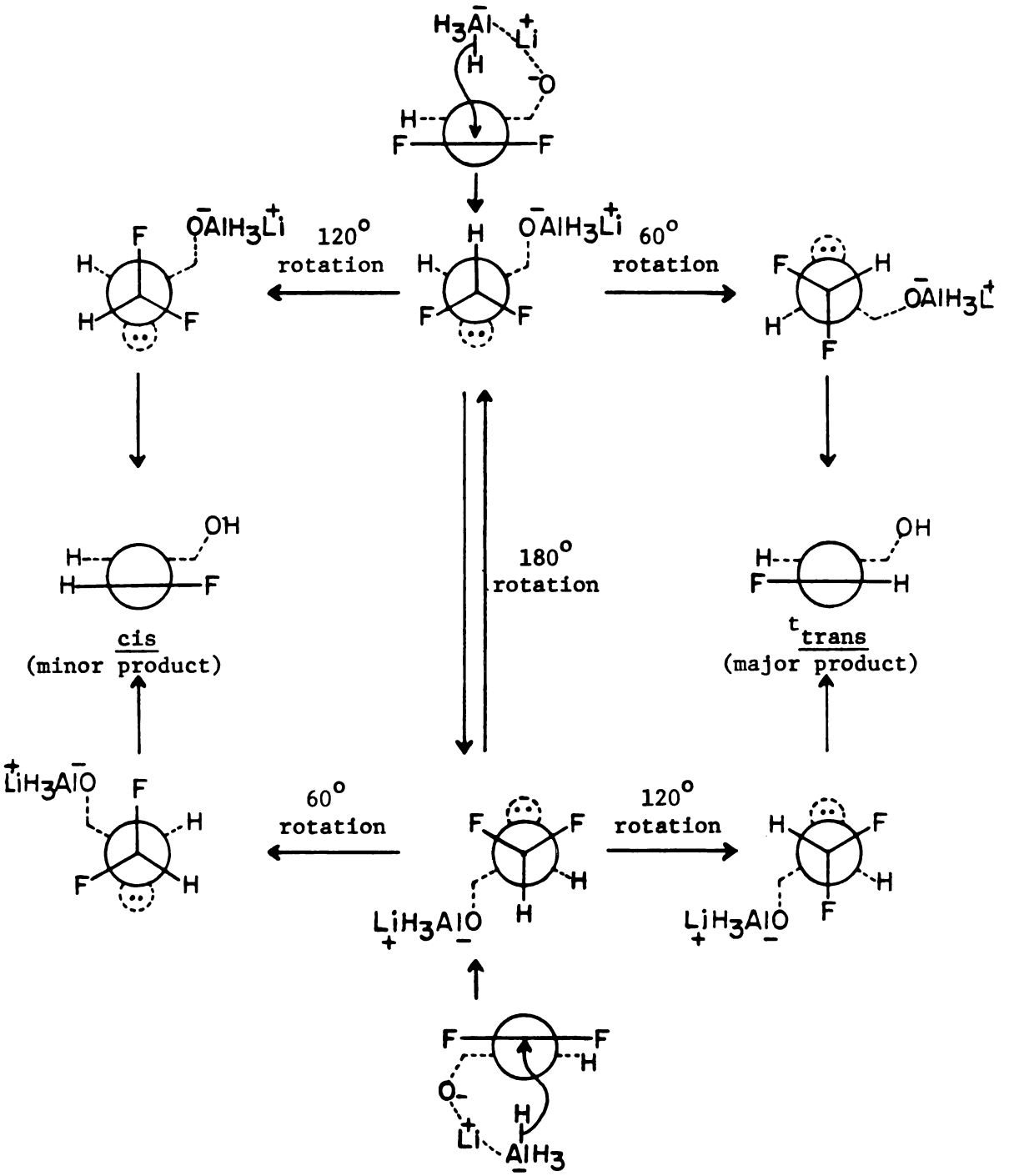


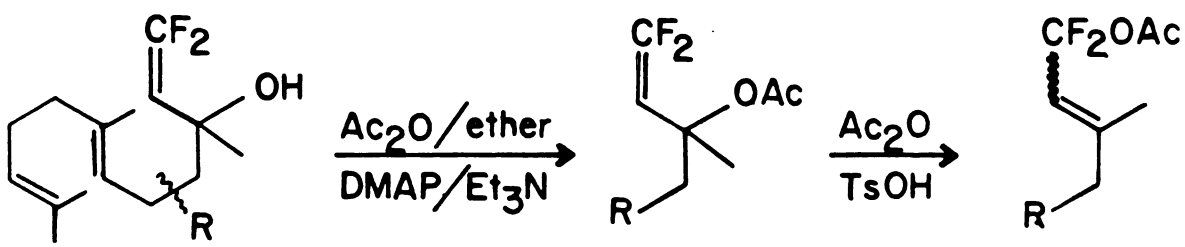
FIGURE 33: Proposed conformers of the intermediate carbanions formed by nucleophilic attack of the LAH complex on difluorovinyl alcohols.

ion and reformation of the double bond, the trans isomer represents the more favorable conformation since it has the least amount of steric and electronic interference. These two effects must in fact be important since in the method of Normant and coworkers (Figure 29) if the C-1' hydrogen is either a fluorine (Sauvetre et al., 1978) or a chlorine (Masure et al., 1978) the isomeric ratio of products is more evenly distributed.

4.2.4. Synthesis of difluoro and monofluoro alkyl esters

Now that both the difluoro and monofluoro precursors to the pivotal intermediate 46 (Figure 26) were prepared, we were ready to attempt the synthesis of the desired carboxylic and phosphate esters of α-fluoro alcohols.

Our initial synthetic objective was to prepare the primary acetate derivative 63. Using a newly developed procedure for the esterification of hindered alcohols (Hofle et

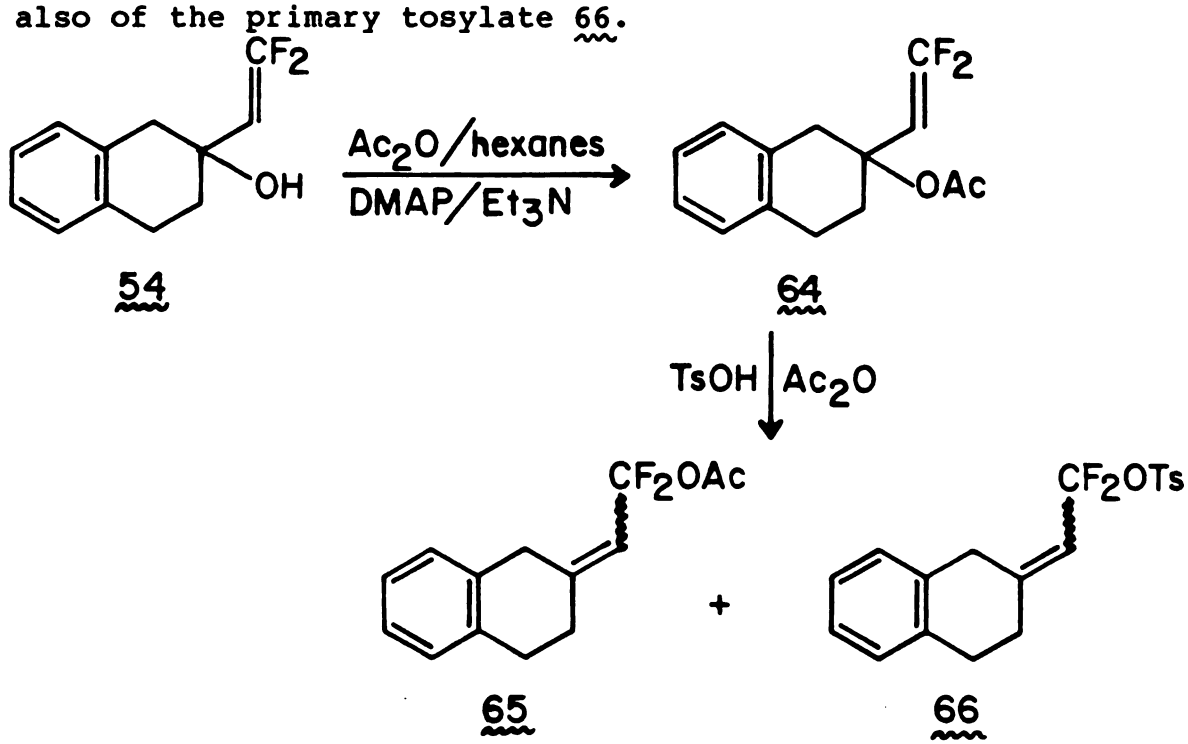


48 62 63

al., 1978; Hassner et al., 1978), alcohol 48 was reacted with acetic anhydride, triethylamine, and 4-

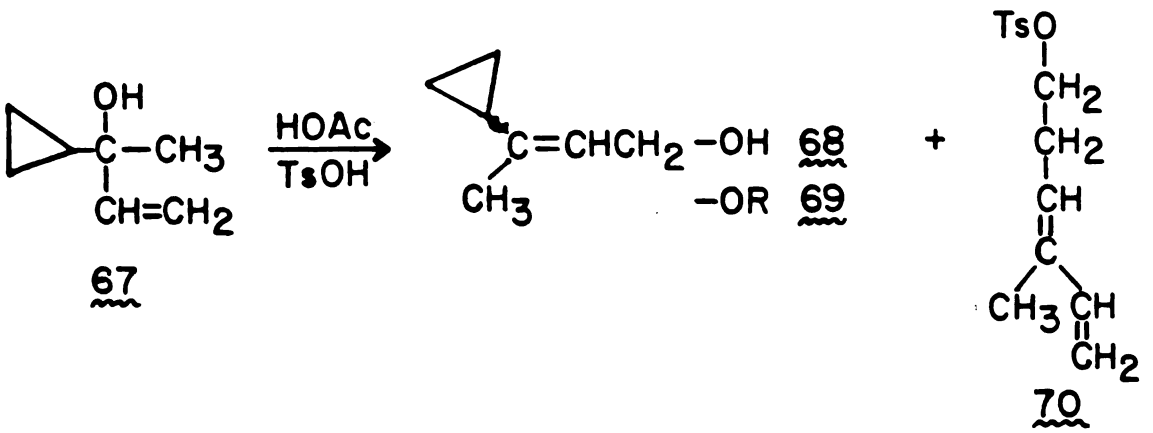
dimethylaminopyridine in ether to afford the tertiary acetate 62 in 98% isolate yield (Vinson, 1978). Although it has been suggested (Hassner et al., 1978) that the reaction proceeds faster in nonpolar solvents, we found in our reactions that the use of either ether or hexanes did not result in a significant difference.

Reaction of the tertiary acetate 62 in acetic anhydride with a catalytic amount of p-toluenesulfonic acid resulted in rearrangement to the primary acetate 63 (75% yield) (Babler and Olsen, 1974; Babler et al., 1977; Ortiz de Montellano and Vinson, 1979). These procedures were subsequently carried out with alcohol 54 and tertiary acetate 64 was prepared. However, because a catalytic amount of p-toluenesulfonic acid was insufficient to permit complete rearrangement of 64 to the primary acetate 65, it was necessary to introduce an additional quantity of the acid. This resulted in formation not only of the primary acetate 65 but also of the primary tosylate 66.



54 64 65 66

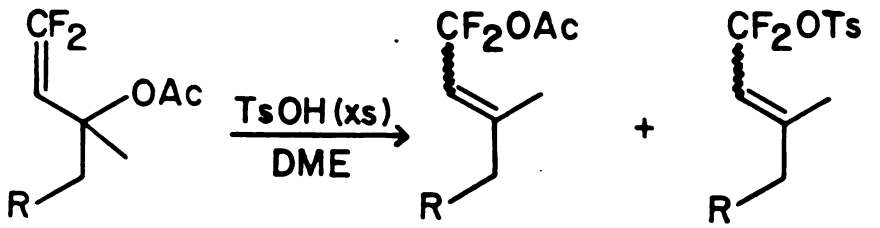
Products 65 and 66 were isolated in 50% combined yield in a 3 : 1 ratio, respectively. Babler and coworkers (1977), in their investigations of the solvolysis of 2-cyclopropyl-3-buten-2-ol 67 (0.02M solution) in acetic acid catalyzed by p-toluenesulfonic acid (0.0025M), obtained a 25 : 1 mixture of the rearranged primary allylic alcohol 68 and its acetate derivative 69 in 68% yield, as well as the ring-opened tosylate 70 in 8% yield.



67 70

If they increased the amount of p-toluenesulfonic acid they observed an increase in the yield of the tosylate product. Since tosylates are good leaving groups in nucleophilic substitution reactions (R-O cleavage is much more likely than S-O cleavage), derivatives like compound 66 offer an alternative route, through reaction with the appropriate nucleophile, for the preparation of fluorinated alkyl or phosphate esters. Therefore, in an attempt to improve the yield of the primary tosylate products, the reaction was run using a two-fold excess of p-toluenesulfonic acid and 1,2-dimethoxyethane (DME) rather than acetic anhydride as the solvent. The result was an increase in the combined yield

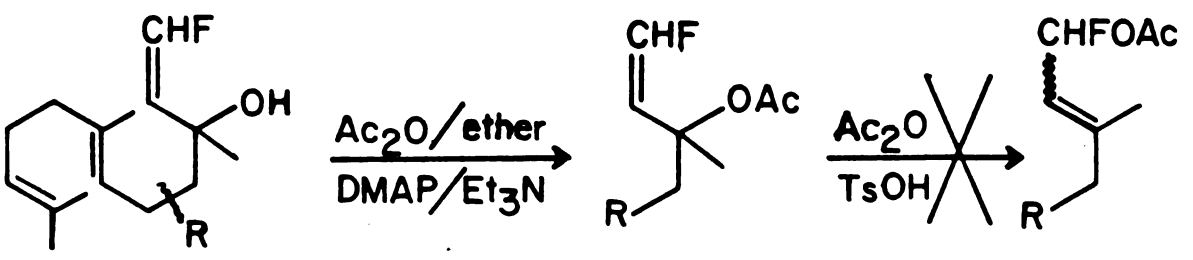
(75%) and an inversion in the ratio of products (1 : 2 of primary acetate to primary tosylate, respectively). When tertiary acetate 62 was allowed to react under these new conditions, the same ratio (1 : 2) of primary acetate 63 to



62 63 71

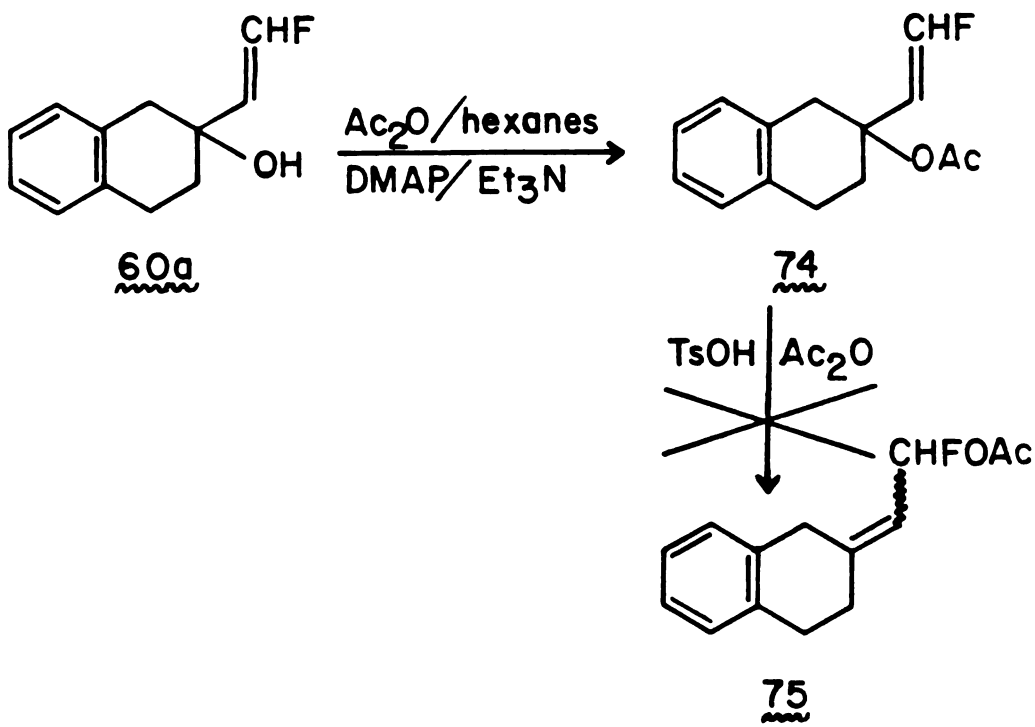
primary tosylate 71 was obtained. The structures assigned to both the pimary acetates and primary tosylates concur with their characteristic proton and fluorine NMR spectra.

We next turned our attention toward the preparation of the monofluoro primary acetate 73. Utilizing the previously described procedures, tertiary acetate 72 was successfully prepared in 99% yield. The subsequent rearrangement step to the primary acetate 73, however, resulted only in a complex mixture of mostly non-polar, low-boiling, uncharacterized products.



56a 72 73

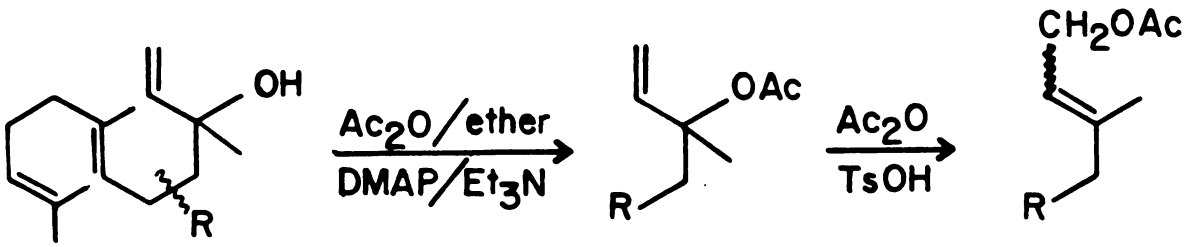
Monofluoroalcohol 60a reacted similarly to give the tertiary



60a 74 75

acetate 74 (75% yield) but not the primary acetate 75. Success with the difluoro compounds and failure with the monofluoro analogs in the rearrangement of the tertiary acetates to the primary acetates prompted us to seek a possible correlation between the ease of reaction and the number of fluorines at the terminal vinyl position. Therefore, using the same methodology, the tertiary acetate (76) of nerolidol, the nonfluorinated analog, was prepared and subjected to the same acetic anhydride/tosic acid conditions.

The primary acetate 77 (identified by comparison with the known compound) was slowly formed but was accompanied by the formation of side products, primarily C_{15} hydrocarbons.



Nerolidol 76 77

The apparent failure of the rearrangement reaction with the monofluoro compounds despite its success with the analogous difluoro and nonfluoro compounds is not easily explained.

To attempt to offer possible explanations for this inconsistent reaction pattern, the mechanism of the reaction and the stability of the products should be evaluated. Acid catalyzed allyl acetate rearrangements are well known in the literature (March, 1977). The acid proton accelerates the SN1 rearrangement of these allylic compounds due to its electrophilic interaction with the acetate. The rate of formation of the intermediate ion pair is increased by electron releasing substituents on either the α or the β carbon atom. This enhancement is probably due to facilitation of the ionization step and stabilization of the resulting carbonium ion by the substituents. The "tightness" of the ion pair intermediate is determined, in part, by the solvating ability of the solvent. Together, these factors control not only the rate of product formation but also the formation of competing side products due to diversion of the intermediate

ion pair along other reaction pathways. A possible mechanism for the rearrangement of the tertiary acetate to the primary acetate, as represented by the difluoro analog 62, is shown in Figure 34.

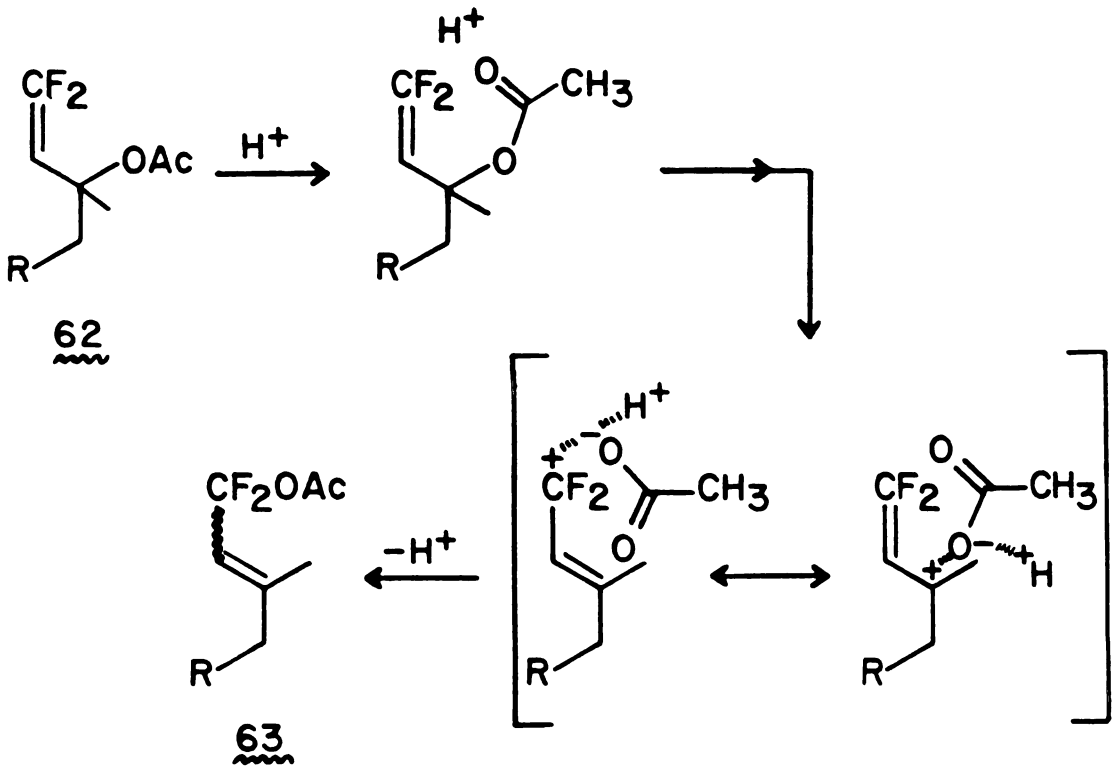
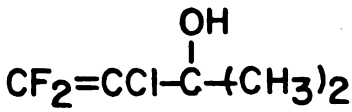


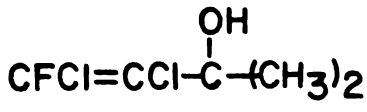
FIGURE 34: Proposed mechanism for rearrangement of tertiary acetate 62 to primary acetate 63.

The primary tosylates are formed by a competing side reaction, in agreement with the finding that their yield is increased by an increased concentration of the p-toluenesulfonic acid catalyst. Babler and coworkers (1977) have also suggested that their reaction (67→68, 69 and 70)

proceeds via an intimate ion pair intermediate which undergoes rapid rearrangement to the observed products. It is known that fluoro olefins with terminal difluoromethylene groups are readily attacked at this position by nucleophiles. However, nucleophilic attack on fluoro olefins with a terminal chlorofluoromethylene group does not occur as easily (Chambers and Mobbs, 1965; Drakesmith et al., 1968). In fact, alcohol 78 has been shown to be stable to rearrangement whereas 79 is not (Drakesmith et al., 1968).



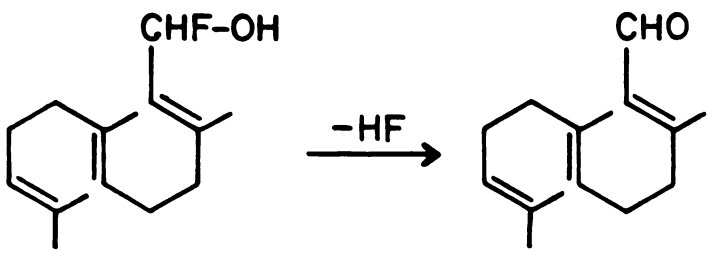
79



78

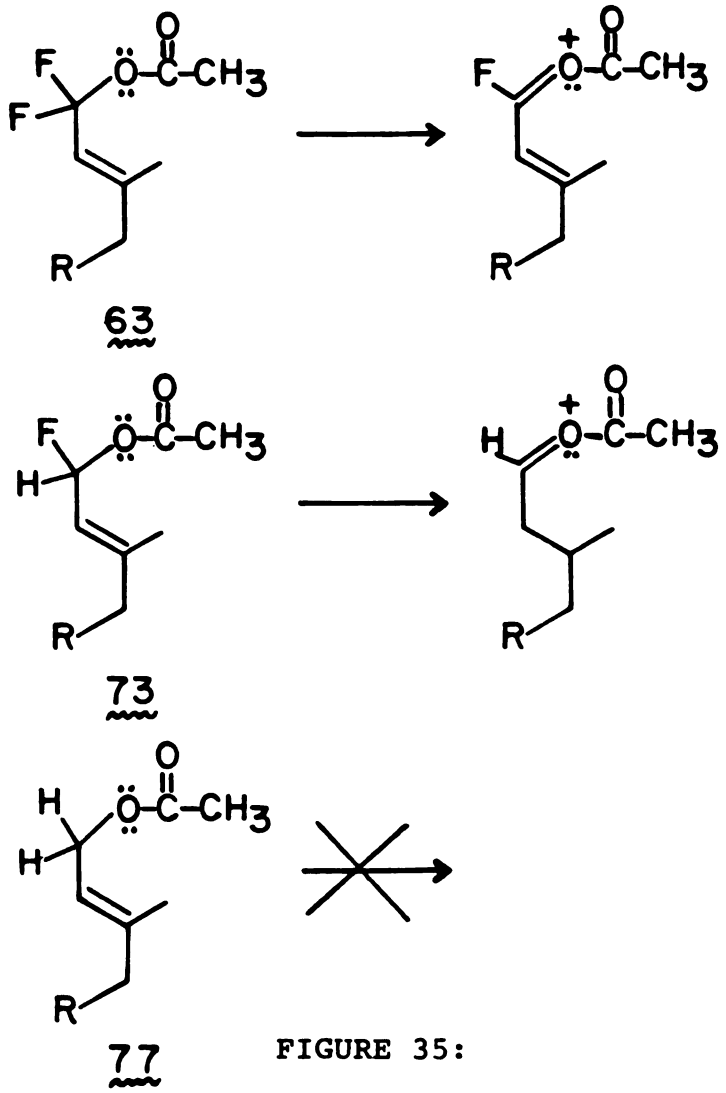
Although these differences in reactivity have been attributed to the less efficient mesomeric electron release by chlorine than by fluorine, the larger size of the chlorine could also have an effect. In our compounds the terminal methylene group contains either two fluorines, one fluorine and one hydrogen, or two hydrogens. Since a fluorine is about the same size as a hydrogen (Table I), steric effects should not be a serious factor, whereas the mesomeric stabilizing influence at this terminal position would be expected to decrease in the order 2F>1H1F>2H. Thus the reactivity of these compounds to SN1 allylic rearrangements would also be expected to decrease in the same order. However, this is not the apparent observation. One possible

explanation is that the presumably less reactive monofluoro compounds may allow side reactions to compete more effectively. One seriously detrimental side reaction is the possible formation of the primary monofluoro alcohol 80, and its subsequent rapid loss of hydrofluoric acid (HF). The acid in turn, could aid in destruction of any primary acetate which may have been formed.



80 81

For the nonfluorinated compounds even though the primary acetate was formed (along with side products), no analogous detrimental side reactions could occur to destroy its presence. An alternate explanation for the observed discrepancy in the formation of the primary acetate products may be due to their inherent stability (or instability). In Figure 35 are represented the three possible primary acetates. The availability of the electron pair on the linked oxygen could be a significant factor in determining if loss of fluoride and subsequent hydrolysis occurs.



63

73

77

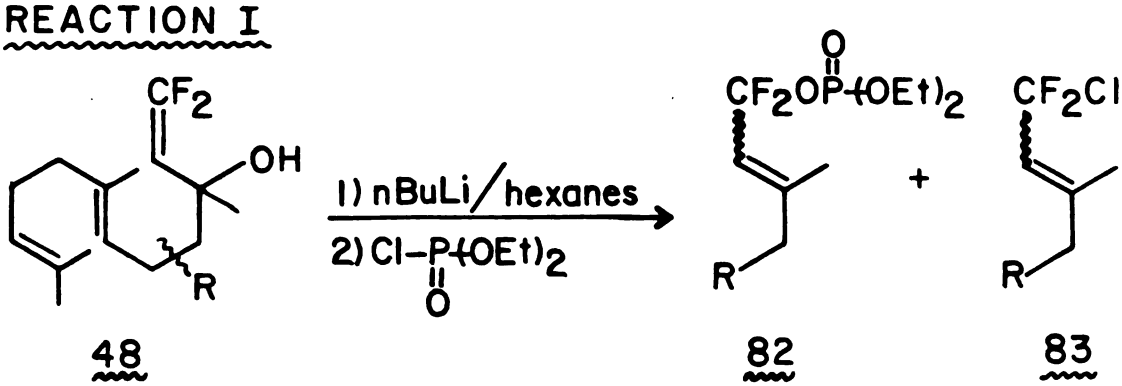
FIGURE 35:

In both 63 and 73 the pi bond conjugation of the carbonyl creates a γ+ charge on the oxygen. On the other side attached to the oxygen is a carbon with either one or two fluorine substituents. The inductive electron withdrawing effect of these fluorines on the C-O sigma bond creates an additional γ+ on the oxygen. The inductive effect of two fluorines versus one fluorine may be a subtle difference which allows 63 to be more stable to decomposition than 73. In contrast, the loss of hydride from 77 is not a likely

possibility so that, even though it is formed more slowly, the product is stable.

The next step in our studies was the attempted preparation of phosphate ester derivatives. The addition of diethyl phosphochloridate to the alkoxide generated from the treatment of difluorovinyl alcohol 48 with n-butyllithium was shown to give the primary phosphate 82 in 30% yield (Vinson, 1978). A major side product of the reaction was the chloro derivative 83.

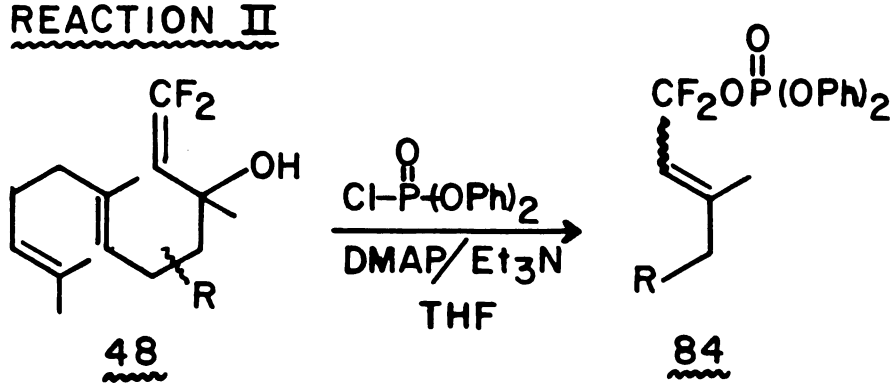
REACTION I



48 82 83

In a later reaction, phosphate ester 84 was prepared in 80% isolated yield from difluorovinyl alcohol 48 using diphenyl phosphochloridate, 4-dimethylaminopyridine (DMAP), and triethylamine in THF (Vinson, 1978).

REACTION II



48 84

Relatively little of the chloro derivative was obtained by this procedure. The 2E and 2Z isomers of 82 and 84 were separable by LOBAR chromatography. Possible improvements in the methodology, extrapolation to the monofluorinated vinyl alcohols, and generalization of the procedures to non-terpenoid systems were undertaken.

Since the conditions of reaction I gave the chloro derivative 83 as a side product, whereas the conditions of rection II did not, the substitution of diethyl phosphochloridate for diphenyl phosphochloridate in reaction II was examined. Unfortunately, the reaction did not proceed at all. If 4-pyrrolidinopyridine, which has been found to be 7-10x more reactive than DMAP as an acylating catalyst (Hassner et al., 1978), is used, the reaction still does not take place. This catalyst also does not give an apparent increase in the rate of reaction II. The effects of several different solvents on reaction I were investigated. Reactions in dry HMPA(7), pentane, benzene, or THF, under otherwise identical conditions, were worked up by a mild procedure to minimize loss of products. Each mixture was then examined by ^{19}F-NMR and the ratio of 82 to 83 was noted. Benzene appeared to be the best solvent for maximization of 82 and minimization of 83, although the overall yield of 82 was still low.

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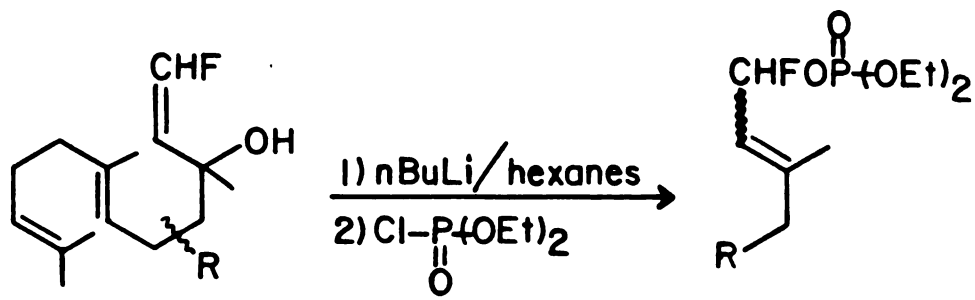
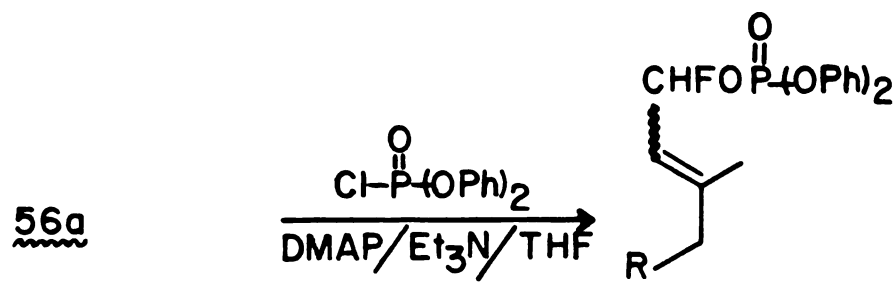
(7) The reaction in this solvent did not proceed at all. This may have been due to reaction of nBuLi with the HMPA (Savignac and Leroux, 1973).

Reactions I and II were attempted with the monofluoro vinyl alcohol 56a. Diethyl phosphate ester 85 was prepared in 15% yield (Vinson, 1978) but reproducibility has proved to be erratic. This may, in part, be due to the low yields and instability of these derivatives.



56a 85

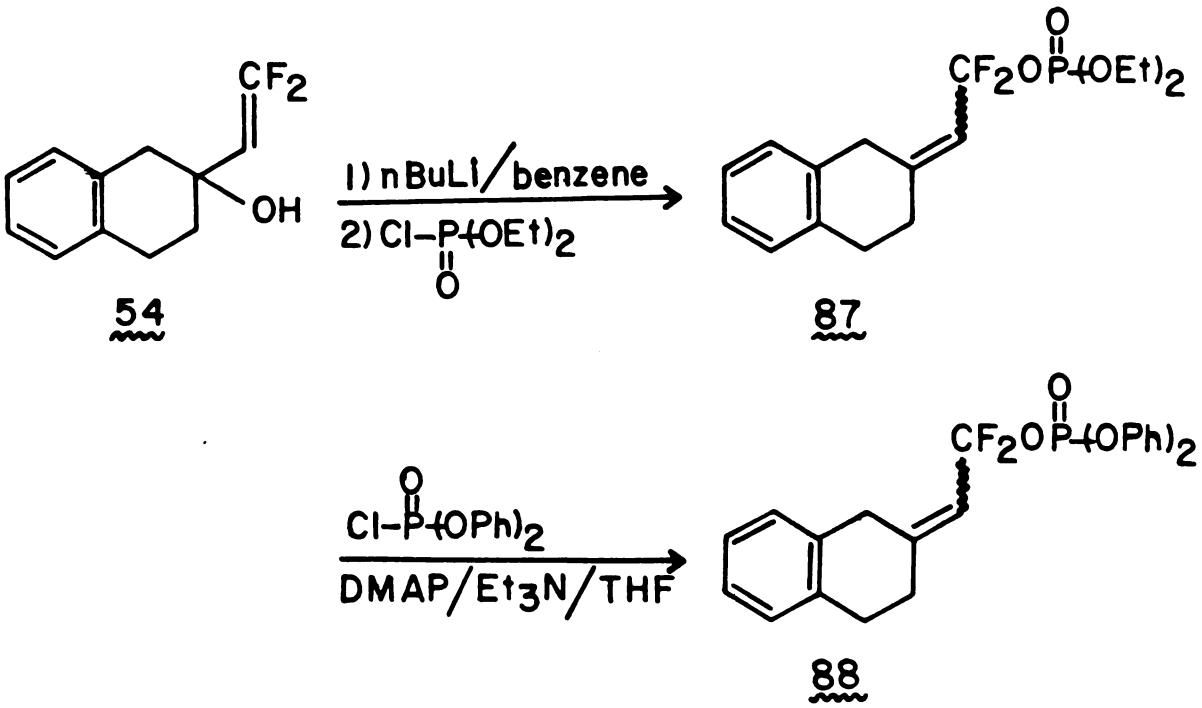
The preparation of the diphenyl phosphate ester 86 gave the product in 30% yield.



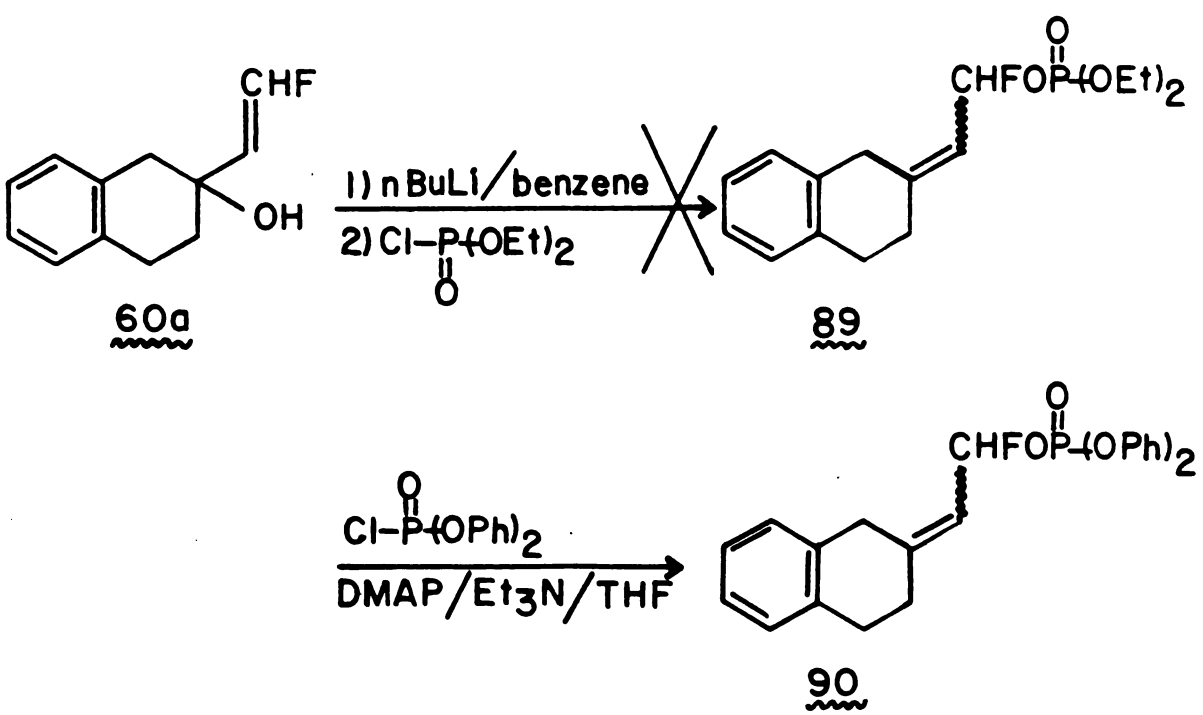
86

The distinctive proton, fluorine, and phosphorus NMR spectra of 86 are discussed in Section 4.3.

The reactions were applied successfully to the difluoro vinyl alcohol 54 to produce phosphate esters 87 and 88.



The monofluoro vinyl alcohol 60a was treated under the conditions of reaction I and II, but only the diphenyl phosphate ester 90 was successfully prepared.



When nerolidol was subjected to these reaction conditions no phosphate ester products were observed.

Possible mechanisms for reactions I and II are shown in Figure 36. Because the rearrangements in these reactions were performed in basic media, an SN1 allylic rearrangement mechanism is not probable. One possible mechanism involves, initial formation of the tertiary ester intermediates 91 and 92 followed by their subsequent concerted rearrangement to the corresponding primary phosphates. The collapse of the pentacoordinate intermediate 91 of reaction I to the chloro derivative 83 may explain not only its formation but also why it is not observed in reaction II. Again, as with the acetates, the differences in reactivity between the difluoro, monofluoro, and nonfluoro compounds may be due to the subtle electronic variations of these terminal methylene groups.

The monofluoro compounds were found to be generally less stable to heat, silica gel purification, and storage as a neat oil than the corresponding difluoro compounds. In some instances, stability could be improved by prewashing the LOBAR columns with a dilute Et_3N/EtOAc solution before purification, and by storing the compounds either as an ether solution or neat over anhydrous potassium carbonate in the refrigerator.

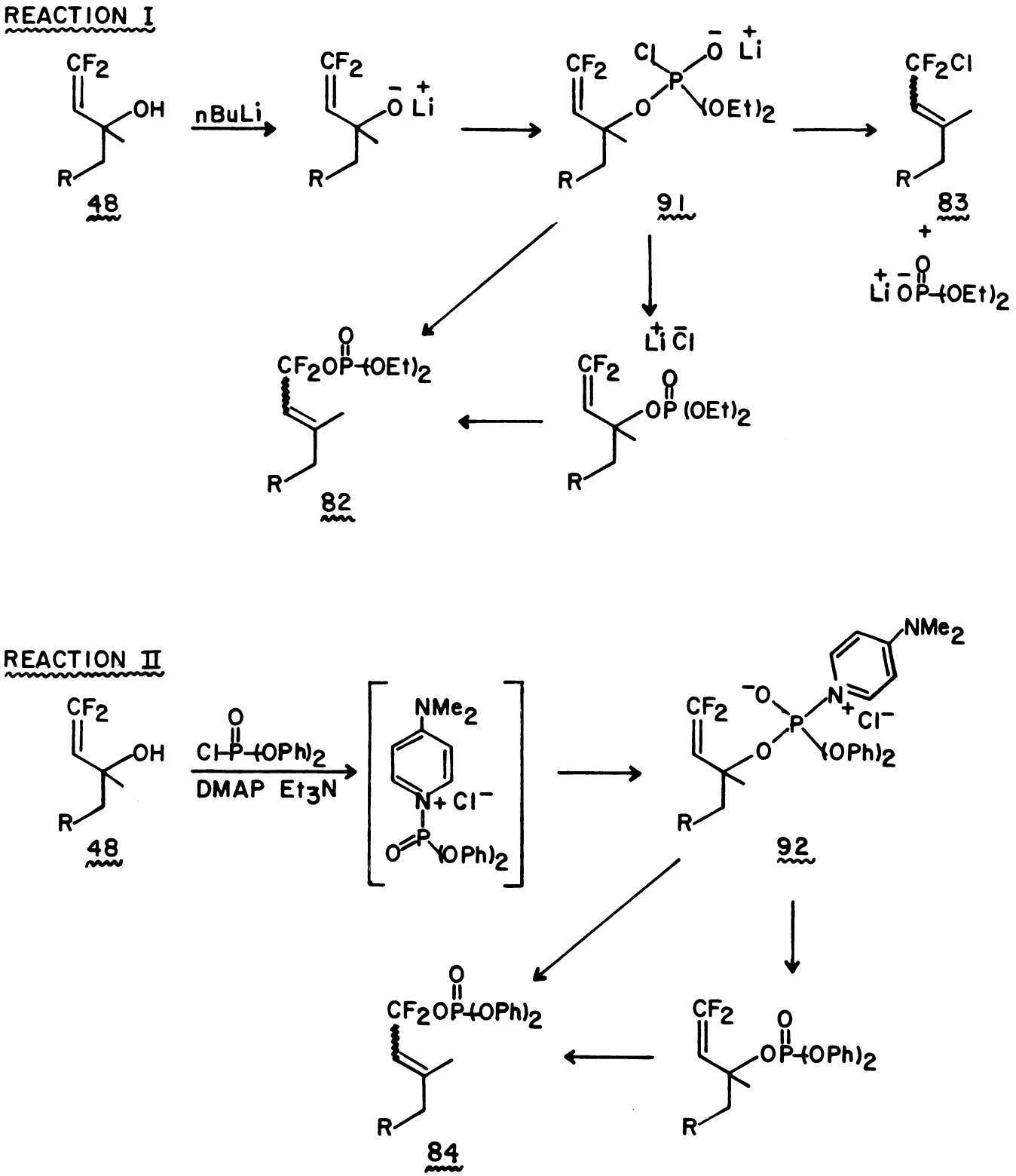


FIGURE 36: Possible mechanisms for reactions I and II.

4.3. PROTON-FLUORINE-PHOSPHORUS NMR OBSERVATIONS[8]

An interesting proton-fluorine-phosphorus coupling phenomena was previously observed in our laboratory with the difrluoro diethyl phosphate ester 82 and the monofluoro diethyl phosphate ester 85 (Vinson, 1978). In addition to normal proton and fluorine coupling, fluorine NMR off-resonance decoupling experiments indicated the presence of F-C-O-P coupling. To further explore and evaluate this observation a series of proton, fluorine, and phosphorus NMR spectra were obtained. The results are summarized in Table VIII.

The chemical shifts and coupling patterns of the difluoro compounds were determined from the difluoro diphenyl phosphate esters 84 and 88. The proton NMR spectra showed a broad triplet at about 5.5ppm (J = 10 Hz) for the C-2 vinyl proton due to coupling with the two C-1 fluorines. The proton coupled fluorine NMR spectra of each iosmer of 84 and 88 gave a multiplet that appeared to be a broad triplet. Proton decoupling reduced these multiplets to two doublets (one for each isomer, J = 6-7 Hz). The proton coupled and decoupled phosphorus NMR spectra shown in Figure 37 reveal a triplet (J = 6-7 Hz), indicative of two equivalent fluorines

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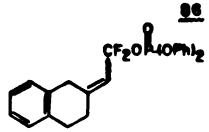
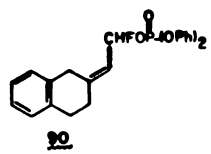
(8) The author wishes to thank the following persons for their technical assistance in obtaining the fluorine and phosphorus NMR spectra: N. Lam, P. Mirau, B. Spahic, K. Kunze, and S. Brown.

TABLE VIII

	^{1}H-NMR		^{19}F-NMR		^{31}P-NMR	
decoupler:	on	off	on	off	on	off
 **84**	———	5.5ppm bt J=10Hz	ϕ 54.2 and 54.7 doublet corresponding to each isomer, J=6-7Hz	*two broad multiplets (~20Hz) (look similar to broad triplet; coupling variable J=6Hz)	sharp triplet J=6Hz	less sharp triplet, J=6Hz
 **86**	doublet of doublets for H_b when irradiate ^{b}Ha J=55 and 7Hz (trans isomer)	6.65ppm doublet of triplets for H_b J=55 and 7Hz ; 5.38ppm bt, H_a J=7Hz	ϕ 112 and 111 two singlets corresponding to each isomer	*two doublet of doublets J=55 and 5-6Hz	for trans isomer singlet	doublet J=7Hz
 **88**	———	5.69ppm bt J=10Hz	ϕ 54.9 and 55.2 doublet corresponding to each isomer, J=7Hz	*two broad multiplets coupling variable, J=8Hz	sharp triplet J=7Hz	less sharp triplet J=7Hz
 **90**	———	6.79ppm doublet of triplets for H_b J=55 and 7 Hz ; 5.58ppm bt, J=8Hz for H_a	ϕ 113.5 amd 113.7 two singlets corresponding to each isomer	*two doublet of doublets J=55 and 5-6 Hz	uneven doublet (two isomers)	uneven doublet

* corresponding to two isomers

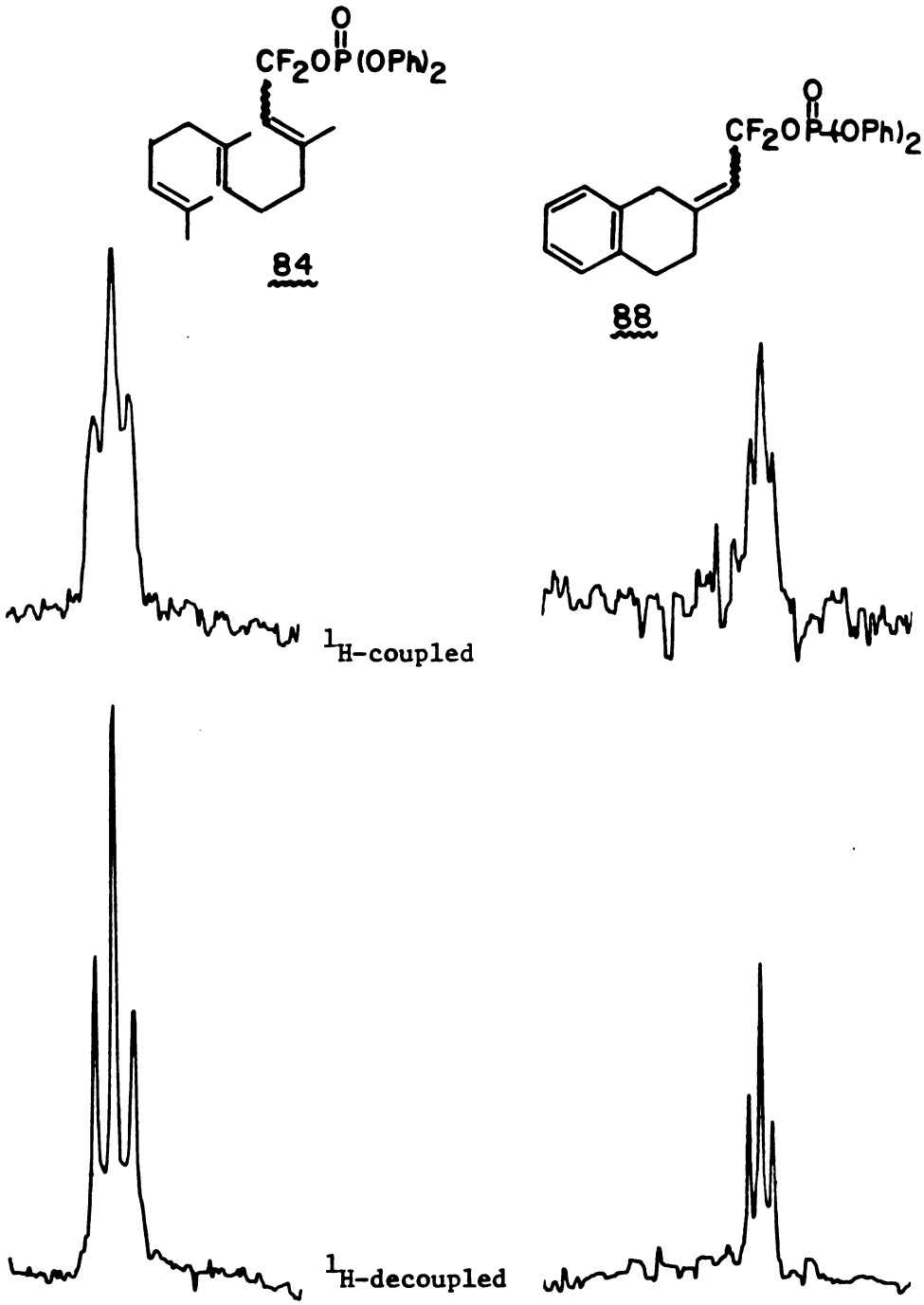
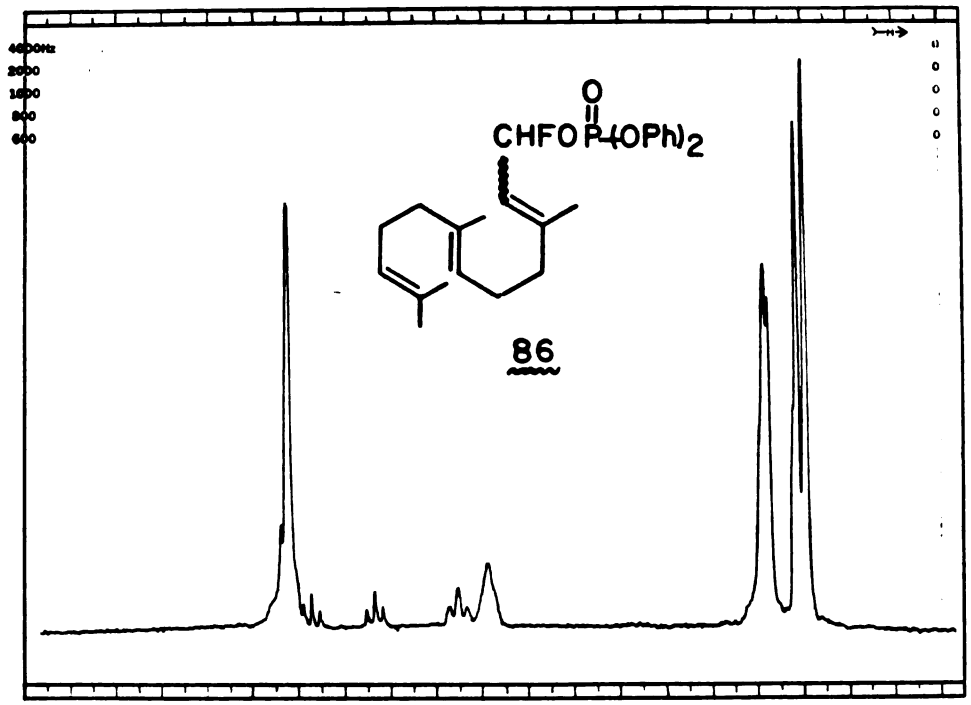


FIGURE 37: Proton coupled and decoupled ^{31}P-NMR spectra of the difluoro diphenyl phosphate esters, 84 and 88.

splitting the phosphorus (spin quantum number, I = 1/2 for fluorine and phosphorus). This agrees with the proton decoupled fluorine NMR spectra. Thus it appears that in the difluoro diphenyl phosphate esters 84 and 88 the proton-fluorine and fluorine-phosphorus nuclei are coupled with each other.

The spectra of the analogous monofluoro diphenyl phosphate esters 86 and 90 was more complicated. Figure 38 illustrates the complete proton NMR spectrum of 86 and the proton coupled and decoupled spectra of the C-1 and C-2 proton regions. As in the difluoro analogs, the C-2 vinyl proton is a broad triplet centered at 5.5ppm (J = 10 Hz). Its splitting pattern is due to equivalent coupling to the C-1 proton and fluorine. The C-1 proton displays a doublet of triplets (6.67ppm, J = 55.5 and 7 Hz) due to geminal coupling to fluorine, vicinal coupling to the C-2 proton, and through bond coupling to phosphrous. The triplets are due to the equivalent coupling with the latter two. The collapse of the triplets to doublets (J = 7 Hz) upon irradiation of the C-2 proton resonance supports proton-phosphorus coupling. The proton coupled fluorine NMR spectra showed two multiplets (one for each isomer) which, when proton decoupled became two singlets, thus indicating the absence of fluorine-phosphorus coupling. The phosphorus NMR spectra of the isomers of 86 and 90 was confusing (Figure 39) until the NMR of one of the resolved isomers was obtained (Figure 40). Apparently, the cis and trans isomers of the



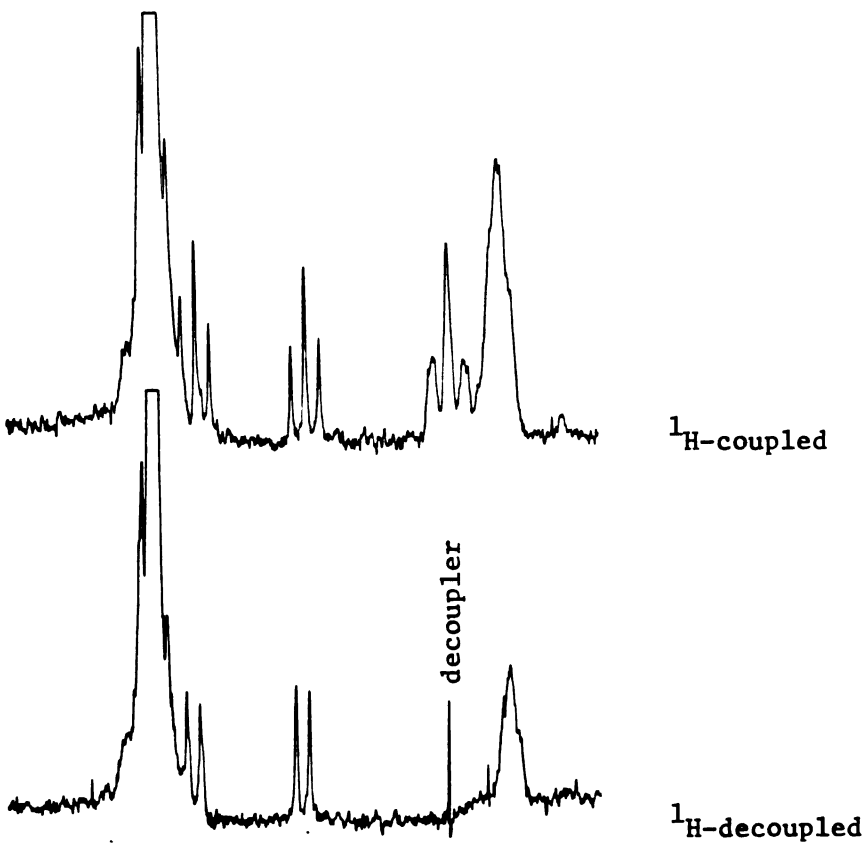


FIGURE 38: Proton coupled and decoupled ^{1}H-NMR spectra of monofluoro diphenyl phosphate ester 86.

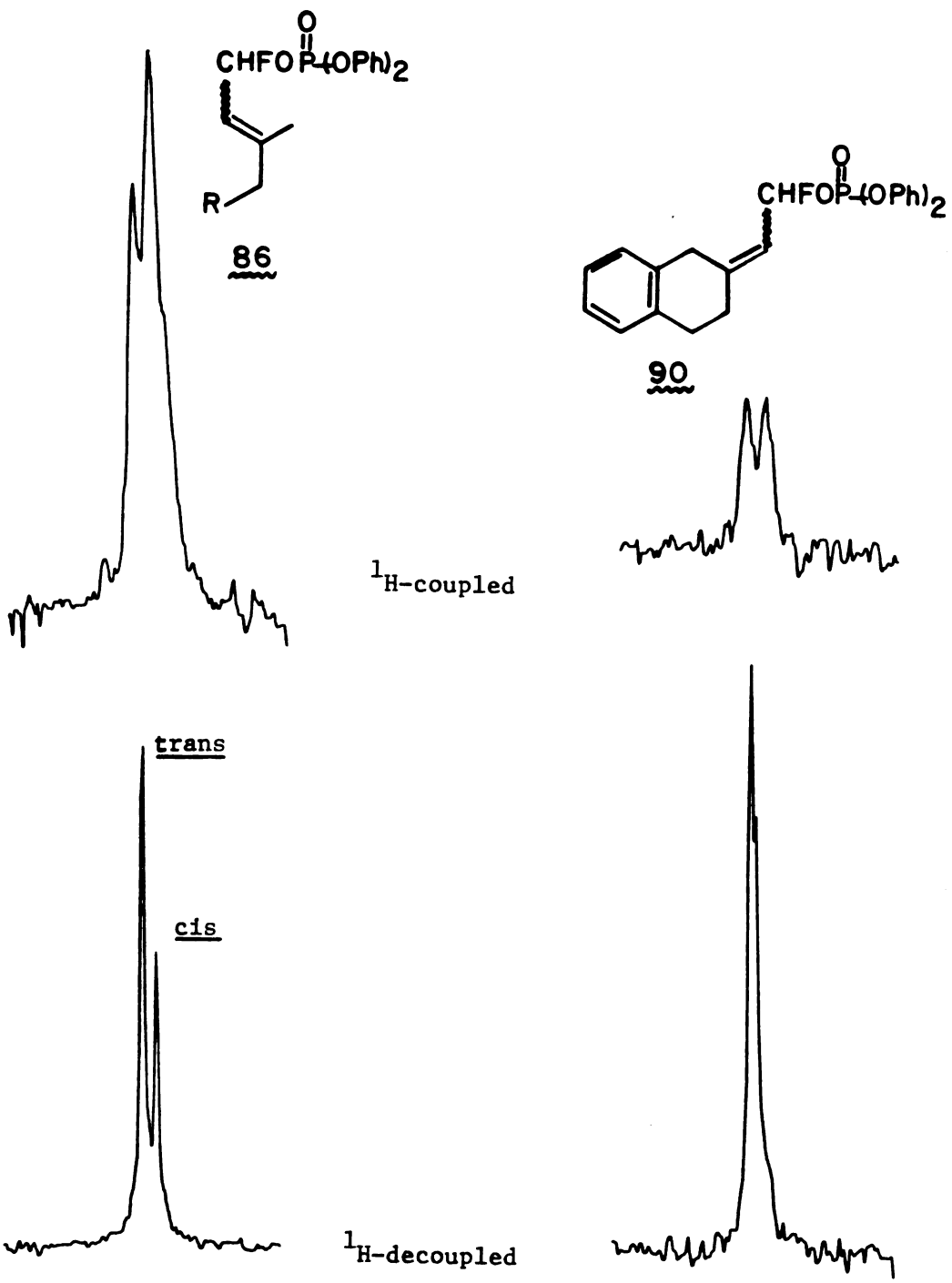
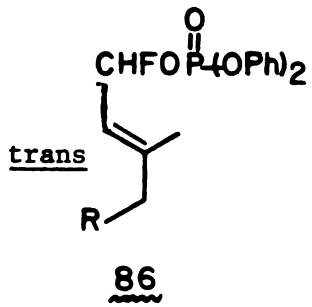


FIGURE 39: Proton coupled and decoupled ^{31}P-NMR spectra of the monofluoro diphenyl phosphate esters 86 and 90.



86

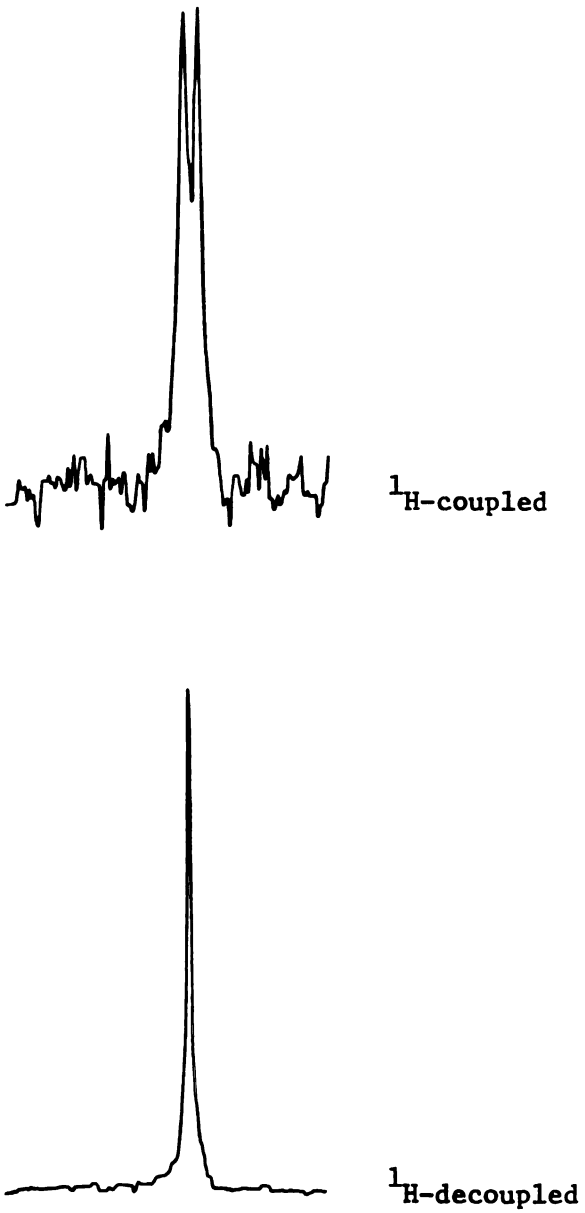


FIGURE 40: Proton coupled and decoupled ^{31}P-NMR spectra of the all trans isomer of monofluoro diphenyl phosphate ester 86.

monofluoro diphenyl phosphate esters have slightly different phosphorus chemical shifts which give rise to anomalous splitting patterns. The proton coupled spectrum of the trans isomer of 86 showed a doublet which collapsed to a singlet when proton decoupled (Figure 40), indicating the absence of fluorine phophorus coupling. It thus seems, for the monofluoro diphenyl phosphate esters 86 and 90, that the proton-fluorine and proton-phosphorus nuclei are coupled but fI0t the fluorine-phosphorus nuclei.

In summary, we have observed fluorine-phosphorus coupling in the difluoro compounds but not in the analogous monofluoro compounds. The origin of this difference between the coupling patterns of the difluoro and monofluoro compounds remains unclear. It has been shown that large variations in phosphorus chemical shifts correlate with the electronegativity of the substituents attached to phosphorus (Grim et al., 1966; Grim and McFarlane, 1965), the angles between them (Letcher and Van Wazer, 1966), the oxidation state of phosphorus (Mather et al., 1973), and the nature of the NMR solvent (Mavel, 1966). Thus it would appear that the perturbations induced in the molecular conformation due to the fluorine substituents may be the reason for the differences in spin-spin coupling.

4.4. DISCUSSION

As the interest for specifically fluorinated molecules

increases so does the need for new synthetic methodology, for better fluorine nuclear magnetic resonance techniques and for improved understanding of the chemical and physical properities of fluorine substituted molecules. In this chapter we have presented new techniques and observations that may be helpful for future use.

CHAPTER FIVE

EXPERIMENTAL PROCEDURES

5.1. GENERAL METHODS AND PROCEDURES

Instrumentation. Proton nuclear magnetic resonance spectra (NMR) were obtained with a Varian A-60A, or a Varian FT-80 spectrometer with signals expressed as parts per million (ppm) downfield from the internal standard tetramethylsilane or expressed as ppm upfield or downfield from the internal chloroform standard set at 7.25 ppm. Fluorine-19 NMR spectra were obtained using a Varian XL-100 spectrometer at 94.1 MHz with signals expressed as parts per million (ϕ) upfield from a trichlorofluoromethane internal standard. Phosphorous-31 NMR spectra were obtained using a Varian XL-100 spectrometer at 40.5 MHz with signals expressed as parts per million upfield from an external standard of trimethyl phosphate. Deuterochloroform was used as the NMR solvent. Infrared spectra (IR) were obtained with a Perkin Elmer 337 grating spectrophotometer using sodium chloride plates or cells. Chemical ionization mass spectra (CIMS) were obtained using an AEI-MS-902 spectrometer adapted to a chem-

ical ionization mode (isobutane gas). A Kratos-MS-A25S spectrometer operated in the electron impact (EI) mode (ionizing voltage, 70eV; source temp., 225^{o}) was used for the acquisition of other mass spectral data. A Packard Tri-Carb Model 3375 scintillation counter was used for radioactivity measurements. Aquasol, obtained from New England Nuclear, Boston, MA was used as scintillation fluid. Counts per minute were converted to disintegrations per minute by use of a quench curve prepared with C-14 toluene. A Buchi Kugelrohr apparatus was employed for bulb-to-bulb distillations. Spinning band distillations were performed using a Nester-Faust 19 inch Teflon band. Optical densities for biochemical experiments were obtained using an Aminco DW-2 spectrophotometer; for protein determination, a Beckman Model B spectrophotometer; and for phosphorus analysis, a Hitachi 100-40 spectrophotometer. A Sorvall Superspeed RC2-B centrifuge (Sorvall SC34 rotor) and a Beckman Model L-2 ultracentrifuge (type 40 rotor) were used for 10,000xG and 100,000xG centrifugations, respectively. A Virtis Unitrap II model was used for general freeze-drying removal of water.

Gas-Liquid Chromatrography (GLC). GLC separations were performed on a Varian 2100 model instrument with flame ionization detectors and 6ft x 2mm i.d. glass column packed with 3% OV-255 on 100/120 mesh chromosorb W, using nitrogen carrier gas at ~20ml/min flow rate.

Thin-Layer Chromatography (TLC). The following systems were used:

A. Analtech pre-coated silica gel GF254 glass plates (2.5 x 10cm; 250 micron thickness) preconditioned by storage over silica gel desiccant in a dessicator.

B. Eastman Kodak plastic-backed silica gel plates with fluorescent indicator.

Column Chromatography. Two general methods were employed for column chromatographic separations:

A. Normal glass columns packed with Merck 70-230 mesh silica-gel 60.

B. Low Pressure Liquid Chromatrogaphy (LOBAR) using Merck prepacked silica gel 60 column, size B and employing an FMI Lab pump (30 psi max. pressure at 40ml/min.flow rate). When using LOBAR for a given separation, the solvent system, the flow rate, and the elution time will be designated. Fractions were generally checked for content using TLC and/or GLC analysis.

Microanalysis. Microanalytical results were obtained from the University of California Microanalytic Laboratory, Berkeley.

General Reaction Procedures. All reactions were conducted

under a nitrogen atmosphere in dry glassware unless specified otherwise. The solvents for reactions were dried and distilled prior to use. Usually the progress of reactions was monitored by TLC and/or GLC analysis. Normal drying and concentration involved shaking the solution with anhydrous magnesium sulfate, filtration through a sintered-glass funnel, and evaporation on a rotary evaporator using water aspirator vacuum.

Presentation of Data. NMR data is presented as follows: chemical shift in ppm or $\emptyset$ (multiplicity, integrated intensity, coupling constant, assignment). Abbreviations used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets; b, broad. Infrared data is presented as follows: position of IR band in cm^{-1} (intensity, assignment). Abbreviations used: s, strong; m, moderate; w, weak; sh, sharp; b, broad. GLC data is presented as follows: temperature, retention time(tr). The presentation of TLC data is as follows: type of plate, solvent system, mobility of compound relative to the solvent front (R_f) . All temperatures are presented as degrees centigrade.

5.2. BIOLOGICAL STUDIES

5.2.1. BIOLOGICAL STUDIES WITH YEAST SQUALENE SYNTHETASE

The procedures outlined in this section may be found in greater detail in references Ortiz de Montellano et al., 1977a; 1977b.

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Squalene synthetase was prepared from baker's yeast by a method of Qureshi et al., 1972 as modified by our laboratories (see ref.).

The standard bioassay procedure was performed with incubations (1ml) containing (concentration units): labeled substrate, 10uM; $MgCl_2$, 10mM; NADPH, 1.6mM; NH_4OH, 1.0mM; protein, 1.34mg; and potassium phosphate buffer (pH 7.5), 50mM. Incubations (37°) , conducted for 10 min. unless specified otherwise, were initiated by addition of the enzyme to the other components prewarmed to 37° and were terminated by addition of ethanol (ethanol: to volume of incubation, 2:1). Control experiments with inactivated enzyme were run by adding ethanol to the incubation mixture before adding enzyme. The nonpolar products from the quenched incubation mixtures were extracted with hexanes and chromatographed through silica gel 60 (10% w/v water deactivated) minicolumns (7cm) and assayed by liquid scintillation counting.

Irreversible inhibition was determined by preincubating (37°) the 2-chloro FPP analog (10uM) for varying times up to 3 hours with the enzyme and all assay components except for the usual substrate. The standard bioassay procedure was

then initiated by addition of (1-^{3}H)-farnesyl pyrophosphate. The percent inhibition, calculated relative to controls with dead enzyme, was plotted versus preincubation times.

Substrate studies to measure the incorporation of labeled (1-^{3}H) -13 into squalene analogs were carried out by preincubating (37^o) varying amounts of (1-^{3}H) -13 and all the assay components with the enzyme for 60 min. In case the analog may be a weak substrate, additional "cold" farnesyl pyrophosphate was added to some incubation samples. All samples were terminated, extracted, and assayed in the usual manner. In order to obtain greater amounts of products(s), the standard incubation mixture was scaled up fifty fold and maintained under nitrogen for a 90 min. incubation period. After terminating the incubation, the total mixture of the hexane soluble compounds was analyzed by silica gel-TLC chromatography, TLC-liquid scintillation counting, and GLC. These methods served to further clarify and identify the presence or absence of any squalene products.

5.2.2. BIOLOGICAL STUDIES WITH RAT HEPATIC MICROSOMAL CYTOCHROME P-450

The procedures outlined in this section may be found in greater detail in references Levin et al., 1973, Ortiz de Montellano et al., 1979, and Mico, 1980.

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Microsomes were isolated by standard procedures from unstarved, 200-250 gram male Sprague - Dawley rats which had been induced with phenobarbital (sodium phenobarbital, 80mg/kg, 80mg/ml aqueous solution) or with 3-methylcholanthrene (20mg/kg, 5mg/ml corn oil) by intraperitoneal injection for four consecutive days. Typical microsomal solutions contained 1.8-2.2 nanomoles of cytochrome P-450 per mg protein.

The standard <u>in vitro</u> assay for cytochrome P-450 destruction was carried out with incubation mixtures containing, in addition to substrates, the following (concentration units): microsomal protein (1mg/ml), NADPH (1mM), KCl (150mM), and EDTA (1.5mM), all in 0.1M phosphate buffer (pH 7.4). Substrates, added without solvent at 2mM (and 10mM for Precocenes I and II) concentration, were preincubated (37^{o}) with the microsomal suspension for 10 min. before NADPH was added to initiate the reaction. In all cases, control incubations were carried out in the absence of added substrates (test for lipid peroxidation) and, for each substrate in the absence of NADPH (test for substrate destructive processes which did not require catalytic turnover of the enzyme). Aliquots at 0, 10, 20, and 30 min. were removed, saturated with carbon monoxide, reduced with sodium dithionite and measured against a reference sample of microsomes which had not been reduced but was saturated with carbon monoxide. The cytochrome P-450 content was determined by difference spectroscopy (450 and 490nm) using an Aminco

DW-2 spectrophotometer.

5.2.3. IN VITRO IN VIVO BIOLOGICAL STUDIES OF ANTI-JUVENILE HORMONE ANALOGS

The *in vitro* and *in vivo* assays of the anti-juvenile hormone analogs (31-35) on the insect, Manduca sexta, were generously performed by the Zoecon Corporation, 975 California Ave., Palo Alto, California 94304.

For their *in vitro* assay, the corpora allata glands were removed from larval insects and incubated with the analogs(4 pairs of CA/4 hr. incubation). Subsequently, (^{14}C-methyl)- methionine, a precursor of the methyl group of the ester of juvenile hormones, was added to the incubation medium and juvenile hormone biosynthesis measured by the ability of the glands to incorporate the methionine into a labeled juvenile hormone product. The percent inhibition was measured as a decrease in juvenile hormone production as compared with control incubations containing no analogs. Also, a similar *in vitro* assay was performed in which the analog was coincubated with farnesoic acid before addition of the labeled methionine and the activity of the glands again correlated with the amount of juvenile hormone production. Farnesoic acid appears to enter the biosynthetic pathway before the last two steps (methylation and epoxidation) of juvenile hormone production. Therefore, if the original percent of inhibition decreases upon coincubation

with farnesoic acid presumably the analog was a competitive inhibitor of one (or both) of these last two steps. For further details on these experimental procedures see Kramer and Law, 1980b.

Their *in vivo* assays utilized the procedures described below.

Evaluation of Contact Activity on Tomato Hornworm, *Manduca sexta* (Assay 41)

Test compounds are diluted in acetone and topically applied in 1 ul drops to the dorsum of 20 IIIrd instar larvae 0-24 hrs postmolting which are retained individually in 1 oz jelly cups with artificial food. Treated larvae are scored after each molt to the subsequent instar until they reach the Vth instar or have died. Test doses range from 250 ug/III down to a dose at which no effect is registered.

Although this assay was developed for JH antagonist research, it is capable of picking up several different types of activities.

1) Toxicity (larval mortality).

2) JH activity (pigmentation changes).

3) Anti-JH activity (pigmentation changes, precocious development of pupal cuticle, premature prepupal

behavior).

4) Growth Inhibition (prolonged developmental time, lack of larval growth).

5) Cuticular abnormalities (chitin inhibition, distortion of cuticle ("scarring", molting difficulties).

Activities of standard insecticides.

	ED_{50} ug/III
sumicidin	0.019
methyl parathion	1.300
DIMILIN	0.370
fluvalinate	4.000
fioresmethrin	0.010

Evaluation of Ingestion/Contact Activity on Tomato Hornworm, Manduca sexta (Assay 108)

Third instar larvae, 0-24 hrs postmolting, are placed in individual 1 oz jelly cups on a meridic diet in which a candidate chemical had been admixed. The assay is incubated at 27°C and runs until the larvae reach the Vth instar. Observations are made during each instar. Test concentrations range from 100ppm down to a concentration at which no

response is registered.

This assay is capable of registering several different types of activites.

1) Toxicity (larval mortality).

2) JH activity (pigmentation changes, at high doses only). IP 3) Anti-JH activity (pigmentation changes, precocious development of pupal cuticle, premature prepupal behavior).

4) Growth inhibition (prolonged developmental time, lack of growth).

5 Cuticular abnormalities (chitin inhibition, distortion of cuticle).

Activities of standard insecticides

	EC_{50} (ppm)
decamethrin	0.065
methyl parathion	0.480
propoxur	31.000
DIMILIN	0.230

5.3. SYNTHESIS

Acetic acid, dichloro(diethoxyphosphinyl)-, ethyl ester (18)

Sulfuryl chloride (39ml, 0.48mole) was added dropwise via an addition funnel to a flask containing acetic acid, (diethoxyphosphinyl)-, ethyl ester (Aldrich, 17.94g, 0.08mole) maintained at 50°. After the addition was complete (4 hrs.), the reaction was heated at 80° for 1.5 hr. The evolved HCl and SO_2 were removed by bubbling through a water trap. After cooling, the reaction mixture was poured into 100ml of vigorously stirred water and then brought to neutral pH by addition of 100ml of a saturated sodium bicarbonate solution. The product was extracted wih methylene chloride, washed with water, dried over magnesium sulfate, and the solvent removed (rotoevaporation) to give 11.5g (49%) of a clear, colorless oil. Bulb-to-bulb distillation (95°, 0.025mm Hg) afforded pure product; TLC (System A, 50% EtoAc/Hexanes, R_f=0.16); GLC(150°, t_r = 16.12 min.); IR (flim)1760 cm^{-1} (s,C=O) and 1475, 1450, 1390, 1370, 1250, 1020 ($(CH_3CH_2O)d2^{-P(O)-}$); NMR ($CDCl_3$) 1.37 and 1.40 (2t, 9H, $-CH_3$), 4.43 (m, 6H, $-CH_2-$).

Acetic acid, chloro(diethoxyphosphinyl)-, ethyl ester (14)

50ml of a 1.0 molar sodium bisulfite solution was added dropwise to a flask containing 18 (11.5g, 39.2mmole) and 50ml of methanol. After addition was complete (1.5 hr.) the reaction was stirred for 15 min. longer. The product was extracted with chloroform, dried over magnesium sulfate, and the solvent removed (rotoevaporation) to give 7.675g (82%)

of a clear, colorless oil. Purification by column chromatography (250g slica gel 60, 42 x 4.1cm column, 50% EtoAc/Hexanes, 2.5ml/min.) gave 4.233g (45.5%). Bulb-to-bulb distillation (85°, 0.05mmHg) of the oil yielded 3.77g (40.5%) of pure product; TLC (System A, 50% EtoAc/Hexanes, R_f=0.25); GLC (150°, t_r=11.25 min); IR (film) 1750cm$_{-1}$ (s,C=O) and 1475, 1450, 1390, 1370, 1265, 1020, ((CH_3CH_2)-$_2$P(O)-); NMR $CDCl_3$) 1.33 and 1.38 (2t, 9H, -CH_3), 4.32 (m, 6H, -CH_2-), 4.4 (d,1H, J_{H-P}=16Hz, -CHCl-); CIMS 259 (MH^+),261(MH^++2, ^{37}Cl).

Anal. Calcd. for $C_8H_{16}O_5ClP$; C 37.17; H, 6.24; Cl, 13.72.

Found: C, 37.14; H, 6.29; Cl, 13.88.

Ethyl 2-chloro-3,7,11-trimethyl-2(Z), 6(E),10-dodecatrienoate (16a) and Ethyl 2-chloro-3,7,11-trimethyl-2(E),6(E), 10-dodecatrienoate (16b).

Product 14 was added to hexane washed sodium hydride (768mg, 16mmole) in 15ml of THF at 0 degree . After addition, the reaction was allowed to warm to room temperature (1.5 hr) and subsequently heated to 50° for 30 min. (until no more H_2 evolved). The reaction mixture was cooled back down to room temperature and all trans geranyl acetone (1.943g, 0.01mole) added all at once. The reaction was heated at 55° for 48 hrs. At the end of that time the reaction was cooled and taken up in 250ml of ether and 50 ml of water. The ethereal layer was separated and washed with water and brine, dried

over magnesium sulfate, and the solvent removed (rotoevaporation) to give 2.7491 g (92%) of crude product (orange-yellow oil). Purification by LOBAR chromatography (1% EtoAc/Hexanes, 6ml/min. 20-28 min. (2E) and 45-58 min.(2Z) elution time) separated the two isomers to furnish 75% combined yield; TLC (System A, 5% EtoAc/Hexanes, R_f = 0.44 and 0.52 for the 2Z and 2E isomers respectively); GLC (180°, t_r = 5.81 and 5.44 min.for the 2Z and 2E isomers respectively); IR (film) 1720 cm^{-1} (s, C=O), 1620 (m, C=C), 1440 (m), 1375 (m), 1250 (s, C-O); 16a NMR ($CDCl_3$) 1.33 (t, 3H, J = 7Hz, $OCH_2\underline{CH_3}$), 1.63 and 1.70 (2s, 9H, vinyl CH_3) 2.17 (s, 3H, C-3 Me), 1.90-2.50 (m, 8H, allylic CH_2), 4.32 (q, 2H, J = 7Hz, $O\underline{CH_2}CH_3$), 5.17 (m, 2H, vinyl H); 16b NMR ($CDCl_3$) 1.33 (t, 3H J = 7Hz, $OCH_2\underline{CH_3}$), 1.63 and .1.70 (2s, 9H, vinyl CH_3), 2.03 (s, 3H, C-3 Me), 1.95-2.75 (m, 8H, allylic CH_2), 4.32 (q, 2H, J = 7Hz, $O\underline{CH_2}CH_3$), 5.17 (m, 2H, vinyl H); CIMS (of each ismore) 299 (MH^+), 301 (MH^+ + 2, ^{37}Cl), 263 (MH^+ - HCl), 253 (MH^+ - HOEt).

Anal. Calcd. for C_{17} H_{27} O_2 Cl; C,68.32; H,9.11; Cl,11.86.

Found 16a: C, 68.45; H, 8.97; Cl, 12.01. 16b: C, 68.27; H, 8.95; Cl, 11.93.

2-Chloro-3,7,11-trimethyl-2(Z), 6(E),10-dodecatrienol (21a) and 2-Chloro-3,7,11-trimethyl-2(E),6(E),10-dodecatrienol (21b)

Ester 16a (or 16b) (350mg, 1.17mmole) was added to a slurry of lithium aluminum hydride (85mg, 2.24mmole) in 10ml of ether cooled to -78°. The reaction was continued at -78° until judged complete by GLC. It was then quenched by the addition of 85ul of water and the mixture warmed to room temperature. The subsequent addition of 85ul of 15% NaOH was followed in 5 min. by 255ul of water. After stirring for several minutes, a small amount of anh. magnesium sulfate was added. Filtration and solvent removal (rotoevaporation) afforded 286mg (95%) of the corresponding alcohol, 21a (or 21b); TLC (System A, 20% EtoAc/Hexanes, R_f = 0.34 and 0.49 for the 2Z and 2E isomers respectively); GLC (180°, t_r = 6.75 and 6.00 min. for the 2Z and 2E isomers respectively); IR (film) 3350cm^{-1} (b,-OH), 1450 (m), 1385 (m), 1010 (m); 21a NMR ($CDCl_3$) 1.63 and 1.70 (2s,9H, vinyl CH_3), 1.85 (s, 3H, C-3 Me), 2.03 (s, 1H, -OH), 1.85-2.30 (m, 8H, allylic CH_2), 4.32 (bs, 2H, C-1 CH_2), 5.18 (m, 2H, vinyl H); 21b NMR ($CDCl_3$) 1.63 and.170 (2s, 9H, vinyl CH_3), 1.90 (s, 3H, C-3Me), 2.03 (s, 1H, -OH), 1.90 - 2.30 (m, 8H, allylic CH_2), 4.32 (bd, 2H, C-1 CH_2), 5.18 (m, 2H, vinyl H); CIMS (of each isomer) 239 (MH^+-H_2O), 203 (MH^+-H_2O-HCl), no MH^+ observed.

Anal. Calcd. for $C_{15}H_{25}OCl$; C, 70.15; H, 9.81; Cl, 13.81.

Found, 21a: C, 70.18; H, 9.68; Cl, 13.78. 21b: C, 69.74; H, 9.67; Cl, 14.03.

A series of NMR spectra were taken for each isomer (21a and 21b) with increasing amounts of the shift reagent, $Eu(fod)_3$. The effect on the 3-methyl protons is presented below and in Figure 11.

2-Chlorofarnesyl pyrophosphate (13)

A solution fo 450.5mg (1.5mmole) of di-(triethylammonium)-phosphate in 40ml of acetonitrile was added over a period of 6 hrs. to 128.5mg (0.5mmole) of purified alcohol 21a and 996mg (6.9mmole) of trichloroacetonitrile in 5ml of acetonitrile. After stirring for 24 hrs. the solvent was removed in vacuo leaving a yellow residue which was dissolved in 10ml of acetone containing 0.01N NH_4OH. Concentrated NH_4OH was added (1.0ml) resulting in a precipitate formation which was subsequently washed thrice with 5ml portions of acetone containing 0.01N NH_4OH. The solid was dissolved in a minimal amount of water (0.01N NH_4OH) and chromatographed on 30g of silica gel using a 9 : 4 : 1 (n-propanol: NH_4OH : water) solvent system, 1.0ml/min., and collecting 3ml fractions. The monophosphate salt eluted out in fractions 16-21 and the pyrophosphate in fractions 24-31. The fractions containing the pyrophosphate 13 (TLC system B, 6 : 3 : 1 of n-propanol : NH_4OH : water, R_f = 0.29) were combined and the solvent removed (rotoevaporation and lyophilization) to provide 45.2mg (20%) of a white solid. Quantitative phosphorus analysis (Bartlett, 1959; Castillo, 1977) indicated 12.91%phosphorus (expected, 13.24%).

Compound with longer t_r (GLC) and lower R_f (TLC), 21a. Concentration 32.7mg/500ul $CDCl_3$ = 0.1273mmole.

Eu(fod)$_3$ mg	Eu(fod)$_3$ mmole	Mole Ratio Eu(fod)$_3$/ Substrate	3-Me shift from TMS
0.0	0.0000	0.0000	112cps
20.5	0.0198	0.155	145
33.4	0.0322	0.253	163
45.2	0.0436	0.342	182
57.3	0.0552	0.434	199
80.8	0.0779	0.612	223

Compound with shorter t_r (GLC) and higher R_f (TLC), 21b. Concentration 34.0mg/500ul $CDCL_3$ = 0.1324mmole.

Eu(fod)$_3$ mg	Eu(fod)$_3$ mmole	Mole Ratio Eu(fod)$_3$/ Substrate	3-Me shift from TMS
0.0	0.0000	0.000	114cps
21.4	0.0206	0.156	139
32.4	0.0312	0.236	152
43.7	0.0421	0.318	164
57.9	0.0558	0.421	180
81.3	0.0784	0.592	201

Ethyl 3,7-dimethyl-10-oxo-2(E),6(E)-decadienoate (36)

This compound was synthesized according to Boparai, 1977 (65% yield); NMR ($CDCl_3$) 1.25 (t, 3H, J = 7Hz, $OCH_2\underline{CH_3}$), 1.63 (s, 3H, C-7 Me), 1.55-2.58 (m, 8H, allylic CH_2), 2.15 (d, 3H, J = 1Hz, C-3Me), 4.12 (q, 2H, J = 7Hz, $O\underline{CH_2}CH_3$), 5.14 (m, 1H, C-6 H), 5.62 (bs, 1H, C-2 H), 9.67 (t, 1H, J = 1.5Hz, aldehydic H).

Ethyl 3,7-dimethyl-2(E),6(E), 10-undecatrienoate(31a) and Ethyl 3,7-dimethyl-2(Z),6(E), 10-undecatrienoate(31b)

A suspension of methyltriphenylphosphonium bromide (750mg, 2.1mmole) in 15ml THF was cooled to 0° and treated with n-butyllithium (945 ul of a 2.38M hexane solution, 2.25mmoles). The reaction was stirred for 5 min at 0° and then the ice-bath was removed and the reaction stirred for an additional 30 min. The resulting orange ylide solution was cooled to -78° and the aldehyde 36 (500mg, 2.1mmole of a mixture of 2E : 2Z isomers, 9 : 1) in 2ml of THF was added dropwise. The mixture was kept at -78° for 5 min., at -25° for 1.5 hr. It was then poured into ice-water and extracted with pentane. The combined pentane extracts were washed with brine, dried over magnesium sulfate and concentrated in vacuo (rotoevaporation). Filtration of the residue with hexanes removed the precipitated phosphine oxides, and the filtrate was concentrated in vacuo. Purification by LOBAR chromatography (5% EtoAc/Hexanes, 6ml/min., 16-19min.(2Z) and 22-28 min (2E) elution time) separated the two isomers

6Hz, C-6H), 5.64 (bs, 1H, C-2 H), 5.60-6.02 (m, 2H, C-10 H and C-11 H); CIMS (of each isomer) 271 (MH^+), 273(MH^+ + 2, ^{37}Cl), 243, ($MH^+ - CH_2 = CH_2$), 235 (MH^+ - HCl), 225 (MH^+ - EtOH).

Anal. Calcd. for $C_{15}H_{23}O_2Cl$; C, 66.53; H, 8.56; Cl, 13.09. Found for mixture of isomers: C, 66.51; H, 8.62; Cl, 12.94.

Ethyl 3,7-dimethyl-10-yne-2(E),6(E) undecadienoate (33) and Ethyl 3-methylene -7-methyl-10-yne-6(E) undecenoate (34)

To a flask containing a solution of chloro olefin 32a (320mg, 1.18mmole) in 19ml of THF at -40° was added 3.6ml of 1.0 molar LDA (see below for preparation of) dropwise. After addition, the reaction was allowed to warm to 0° and maintained for 30 min.at this temperature. It was then hydrolyzed by quickly pouring into 6ml of cold 2N sulfuric acid. The alkynes were extracted with ether and the extracts washed with water and brine, dried over magnesium sulfate, and the solvent removed (rotoevaporation) to give a slightly yellow oil which was purified by LOBAR chromotography (5% EtoAc/Hexanes, 6ml/min., 28-31 min.(33) and 33-37 min.(34) elution times) to yield 130mg (47%) of 33 and 141mg (51%) of 34; TLC (System A, 5% EtoAc/Hexanes, R_f = 0.25 (33) and R_f = 0.22 (34)); GLC (175°, t_r = 2.55 min (33) and t_r = 1.99 min.(34)); 33 IR (film) 3300cm^{-1} (s, ≡C-H), 2110 (w, C≡C), 1700 (s, C = O), 1650 (m, C = C), 1450, 1375, 1220(s), 1145(s), 630 (s, ≡C-H bending); 34 IR (film) 3300 cm^{-1} (s, ≡C-H), 2110(w, C≡C), 1730 (s, C=O), 1650 (vw, C=C), 1450,

1375, 1150 (m) 1030 (m), 630 (s, ≡C-H bending); 33 NMR ($CDCl_3$) 1.25 (t, 3H, J = 7Hz, $OCH_2\underline{CH_3}$), 1.60 (bs, 3H, C-7Me), 1.92 (m, 1H, C≡C-H), 2.14 (d, 3H, J = 1Hz, C-3Me), 1.95-2.45 (m, 8H, allylic CH_2), 4.12 (q, 2H, J = 7Hz, $O\underline{CH_2}CH_3$), 5.15 (m, 1H, C-6 H), 5.63 (bs, 1H, C-2 H); 34 NMR ($CDCl_3$) 1.25 (t, 3H, J = 7Hz, $OCH_2\underline{CH_3}$), 1.60 (s, 3H, C-7 Me), 1.92 (m, 1 H, C≡C-H), 1.95-2.45 (m, 8H, allylic CH_2), 3.02 (s, 2H, C= CH_2), 4.12 (q, 2H, J = 7Hz, $OCH_2\underline{CH_3}$), 4.90 (s, 2H, $-CH_2COO$ 5.17 (m, 1H, C-6H); 33 EI 234 (M), 195 (M-$CH_2C{=}CH$), 189 (M-OEt), 161 (M-COOEt); 34 EI 234 (M), 219 (M-CH_3), 161 (M-COOEt), 146 (M- CH_3 - COOEt).

Anal. Calcd. for $C_{15}H_{22}O_2$; C, 76.88; H, 9.46.

Found 33: C, ; H, 34; C, 76.66; H, 9.34.

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1.0M Lithium Diisopropylamide. Solutions of LDA in THF were prepared as needed in the following manner: a dry septum-capped flask was flushed with nitrogen and cooled to -78° THF (1.0ml) and diisopropylamine (0.75ml) were added, followed by 3.25ml of 1.54M n-butyllithium in hexane. The 1M LDA solution was warmed to 0^{degree} and kept at this temperature for immediate use.

Ethyl 11,11-difluoro-3,7-dimethyl-2(E), 6(E), 10-undecatrienoate (35)

Dibromodifluoromethane (420mg, 2.0mmole) was condensed into a cooled -78° solution of THF (8ml), followed by addition of 727ul (4.0mmole) of hexamethylphosphorus triamide. The resulting suspension was allowed to warm to room temperature and stirred for 30 min. before introducing aldehyde 36 (230mg, 0.96mmole). After 24 hrs. at room temperature the reaction had not progressed any further and so was worked up by adding 10ml of water and then transferring the mixture to a separatory funnel and extracting with pentane. The combined organic extracts were washed with 0.1N HCl, water, and brine; dried over magnesium sulfate; and the solvent removed (rotoevaporation) to give a clear, colorless oil (174mg) which was purified by LOBAR chromatography (5% EtoAc/Hexanes, 6ml/min., 28-31 min. elution time) to yield 123mg (47%) of product (approx. 15% starting aldehyde also recovered); TLC (System A, 15% EtoAc/Hexanes, R_f = 0.54); GLC (150°), t_r = 3.37 min); IR (film) 1750 cm^{-1} (s, C = O), 1710 (s, C = CF_2), 1650 (m, C = C) 1450 (m), 1375 (m), 1210, 1140; ^{1}H-NMR($CDCl_3$) 1.25 (t, 3H, J = 7Hz, OCH_2OCH_3), 1.60 (bs, 3H, C-7 Me), 1.95 -2.35 (m, 8H, allylic CH_2), 2.14 (s, 3H, C-3 Me), 4.14 (q, 2H, J = 7Hz, $O\underline{CH_2}CH_e$), 3.80-4.30 (m, 1H, CH = CH_2), 5.09 (m, 1H, C-6H), 5.65 (bs, 1H, C-2H); ^{19}F-NMR ($CDCl_3$) ϕ 90(d, 1F, J = 49Hz, C = CF trans), 92.2 (dd, 1F, J = 49 and 26Hz, C = CF cis); EI 272 (M), 252 (M-HF), 227 (M-OEt), 199 (M-COOEt).

Anal. Calcd. for $C_{15}H_{22}O_2F_2$; C, 66.15; H, 8.14

Found: C, 66.32; H, 8.13.

1-(2',2'-difluoroethenyl)-1,5,9-trimethyl-4, 8-decadienol (48)

This compound was synthesized according to Vinson (1978) (75% yield); ^{1}H-NMR ($CDCl_3$) 1.43 (d, 3H, J = 2Hz, C-1 Me), 1.63 and 1.72 (m, 9H, vinyl Me), 2.05 (bm, 8H, allylic CH_2), 2.15 (s, 1H, OH), 4.43 (dd, H, J = 26 and 6Hz, $CH = CF_2$), 5.20 (m, 2H, vinyl H); ^{19}F-NMR ($CDCl_3$) ϕ 84.9 (dd, 1F, J = 46 and 26Hz, C = CF cis), 86.3 (dd, 1F, J = 46 and 6Hz, C = CF trans).

1-(2',2'-difluoroethenyl)-1-methyl-decanol (52)

This compound was prepared from ketone 49 by Spahic (1981) according to the procedure of Vinson (1978).

3,3-Difluoro-1-methyl-1-(17'-5' β - Androstan-3' β-ol)-2-propenol (53)

This compound was prepared from ketone 50 by Spahic (1981) according to the procedures of Vinson (1978).

2-(2',2'-difluoroethenyl)-1,2,3,4- tetrahyro-2-napthol (54).

This compound was prepared via the synthetic scheme shown in Figure 27. The specific procedures and characterizations of the intermediates and product (54) are described below.

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2-Trimethylsiloxyl,2,3,4- tetrahydronapthyl-6-al.

The catalyst, HgI_2 (30mg) was added to a neat solution of β -tetralone (51) (Aldrich, 15.94g, 109mmole) and stirred for 20 min. before adding the freshly distilled TMS-CN (exothermic reaction). The mixture was subsequently heated (80°) for 5 hrs., then cooled to room temperature and the excess TMS-CN removed in vacuo (water aspiration). After adding 150ml of THF and cooling to -78°, the diisobutylaluminum hydride (142ml fo a 1.0M hexane solution) was added dropwise. The reaction was allowed to warm to room temperature and the progress monitored by GLC analysis. When complete, the mixture was carefully poured into 600ml of ice-cold 5% H_2SO_4 and vigorously stirred. 300ml of ether was added and the organic layer separated and concentrated on the rotovap. The aqueous layer was extracted 4X with ether and all the organic portions combined, washed with brine, dried over magnesium sulfate, and concentrated (rotoveaporation) to give 24.35g (90%) of a clear, pale yellow oil. Purification was by LOBAR chromatography (15% EtOAc/Hexanes, 8ml/min., 11-14 min. elution time); TLC (System A, 10% EtOAc/Hexanes, R_f = 0.47); GLC (150°, t_r = 3.75 min.); IR(film) 1740 cm^{-1} (S, C=O), 1250 (s, Si-CH_3), 1100 (s, Si-O), 840, 750 (s, four adjacent aromatic hydrogen atoms); NMR ($CDCl_3$) 0.06 (s, 9H, $OSiMe_3$), 1.57-1.96 (m, 2H, C-3 CH_2), 2.63-3.36 (m, 4H, C-4 CH_2 and C-1 CH_2), 7.11 (s, 4H, aromatic H's), 9.67 (s, 1H, CHO); EI 248 (M), 233 (M-CH_3), 220 (M-CO), 219 (M-CHO), 129 (M-CHO-HOTMS), 115, 75 (HO-$SiMe_2$) 73 ($SiMe_3$).

Anal. Calcd. for $C_{14}H_{20}O_2Si$; C, 67.69; H, 8.12.

Found: C, 67.33; H, 8.13.

2-(2',2'-difluoroethenyl)-2,-trimethylsiloxy- 1,2,3,4-tetrahydronapthalene.

Dibromodifluoromethane (about 15g, 70mmoles) was condensed into 150ml of THF at -78°. Then, 25.5ml (140mmoles) of hexamethylphosphorus triamide was added (white-colored suspension forms immediately) and the mixture allowed to warm to room temperature. The previously prepared aldehyde (8.69g, 35mmole) was subsequently added and the reaction continued until judged complete by GLC. The slurry was transferred to a separatory funnel using 150ml of water and 300ml of pentane. The aqueous layer was twice extracted with pentane and then all the organic portions combined and[9] washed with water and brine, dried over magnesium sulfate, and concentrated (rotoevaporation) to give 8.38g (85%) of a clear orange oil. Purification by silica gel chromatography (column or LOBAR) always resulted in some decomposition. For analytical work, a small amount was purified by LOBAR chromatography (15% EtOAc/Hexanes, 8ml/min., 10-19 min. elution time); TLC (System A, 15% EtOAc/Hexanes, R_f = 0.67); GLC (130°, t_r = 2.7 min.); IR (film) 1740 cm^{-1} (s, C=CF_2),

(9) Subsequently, it was discovered that if part of the workup included a 5% HCl solution wash, then cleavage of the O-TMS bond occurred to give 54 in nearly quantitative yield.

1250 (s, Si-CH_3), 1080 (s, Si-O), 840, 740 (s, four adjacent aromatic hydrogen atoms); ^{1}H-NMR ($CDCl_3$) 0.08 (s, 9H, $SiMe_3$), 2.00 (t, 2H, J = 7Hz, C-3 CH_2), 2.83 (t, 2H, J = 7Hz, C-4 CH_2), 3.04 (s, 2H, C-1 CH_2), 4.40 (dd, 1H, J = 27 and 5Hz, $CH=CF_2$), 7.08 (s, 4H, aromatic H's); ^{19}F-NMR ($CDCl_3$) ϕ 82 (dd, 1F, J = 42 and 27Hz, C=CF cis), 84.6 (dd, 1F, J = 42 and 5Hz, C=CF trans); EI 282 (M), 267 (M-CH_3), 192 (M-HOTMS), 115, 77 (Ph), 75 (HO-$SiMe_2$), 73 ($SiMe_3$).

Anal. Calcd. for $C_{15}H_{20}OF_2Si$; C, 63.79; H, 7.14.

Found: C, 64.00; H, 7.06.

2-(2',2'-difluoroethenyl)- 1,2,3,4-tetrahydro-2-napthol (54)

Addition of the requisite amount of 15% NaOH (3.1ml) to a solution of the previously prepared difluorovinyl-TMS ether (2.5g, 8.8mmoles) in 12.5ml of methanol was begun, however after addition of only 1.0ml the reaction turned a dark blue color and so subsequent addition was ceased. After stirring for 12 hrs. the reaction was worked up by transferring to a separatory funnel with pentane and water. After separation, the aqueous layer was again extracted with pentane. The combined organic extracts were washed with water and brine, dried over magnesium sulfate, and the solvent removed (rotoevaporation) to afford 1.57g (85% yield) of a clear yellow oil. Purification of an analytical amount was done by LOBAR chromatography (20% EtOAc/Hexanes, 8ml/min., 25-38

min. elution time); TLC (System A, 20% EtOAc/Hexanes, R_f = 0.21); GLC (150°, t_r = 3 min.); IR (film) 3375 cm^{-1} (b, OH), 1740 (s, $C=CF_2$), 1170, 920, 740 (s, four adjacent aromatic hydrogen atoms); ^{1}H-NMR ($CDCl_3$) 1.80 (s, 1H, OH), 2.03 (t, 2H, J = 7Hz, C-3 CH_2), 2.90 (t, 2H, J = 7Hz, C-4CH_2), 3.02 (s, 2H, C-1 CH_2), 4.48 (dd, 1H, J = 27 and 5Hz, $CH=CF_2$), 7.10 (s, 4H, aromatic H's); ^{19}F-NMR($CDCl_3$) ϕ 82.6 (dd, 1F, J = 42 and 27Hz, C=CF cis), 84.6 (dd, 1F, J = 42 and 5Hz, C=CF trans); EI 210 (M) 192 (M-H_2O), 128 (M-H_2O-$CH_2=CF_2$), 115.

Anal. Calcd. for $C_{12}H_{12}OF_2$; C, 68.56; H, 5.76.

Found: C, 68.94; H, 6.16.

1-(2'-fluoroethenyl)-1,5,9-trimethyl-4, 8-decadienol (56a, 2'-E and 56b, 2'-Z)

To a cooled (0°) solution of the difluoro analog 48 (1.03g, 4.0mmoles) in 20ml of ether was added 1.39ml (4.16mmole) of n-butyllithium (3.0M in hexane). After stirring for 15 min. at 0° the lithium aluminum hydride (227.7mg, 6.0mmole) was added all at once and the reaction allowed to warm to room temperature and then refluxed (35-40°) for 24 hrs. After cooling to room temperature the reaction was quenched by the addition of 228ul of water, followed by 228ul of 15% NaOH and then an additional 684ul of water. After stirring several min., a small amount of anh. magnesium sulfate was added, the mixture filtered and the solvent removed (rotoevaporation) to give 940.8mg (98%) of a clear, slightly yel-

low oil. Purification and separation of isomers by LOBAR chromatography (15% EtOAc/Hexanes, 8ml/min. 24-32 and 36-40 min. elution time for 2'-Z and 2'-E isomers respectively) gave 90% combined yield in a 9 : 1 ratio of E : Z; TLC (System A, 20% EtOAc/Hexanes, R_f = 0.37 and .045 for the 2'-Z and 2'-E isomers respectively); GLC (150°, t_r = 3.98 and 5.63 min. for 2'-Z and 2'-E isomers respectively); IR(film) 3400 cm^{-1} (b, OH), 1670 (s, C=CHF); 56a ^{1}H-NMR ($CDCl_3$) 1.32 (s, 3H, C-1 Me), 1.63 and 1.70 (2s, 9H, vinyl CH_3), 1.73 (m, 2H, C-2CH_2), 2.02 (m, 6H, allylic CH_2), 5.17 (m, 2H, vinyl H), 5.50 (dd, 1H, J = 21 and 10Hz, C-1' vinyl H), 6.75 (dd, 1H, J = 86 and 10Hz, C-2' vinyl H), ^{19}F-NMR($CDCl_3$) ϕ 136 (dd, 1F, J = 86 and 21Hz, C=CHF); 56b ^{1}H-NMR ($CDCl_3$) 1.42 (d, 3H, J 1.5Hz, C-1 Me), 1.62 and 1.68 (2s, 9H, vinyl CH_3), 1.75 (m, 2H, C-2 CH_2), 2.05 (m, 6H, allylic CH_2), 5.08 (dd, 1H, J = 47 and 5Hz, C-1' vinyl H), 5.17 (m, 2H, vinyl H), 6.13 (dd, 1H, J = 85 and 5Hz, C-2' vinyl H), ^{19}F-NMR ($CDCl_3$) ϕ 127 (dd, 1F, J = 86 and 47Hz, C=CHF); CIMS (of each isomer) 223)MH+ -$H_2$0), 203 (MH+ -H_2O-HF), no MH+ observed.

Anal. Calcd. for $C_{15}H_{25}OF$; C, 74.95; H, 10.48.

Found 56a: C, 74.74; H, 10.36. 56b: C, 75.09; H, 10.49.

1-(2'2'-deuterofluoroethenyl)-1,5,9-trimethyl-4, 8-decadienol (61a, 2'-E and 61b 2'-Z).

Reaction and purification procedures were essentially ident-

ical to those for the preparation of 56a and 56b except for the addition of 2X mole equivalents of lithium aluminun deuteride rather than 1.5X mole equivalents of lithium aluminum hydride. Both isomers were observed by GLC in the same 9 : 1/E : Z ratio. Analysis of only the E isomer gave; TLC (System A, 20% EtOAc/Hexanes, R_f = 0.45); GLC (150°, t_r = 5.63 min.); IR (film) 3400 cm^{-1} (b, OH), 1670 (s, C=CDF); ^{1}H-NMR ($CDCl_3$) 1.32 (s, 3H, C-1 Me), 1.63 and 1.70 (2s, 9H, vinyl CH_3), 1.73 (m, 2H, C-2 CH_2), 2.02 (m, 6H, allylic CH_2), 5.18 (m, 2H, vinyl H), 5.50 (bd, 1H, J = 22Hz, C-1' vinyl H); ^{19}F-NMR ($CDCl_3$) ϕ 137 (dt, 1F, J = 20 and 13Hz, C=CDF); CIMS 224 (MH+ $-H_2O$), 204 (MH+ $-H_2O$-HF), no MH+ observed.

Anal. Calcd. for $C_{15}H_{24}DOF$; C, 74.64; H, 10.85.

Found: C, 74.54; H, 10.46.

1-(2'-fluoroethenyl)-1-methyl-decanol (58).

This compound was prepared from the difluoro analog 52 by Spahic (1981) according to the procedures established for the preparation of 56a and 56b.

3-Fluoro-1-methyl-1(17'-5' β -androstan-3' β -ol)-2-propenol (59)

This compound was prepared from the difluoro analog 53 by Spahic (1981) according to the procedures established for the preparation of 56a and 56b.

2-(2'-fluoroethenyl)-1,2,3,4-tetrahydro-2-napthol (60a, 2'-E and 60b, 2'-Z).

To a cooled (0^{o}) solution of the difluoro analog 54 (315mg, 1.5mmole) in 6ml of ether was added 715ul (1.7mmole) of n-butyllithium (2.38M in hexane). The reaction was then allowed to warm to room temperature and stirred for 15 min. before cooling back down to 0^{o} and adding the lithium aluminium hydride (85mg, 2.25mmole) all at once. After stirring for 24 hrs. the reaction was judged complete by GLC, and subsequently quenched with 85ul of water followed by 85ul of 15% NaOH and an additional 255ul of water. After stirring for several min., a small amount of anh. magnesium sulfate was added, the mixture filtered, and the solvent removed (rotoevaporation). Purification by LOBAR chromatography (20% EtOAc/Hexanes, 8ml/min., 33-45 and 51-55 min. elution time for the 2'-E and 2'-Z isomers respectively) gave 85% combined yield in a 9 : 1 ratio of E : Z; TLC (System A, 20% EtOAc/Hexanes, R_f = 0.23 and 0.31 for the 2'-Z and 2'-E isomers respectively); GLC (150^{o}, t_r = 4.30 and 5.63 min. for 2'-Z and 2'-E isomers respectively); IR (film) 3400 cm^{-1} (b, OH), 1670 (s, C=CHF), 1500,, 1450, 1100, 750 (s, four adjacent aromatic hydrogen atoms); 60a ^{1}H-NMR ($CDCl_3$) 1.53 (s, 1H, OH), 1.83 - 2.09 (m, 2H, C-3 CH_2), 2.81-3.20 (m, 4H, C-1 CH_2 and C-4 CH_2), 5.60 (dd, 1H, J = 20.5 and 11Hz, C-1' vinyl H), 6.79 (dd, 1H, J = 84 and 11Hz, C-2' vinyl H), 7.11 (s, 4H, aromatic H's), ^{19}F-NMR ϕ 135 (dd, 1F, J = 84 and 21Hz, C=CHF); 60b ^{1}H-NMR 1.98 - 2.18 (m,

3H, OH and C-3 CH_2), 2.82 - 3.14 (m, 4H, C-1 CH_2 and C-4 CH_2), 4.97 (dd, 1H, J = 47 and 5Hz C-1' vinyl H), 6.42 (dd, 1H, J = 84 and 5Hz, C-2' vinyl H), 7.11 (s, 4H, aromatic H's,) ^{19}F-NMR ϕ 125 (dd, 1F, J = 84 and 47Hz, C=CHF); EI (of each isomer) 192 (M), 174 (M-H_2O), 128 (M-H_2O-CH_2=CHF), 115.

Anal. Calcd. for $C_{12}H_{13}FO$; C, 74.98; H, 6.81.

Found for isomeric mixture: C, 74.84; H, 7.02.

2-(2',2'-difluoroethenyl)-2-acetoxy-1,2,3,4-tetrahyhronaphalene (64).

Difluoro vinyl alcohol 54 (105mg, 0.5mmole)in 3ml of ether was combined with triethylamine (105ul, 0.75mmole), 4-dimethylamino pyridine (61mg, 0.5mmole) and acetic anhydride (118ul, 1.25mmole), and stirred for 24 hrs. at room temperature. The reaction as judged complete by GC and TLC was subsequently taken up in ether and washed with 5% NaOH, 0.1N HCl, and water. Drying over magnesium sulfate and solvent removal afforded 123mg of a clear, yellow oil. Purification by LOBAR chromatography (15% EtOAc/Hexanes, 8ml/min., 18-21 min. elution time) gave 66mg of a clear colorless oil; TLC (System A, 20% EtOAc/Hexanes, R_f = 0.64); GLC (150°, t_r = 2.63 min.); IR (film) 1750 cm^{-1} (bvs, C=O and $CH=CF_2$), 1230, 1010, 740 (s, four adjacent aromatic hydrogen atoms); ^{1}H-NMR ($CDCl_3$) 1.96 (s, 3H, COMe), 2.00 - 2.95 (m, 4H, C-3 and C-4 CH_2), 3.29 (bs, 2H, C-1 CH_2), 4.72 (dd, 1H, J = 27 and 5Hz, $CH=CF_2$), 7.10 (s, 4H, aromatic H's); ^{19}F-NMR ($CDCl_3$) ϕ 80.7

(dd, 1F, J = 37 and 27Hz, C=CFcis), ϕ 83.8 (dd, 1F, J = 37 and 5Hz, C=CF trans; EI 192 (M-HOAc), 189 (M-HC=CF_2), 141 (M-HOAc-HCF_2), 129 (M-HOAc-HC=CF_2), 115.

Anal. Calcd. for $C_{14}H_{14}F_2O_2$; C, 66.66; H, 5.59.

Found: C, 66.51; H, 5.63.

2-(2'-acetoxy-2',2'-difluorothylidenyl)-1,2,3,4-tetrahydronapthalene (65, E and Z isomers) and 2(2'-tosyl-2',2'-difluoroethylidenyl)-1,2,3,4-tetrahydronaphalene (66, E and Z isomers)

p-Toluenesulfonic acid (150mg) was added to a solution of tertiary acetate 64 (378mg, 1.5mmole) in 3.5ml of acetic anhydride at room temperature. When the reaction was complete by GLC analysis, the mixture was taken up in pentane and washed with 5% NaOH, water, and brine, dried over magnesium sulfate, and the solvent removed to give 218mg of a clear colorless oil. Purification by LOBAR chromatography (20% EtOAc/Hexanes, 6ml/min., 20-23 min. (65) and 26-31 min. (66) elution times) yielded 140mg (37%) of 65 and 71mg (13%) of 66; TLC (System A, 20% EtOAc/Hexanes, R_f = 0.55 (65) and R_f = 0.50 (66)); GLC (160°, t_r = 7.12 and 8.62 for 65 (isomers), 66 not stable to GLC); 65 IR (film) 1750 cm^{-1} (bvs, C=O), 1380(s), 1250, 1200, 1050(s), 740 (s, four adjacent aromatic hydrogen atoms); 66 IR (film) 1410 (s), 1250, 1200(s), 1180, 1080(s), 1125(s), 890(s),740, 710, 660; 65 (both isomers together) ^{1}H-NMR ($CDCl_3$) 2.11 and 2.13 (2s,

each peak corresponds to COMe of each isomer), 2.42 - 2.95 (m, 4H, C-3 and C-4 CH_2), 3.52 and 3.77 (2d, J = 2Hz, each doublet corresponds to C-1 CH_2 of each isomer), 5.73 (bt, 1H, J = 10Hz, C-1' vinyl H), 7.13 (s, 4H, aromatic H's), ^{19}F-NMR ($CDCl_3$) ϕ 63.8 and 64.2 (2d, J = 10Hz, each doublet corresponds to C-2' fluorines of each isomer); 66 (both isomers together) ^{1}H-NMR ($CDCl_3$) 2.43 (s, 3H, aromatic Me), 2.35 - 2.88 (m, 4H, C-3 and C-4 CH_2), 3.46 and 3.60 (2d, J = 2Hz, each doublet corresponds to C-1 CH_2 of each isomer), 5.60 (bt, 1H, J = 10Hz, C-1' vinyl H), 7.13 and 7.10 (2s, each peak corresponds to the 4 aromatic hydrogens of ring system), 7.57 (dd, 4H, J = 44 and 8Hz, A_2B_2 aromatic hydrogens), ^{19}F-NMR ϕ 58.6 and 58.9 (2d, J = 10Hz, each doublet corresponds to C-2' fluorines of each isomer); 65 EI 252 (M), 192 (M-HOAc), 141 8M-HOAc-HCF_2), 129 (M-HOAc-$CHCF_2$), 115; 66 EI 192 (M-TsOH), 172 (TsOH), 141, 115, 91 (tropylium), no M observed.

Anal. 65 Calcd. for $C_{14}H_{14}F_2O_2$; C, 66.66; H, 5.59.

Found, C, 66.33; H, 5.59.

Anal 66 Calcd. for $C_{19}H_{18}F_2O_3S$; C, 62.62; H, 4.98.

Found: C, 62.38; H, 4.99.

Preparation of 65 and 66 by an alternate synthesis

Tertiary acetate 64 (126mg, 0.5mmole) in 3ml of 1,2-diemthoxyethane was treated with an excess of p-

toluenesulfonic acid (190mg, 1.0mmole) at room temperature. After completion of the reaction it was worked up by adding 1ml of water and then concentrating the reaction mixture by rotoevaporation. Subsequently it was taken up in ether and washed with saturated $NaHCO_3$, water and brine, dried over magnesium sulfate, and the solvent removed to give a clear yellow oil. Purification by LOBAR chromatography (15% EtOAc/Hexanes, 6ml/min., 24-29 min. (65) and 31-38 min. (66) elution times) yielded 32mg (25%) of 65 and 83mg (49%) of 66. Both products (65 and 66) were identical to those prepared in the previous reaction.

1-(2',2'-difluoroethenyl)-1,5,9-trimethyl-4,8-decadiene acetate (62)

This compound was prepared from the difluoro vinyl alcohol 48 according to the procedures of Vinson (1978) in 93% yield; ^{1}H-NMR ($CDCl_3$) 1.63 and 1.70 (2bs, 12H, vinyl Me), 1.97 (m, 8H, allylic CH_2), 2.02 (s, 3H, COMe), 4.67 (dd, 1H, J = 27 and 5Hz, $CH=CF_2$), 5.17 (m, 2H, vinyl H).

1,Difluoro-3,7,11-trimethyl-2(E and Z), 6(E), 10-dodecatriene acetate (63) and 1,1-Difluoro-3,7,11-trimethyl-2(E and Z), 6(E), 10-dodecatriene p-toluenesulfonate (71)

Tertiary acetate 62 (150mg, 0.5mmole) in 3ml of 1,2-dimethoxyethane was treated with an excess of p-toluenesulfonic acid (247mg, 1.3mmole) at room temperature.

The progress of the reaction was monitored by GLC and after 24 hrs the reaction was worked up by taking up in ether and washing with saturated $NaHCO_3$, water, and brine, drying over magnesium sulfate, and the solvent removed to give 173mg of a clear yellow oil. Purification by LOBAR chromatography (10% EtOAc/Hexanes, 6ml/min., 21-25 min. (63) and 28-35 min. (71) elution times) gave 35mg (23%) of 63 and 93mg (45%) of 71. Product 63 was identical to that prepared by Vinson (1978). 71 TLC (System A, 10% EtOAc/Hexanes, R_f = 0.54); GLC (150°, not stable); IR (cells) 1390cm^{-1}, 1250, 1200, 1180, 1100, 1020, 650, 570; ^{1}H-NMR ($CDCl_3$) 1.58 and 1.67 (2s, 9H, vinyl CH_3), 1.75 (m, 3H, C-3Me), 1.86 - 2.22 (m, 8H, allylic CH_2), 2.45 (s, 3H, aromatic Me), 5.05 (m, 2H, vinyl H), 5.40 (bt, 1H, J = 10Hz, C-2 vinyl H), 7.60 (dd, 4H, J = 40 and 8Hz, A_2B_2 aromatic hyrogens); ^{19}F-NMR ($CDCl_3$) ϕ 58.4 and 58.6 (2d, J = 10Hz, each doublet corresponds to the C-1 fluorines of each isomer); EI 412 (M), 240 (M-TsOH), 197, 172 (TsOH), 91 (tropylium).

Anal. Calcd. for $C_{22}H_{30}O_3F_2S$; C, 64.05; H, 7.33.

Found: C, 63.84; H, 7.36.

1-(2'-fluoroethenyl)-1,5,9-trimethyl-4,8-decadiene acetate (72).

Alcohol 56a (240mg, 1.0mmole), acetic anhydride (2.5mmole), 4-dimethylamino pyridine (122mg, 1.0mmole), and triethylamine (1.5mmole) were combined in 5ml of ether and stirred for

2 days at room temperature under N_2. The reaction was judged complete by TLC and GLC analysis. The reaction mixture was then taken up in 100ml of ether, washed with 5% NaOH, and 0.1N HCl, dried over magnesium sulphate, and the solvent removed to provide 280mg (99%) of a clear yellow oil. Although fairly homogenous by GLC and TLC analysis, LOBAR chromatography (5% EtOAc/ Hexanes, 8ml/min., 21-26 min. elution time) gave a clear colorless oil; TLC (System A, 5% EtOAc/Hexanes, R_f = 0.37); GLC (150°, t_r = 7,5 min.); IR (film) 1750cm^{-1} (s, C=O) and 1680 (s, C=CHF); ^{1}H-NMR ($CDCl_3$) 1.57 (s, 3H, C-1Me), 1.63 and 1.70 (2s, 9H, vinyl CH_3), 1.98 (s, 3H, COMe), 2.03 (bm, 8H, allylic CH_2), 5.17 (m, 2H vinyl H), 5.50 (dd, 1H, J = 21 and 12Hz, C-1' vinyl H), 6.75 (dd, 1H, J = 84, and 12Hz, C-2' vinyl H); ^{19}F-NMR ($CDCl_3$) ϕ 133 (dd, 1F, J = 84 and 21Hz, C=CHF); CIMS 283 (MH+), 263 (MH+-HF), 223 (MH+ -HOAc), 203 (MH+ -HF-HOAc).

Anal. Calcd. for $C_{17}H_{27}O_2F$; C, 72.30; H, 9.64.

Found: C, 72.55; H, 9.59.

2-(2'-fluoroethenyl)-2-acetoxy-1,2,3,4-tetrahydronapthalene (74)

To monofluoro vinyl alcohol 60a (105mg, 0.5mmole) in 3ml of anhyd. ether was added triethylamine (105ul, 0.75mmole), 4-diemthylamino pyridine (61mg, 0.5mmole), and acetic anhydride (118ul, 1.25mmole) in that order. After stirring for 24 hrs. the reaction was judged complete and taken up in

ether, washed with 5% NaOH, 0.1NHCl, and water, dried over magnesium sulfate and the solvent removed to give 157mg of a clear yellow oil. LOBAR chromatography (15% EtOAc/Hexanes, 8ml/min., 17-19 min. elution time) afforded 88mg (75%) clear, colorless oil; TLC (System A, 15% EtOAc/Hexanes, R_f = 0.40; GLC (150°, t_r = 6.75 min.); IR (film) 1740cm^{-1} (s, C=O), 1670 (s, C=CHF), 1490, 1230 (s), 740 (s, four adjacent aromatic hydrogen atoms); ^{1}H-NMR($CDCl_3$) 1.97 (s, 3H, COMe), 1.93 - 2.98 (m, 4H, C-3 and C-4 CH_2), 3.26 (bs, 2H, C-1 CH_2), 6.77 (dd, 1H, J = 83 and 12Hz C-2' vinyl H), 5.89 (dd, 1H, J = 21 and 12Hz, C-1' vinyl H), 7.14 (s, 4H, aromatic hydrogens); ^{19}F-NMR ($CDCl_3$) ϕ 130 (dd, 1F, J = 83 and 21Hz, C=CHF); EI 174 (M-HOAc), 129 (M-HOAc-HC=CHF), 115, no M observed.

Anal. Calcd. for $C_{14}H_{15}O_2F$; C, 71.77; H, 6.45.

Found: C, 72.23; H, 6.54.

Nerolidol acetate(76)

This compound was prepared from nerolidol by the same procedures used to prepare product 64. Purification by LOBAR chromatography (5% EtOAc/Hexanes, 8ml/min., 15-19 min. elution time) gave a clear, colorless oil; TLC (System A, 5% EtOAc/Hexanes, R_f = 0.45); GLC (150°, t_r = 4.69 and 5.63 min. for the two isomers); IR (film) 1750cm^{-1} (s, C=O), 1370 (m), 1240 (s); ^{1}H-NMR ($CDCl_3$0) 1.55 (bs, 3H, C-3Me), 1.62 and 1.70 (2s, 9H, vinyl CH_3), 1.92 (m, 8H, allylic

CH_2), 2.00 (s, 3H, COMe), 5.20 (m, 4H, vinyl H), 6.08 (dd, 1H, J = 18 and 10Hz, C-2 vinyl H); CIMS 205 (MH+ -HOAc), no parent ion observed.

3,7,11-trimethyl-2(E and Z), 6(E and Z), 10-dodecatriene acetate (77)

A mixture of isomers of tertiary acetate 76 (132mg, 0.5mmole) was stirred in 1ml of acetic anhydride at room temperature and then about 10mg of p-toluenesulfonic acid added. After 4 hrs. the reaction was judged complete by TLC and GLC analysis. The product was taken up in pentane and washed with 5% NaOH, water, and brine dried over magnesium sulfate and the solvent removed to furnish 106mg of a pale yellow oil. Purification by LOBAR chromatography (5%, EtOAc/Hexanes, 6ml/min., 12-15 min. and 15-33 min. elution times) gave essentially two fractions. The first fraction was composed of hydrocarbons (TLC, GLC, IR and NMR analysis), and the second fraction (20% yield) was a mixture of isomers of 77; TLC (System A, 5% EtOAc/Hexanes, R_f = 0.40); GLC (150°, t_r = 8.44, 9.84, and 11.25 min. for the four isomers); IR (film) 1750 cm^{-1} (s, C=O), 1230 (s), 1120; ^{1}H-NMR ($CDCl_3$) 1.62 and 1.70 (2s, 12H, vinyl CH_3), 2.05 (s, 3H, COMe), 2.08 (m, 8H, allylic CH_2), 4.62 (d, 2H, J = 7Hz, C-1 allylic CH_2), 5.25 (m, 3H, vinyl H); CIMS 205 (MH+ -HOAc), no parent ion observed.

Diethyl 1,1-difluoro-3,7,11-trimethyl-2(E and Z), 6(E), 10-dodecatriene phosphate 82 and 1,1-difluoro-1-chloro-3,7,11-

trimethyl-2(E and Z) 6(E), 10-dodecatriene (83)

To study the effects of solvents on the reaction for the preparation of these compounds (and 87) the following procedures were followed. To 0.25mmole of alcohol 48 (or 54) in 1ml of solvent (HMPA, pentane, benzene, or THF) at 0° was added 0.26mmole of n-butyllithium (1.54M in hexane). After stirring for 15 min., 0.2mmole of diethyl phosphochloridate was introduced and the reaction allowed to equilibrate to room temperature. Twelve hours later the reaction was worked up by taking up in ether, washing with water and brine, drying over magnesium sulfate and the solvent removed (rotoevaporation). Fluorine NMR were subsequently run and the peaks representing 82 (ϕ 55) and 83 (ϕ 42) compared. For analytical and spectroscopic chracterization of 82 and 83 see Vinson (1978, pg. 119).

Diphenyl 1,1-difluoro-3,7,11-trimethyl-2(E and Z), 6(E), 10-dodecatriene phosphate (84)

This compound was prepared according to the procedures of Vinson (1978, pg. 120); ^{1}H-NMR ($CDCl_3$) of the 2E and 2Z isomers together 1.60 and 1.70 (2s, 9H, vinyl CH_3), 1.80 (m, 3H, C-3Me), 1.97 -2-.26 (bm, 8H, allylic CH_2), 5.03 (m, 2H, vinyl H), 5.50 (bt, 1H, J = 10Hz, C-2 vinyl H), 7.23 (s, 10H, aromatic H's), ^{19}F-NMR ($CDCl_3$) ϕ 54.2 and 54.7 (2vbm, each multiplet corresponds to C-1 fluorines of each isomer). For further analytical and spectroscopic characterization see Vinson (1978, pg. 120).

Diethyl 1-fluoro-3,7,11-trimethyl-2(E and Z), 6(E) 10-dodecatriene phosphate (85)

This compound was prepared from monofluoro vinyl alcohol 56a by Vinson (1978, pg. 119). Attempts to repeat these procedures always resulted in significantly low yields (<5% isolated).

Diphenyl 1-fluoro-3,7,11-trimethyl-2(E and Z), 6(E) 10-dodecatriene phosphate (86)

Monofluoro vinyl alcohol 56a (240mg, 1.0mmole) in 5ml of THF was stirred with triethylamine (279ul, 2.0mmole) and 4-dimethylaminopyridine (305mg, 2.5mmole) added in that order, 10 min. apart. After 45 min. the reaction was cooled to 0^{o} and then the diphenyl phosphocloridate (518ul, 2.5mmole), which had been briefly treated with anhydrous potassium carbonate, was slowly added. The reaction was allowed to warm to room temperature and continued for 5 days before complete. Workup involved taking the mixture up in ether, washing with 0.1N HCl, 5% NaOH, water, and brine, drying over magnesium sulfate, and the solvent removed (rotoevaporation). Purification by LOBAR chromatography (10% EtOAc/Hexanes, 6ml/min., 36-39 min. (2Z) and 46-50 min., (2E) elution time) gave separation of the isomers as clear, colorless oils (30% combined yield, predominantly the 2E isomer); TLC (System A, 20% EtOAc/Hexanes, R_f = 0.38 and 0.44 for the 2E and 2Z isomers respectively); IR (film) 2970, 2910, 2850, 1680, 1600, 1500, 1280, 1180, 1040, (vbs)

940 (vbs), 770, 680; 2E isomer ^{1}H-NMR ($CDCl_3$) 1.57 and 1.66 (2s, 12H, vinyl CH_3), 2.00 (m, 8H, allylic CH_2), 5.05 (m, 2H, vinyl H), 5.38 (bt, 1H, J = 8Hz, C-2 vinyl H), 6.65 (dt, 1H, J = 55 and 8Hz, C-1 allylic H), 7.24 and 72.6 (2s, 10H, aromatic H's), ^{19}F-NMR ($CDCl_3$) ϕ 112.5 (bd, 1F, J = 55Hz, C-1 F); 2Z isomer ^{1}H-NMR (CDCld3) 1.59 and 1.68 (2s, 9H, vinyl CH_3), 1.78 (dd, 3H, J = 4.6 and 1.0Hz, C-3 Me) 1.99 - 2.27 (m, 8H, allylic CH_2), 5.07 (m, 2H, vinyl H), 5.41 (bt, 1H, J = 8Hz, C-2 vinyl H), 6.66 (dt, 1H, J = 55 and 8Hz, C-1 allylic H), 7.28 (s, 10H, aromatic H's), ^{19}F-NMR ($CDCl_3$) ϕ 111.3 (bd, 1F, J = 55Hz, C-1F); EI (of each isomer) 452 (M-HF), 250 (HO-P(O)$(OPh)_2$), 222 (M-HO-P(O)$(OPh)_2$), 202 (M-HF-HO-P(O)$(OPh)_2$), no parent ion observed.

Anal. Calcd. for $C_{27}H_{34}O_4FP$; C, 68.62; H, 7.25.

Found: 2E isomer C, 68.73; H, 7.34; for mixture of isomers C, 68.93; H, 7.46.

2-(2'-oxydiethylphosphono-2',2'-difluorethylidenyl)-1,2,3,4-tetrahydronapthalene (87 E and Z isomers)

n-Butyllithium (498ul of a 2.38M hexane solution, 1.2mmole) was introduced to a cooled (0°) solution of difluoro vinyl alcohol 54 in 5ml of benzene. The reaction was allowed to warm to room temperature and stirred for 45 min. before cooling back down to 0° and the diethyl phosphochloridate (173ul, 1.2mmole) added. The reaction was maintained at 0° before allowing to return to room temperature. After 24

hrs. the mixture was transferred to a separatory funnel, extracted with pentane, washed with water, saturated sodium bicarbonate and brine, dried over magnesium sulfate, and the solvent removed to give 400mg of a clear yellow oil. Purification by LOBAR chromatography (30% EtOAc/Hexanes, 8ml/min., 31-37 min. elution time) afforded 69mg (19% yield of a clear, colorless oil (the Z and E isomers were not separable); TLC (System A, 20% EtOAc/Hexanes, R_f = 0.13); IR (film) 2970cm^{-1}, 2930, 2900, 1740, 1670, 1360, 1280, 1250, 1120, (vbs), 740; ^{1}H-NMR ($CDCl_3$) 1.33 (t, 6H, J = 7Hz, $POCH_2\underline{CH_3}$), 2.47-2.90 (bm, 4H, C-3 and C-4 CH_2), 3.51 and 3.80 (2d, J = 2Hz, each peak corresponds to C-2' CH_2 of each isomer), 4.18 (p, 4H, J = 7Hz, $PO\underline{CH_2}CH_3$), 5.72 (bt, 1H, J = 7Hz, C-1' vinyl H), 7.12 (s, 4H, aromatic H's); ^{19}F-NMR ϕ 55.1 and 55.3 (2vbm, each multiplet corresponds to C-2' fluorines of each isomer); EI 326 (M-HF), 192 (M-HO-P(O)$(OEt)_2$), 155 (HOP(OH+)$(OEt)_2$), 115, no parent ion observed.

Anal. Calcd. for $C_{16}H_{21}F_2O_4P$; C, 55.49; H, 6.11.

Found for mixture of isomers: C, 55.39; H, 6.03.

2-(2'-oxydiphenylphosphono-2',2'-difluorothylidenyl)-1,2,3,4-tetrahydronapthalene (88 E and Z isomers).

Triethylamine (140ul, 1.0mmole) and 4-dimethylamino pyridine (153mg, 1.25mmole) were added to difluoro vinyl alcohol 54 (105mg, 0.5mmole) in 3ml of THF at room temperature. The

reaction was stirred for 45 min. before cooling to 0^{o} and subsequently adding the diphenyl phosphochloridate (259ul, 1.25mmole). The resulting slurry was allowed to gradually warm to room temperature and stirred for 24 hrs. before working up by taking up in ether, washing with 0.1N HCl, 5% NaOH, water and brine, drying over magnesium sulfate and the solvent removed to give 348mg of a clear yellow oil. Purification by LOBAR chromatography (15% EtOAc/Hexanes, 6ml/min., 32-41 min. elution time) afforded 132mg (of a clear colorless oil[10]; TLC (System A, 15% EtOAc/Hexanes, R_f = 0.20); IR (film) 3050 cm^{-1}, 2930, 2840, 1760, 1600, 1490, 1310 (vbs), 1180 (vbs), 1060, 960, 750, 690; ^{1}H-NMR ($CDCl_3$) 2.17 - 2.83 (m, 4H, C-3 and C-4 CH_2), 3.44 and 3.72 (2d, J = 2Hz, each doublet corresponds to C-1 CH_2 of each isomer), 5.69 (bt, 1H, J = 10Hz, C-1' vinyl H), 7.10 and 7.12 (2s, 4H, each peak corresponds to aromatic H's of each isomer), 7.26 (s, 10H, aromatic (phenyl) hydrogens); ^{19}F-NMR ($CDCl_3$) ϕ 54.9 and 55.2 (2vbm, each multiplet corresponds to C-2' fluorines of each isomer); EI 422 (M-HF), 251 (M-HOP(OH+)$(OPh)_2$), 192 (M-HOP(O)$(OPh)_2$), 115, no parent ion observed.

Anal. Calcd. for $C_{24}H_{21}O_4F_2P$; C, 65.12; H, 4.79.

(10) A small amount of the E and Z isomeric mixture was chromatographed again (LOBAR) and gave separtion of the isomers. The proton NMR of one of the isomers confirms the previous assignments of the C-1 CH_2; ^{1}H-NMR ($CDCl_3$ 2.59 - 2.84 (m, 4H, C-3 and C-4 CH_2), 3.46 (d, 2H, J = 2Hz, C-1 CH_2), 5.71 (bt, 1H, J = 10Hz, C-1' vinyl H) 7.11 8s, 4H, aromatic H's), 7.26 (s, 10H, aromatic (phenyl) hydrogens).

Found for mixture of isomers: C, 64.96; H, 4.95.

2-(2'-oxydiphenylphosphono-2'-fluoroethylidenyl); 1,2,3,4-tetrahydronapthalene (90 E and Z isomers).

To monofluoro vinyl alcohol 60a (163mg, 0.85mmole) in 5ml of THF was added triethylamine (237ul, 1.7mmole) and 4-dimethylaminopyridine (259mg, 2.12mmole) at 10 min. intervals. After stirring 15 min. at room temperature the diphenyl phoshochloridate (439ul, 2.12mmole) which had been briefly treated with anhydrous potassium carbonate, was slowly added. An immediate white slurry resulted. The reaction was continued for 3 days before complete. Workup included taking the mixture up in ether and water, and washing the organic phase with 0.1N HCl, 5% NaOH, water and brine. Normal drying with magnesium sulfate and solvent removal (rotoevaporation) gave 278mg of a clear, yellow oil Purification by LOBAR chromatography (15% EtOAc/Hexanes, 6ml/min., 41 - 50 and 52 - 62 min. elution for the E and Z isomers) provided 109mg (30% combined yield) of clear, colorless oils; TLC (System A, 15% EtOAc/Hexanes, R_f = 0.17 and 0.14); IR (film) 3050cm^{-1}, 2930, 2900, 1600, 1500, 1300, 1280, 1180, 950, 750; 52-62 ^{1}H-NMR ($CDCl_3$) 2.41 - 2.83 (m, 4H, C-3 and C-4 CH_2), 3.47 (bd, 2H, J = 2Hz, C-1 CH_2), 5.58 (bt, 1H, J = 8Hz, C-1' vinyl H), 6.79 (dt, 1H, J=55 and 8Hz, C-2' allylic H), 7.11 (s, 4H, aromatic H's), 7.26 (s, 10H, aromatic (phenyl) hydrogens); ^{19}F-NMR ($CDCl_3$) ϕ 113.5 and 13.7 (2bd, 1F, J = 55, each doublet corresponds to C-2'

fluorine of each isomer); EI 404 (M-HF), 250 ($HOP(O)(OPh)_2$), 154 (M-HF-$HOP(O)(OPh)_2$), 115, no parent ion observed.

Anal. Calcd. for $C_{24}H_{22}O_4FP$; C, 67.91; H, 5.23.

Found for mixture of isomers: C, 67.64; H, 5.27.

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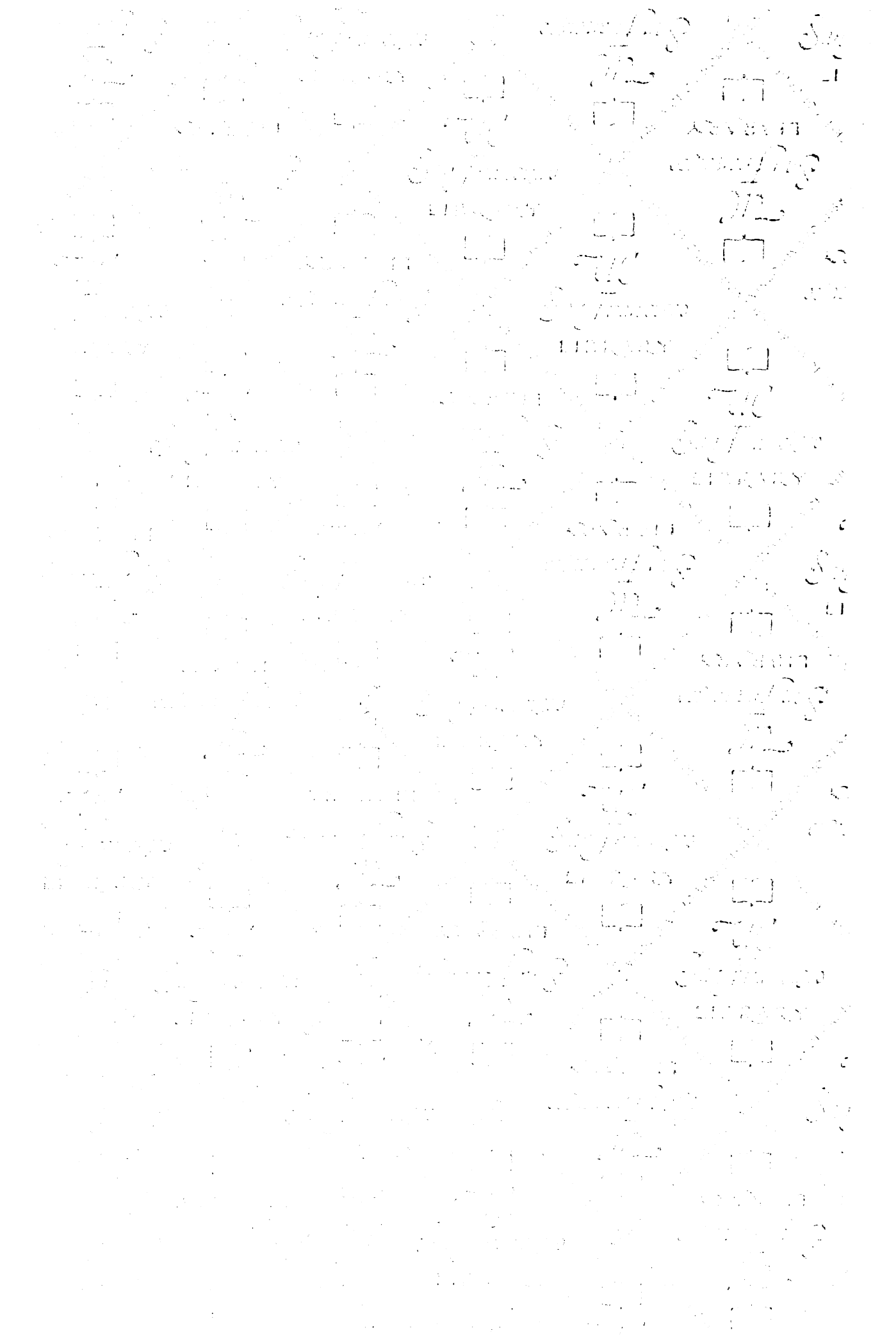

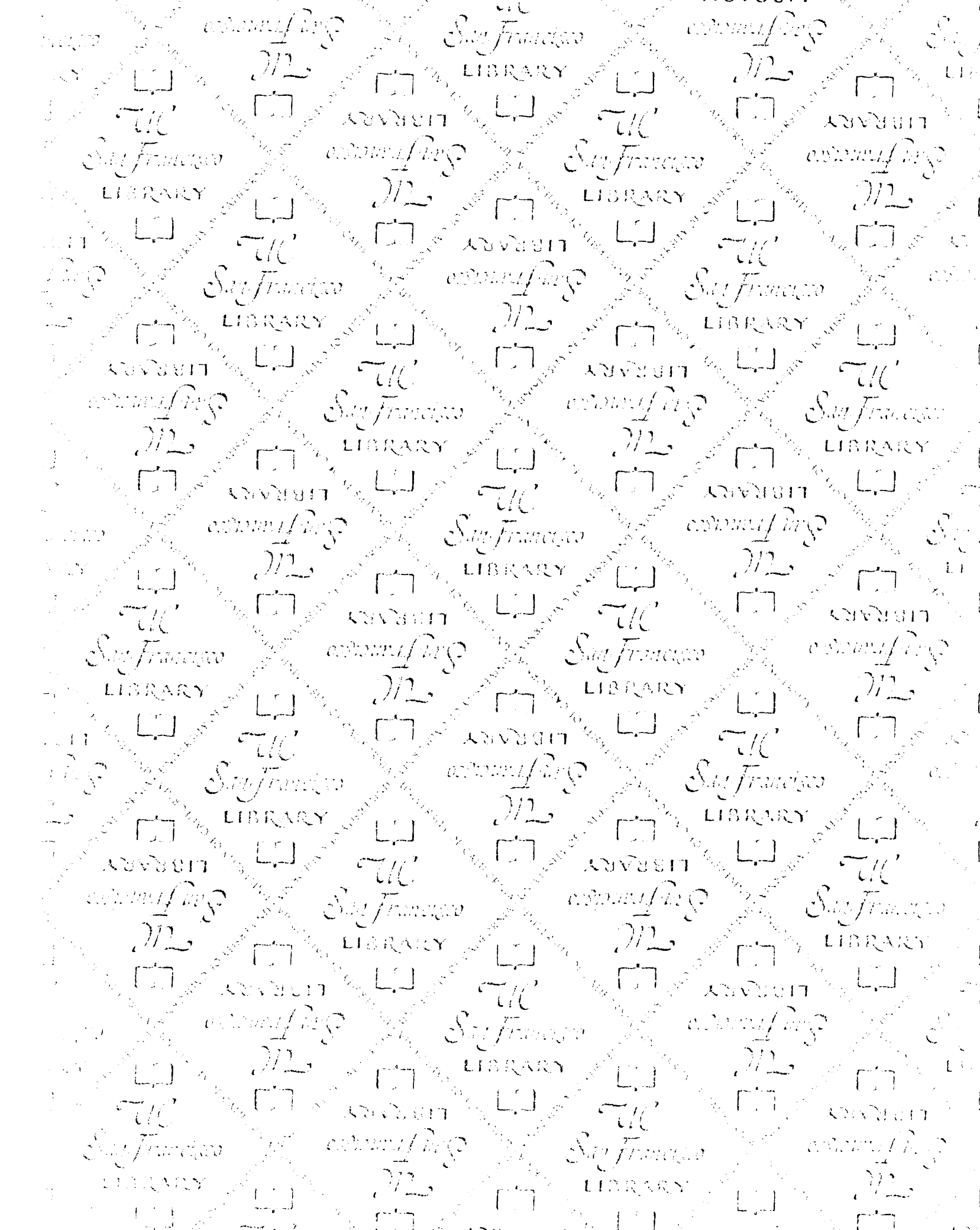
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