1988

Aroyl phosphates : The Mechanism of Isomerization

Sandra Xiu Yan Ning

AROYL PHOSPHATES:
THE MECHANISM OF ISOMERIZATION

BY

SANDRA XIU YAN NING

A thesis submitted

in partial fulfillment of the requirments for

the degree Master of Science

Major in Chemistry

South Dakoda State University

1988

AROYL PHOSPHATES:

THE MECHANISM OF ISOMERIZATION

This thesis is a pproved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirments for this degree. Acceptance of this thesis does not imply that the conclutions reached by the candidate are necessarily the conclusions of the major department.

William s. Wadsworth, Jr Date
Thesis Adviser

David C Hilderbrand Date
Head, Chemistry Department

ACKNOWLEGEMENTS

I like to give my special thanks to my adviser Dr. wadsworth whose patience and guidance made this all possible.

I would also like to express my gratitude to all my professors and friends who made my years at SDSU an enjoyable and rewarding experience.

To my sponsors, uncle and aunt Z. C. Wu

and my parents Mr. and Mrs. Rou-Ren Ning

TABLE OF CONTENTS

AROYL PHOSPHATES:
THE MECHANISM OF ISOMERIZATION

INTRODUCTION

The transfer of energy and its conversion from one form to another are very important in biological systems. Mammals use energy to control their environment, i.e. keeping warm and physical activity, whereas bacteria use energy primarily in chemical synthesis for their very existence as a life form depends upon rapid reproduction.

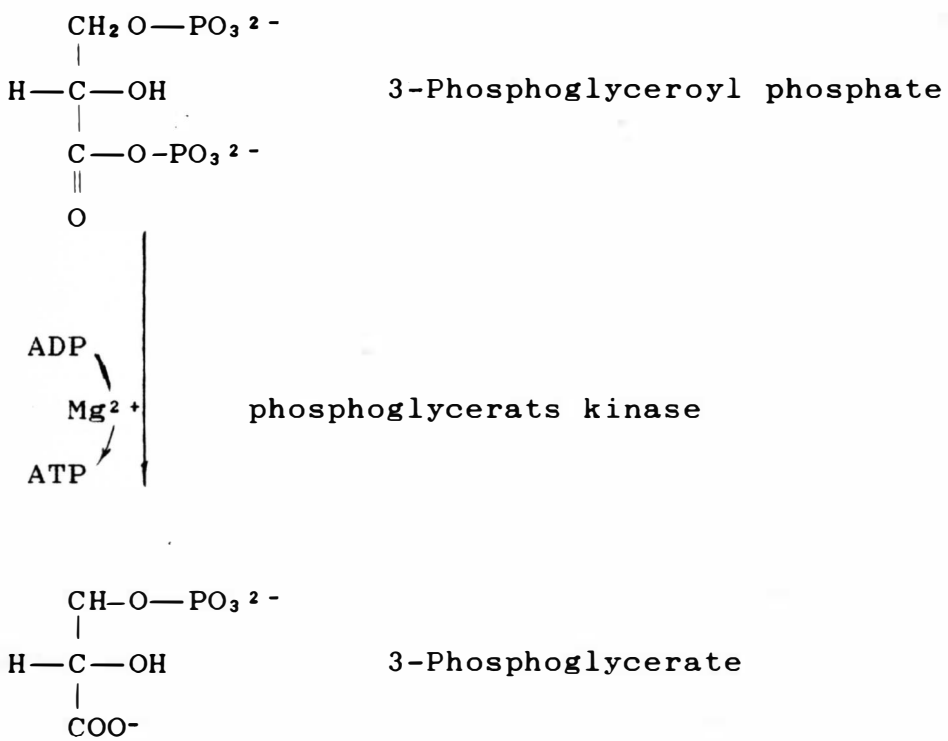
There are two original sources of energy: light and oxygen, O_2. Photosynthesis and oxidative metabolism convert these energy sources via photosynthetic phosphorylation and oxidative phosphorylation into ATP (Adenine Triphosphate), which is in effect the molecular repository of energy. Thus, high energy phosphates(compounds which hydrolyze with a large negative free energy) are very important to biological systems.

Acyl phosphates are mixed anhydrides of carboxylic acids and phosphoric acids. They are very important members of the group of high energy phosphate compounds found in biological system. They act as mediators in the transfer of

energy and are important biological acylating and phosphorylating agents. A study of the chemistry of this group of compounds is important if we are to understand how cells creat and use energy.

A typical example of a reaction of an acyl phosphate is outlined in scheme.1.[1]

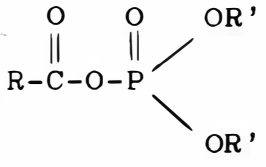
Scheme.1.



In this reaction, 3-phosphoglyceroyl phosphate reacts with ADP (adenine diphosphate) catalyzed by Mg2+ and 3-phosphoglyceroyl phosphate kinase to give ATP and 3-phosphoglycerate. Although the reaction begins with two phosphoryl groups, only one, the acyl phosphate reacts during the reaction, the acyl phosphate. Obviously acyl phosphates are a high energy moiety, more reactive than a simple alkyl phosphate. The energy change for this reaction is -4.5kj/mol which is fairly high, and not unexpected for the reaction of a high energy compound.

The general structure of an acyl phosphate is shown in Fig.1.

Fig.1.



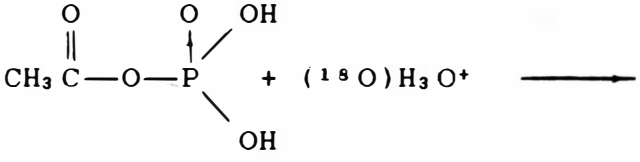
From the structure it is not obvious as to why an acyl phosphate is highly reactive. It should be pointed out that these is no relationship between reactivity, a kinetic parameter,and total energy change, a thermodynamic parameter.

HISTORICAL

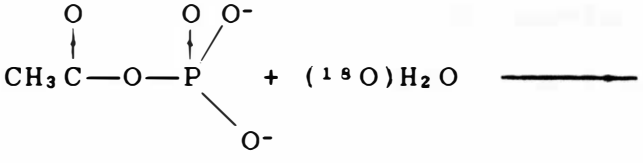
Research on acyl phosphates was started in the early 1900's. Progress was very slow and a study of mechanisms by which acyl phosphates react was only recently undertaken.

In 1971, Klinman and Samuel reported the hydrolysis of acetyl phosphates[2]. By isotope labeling experiments, they discovered that these compounds undergo both C-O and P-O cleavage depending on the pH or the presence of a metal catalyst, Scheme.2.

Scheme.2.



$$(^{18}O)CH_3COOH + H_3PO_4 + H_3O^+$$



$$CH_3CO_2^- + (^{18}O)H_2PO_4^-$$

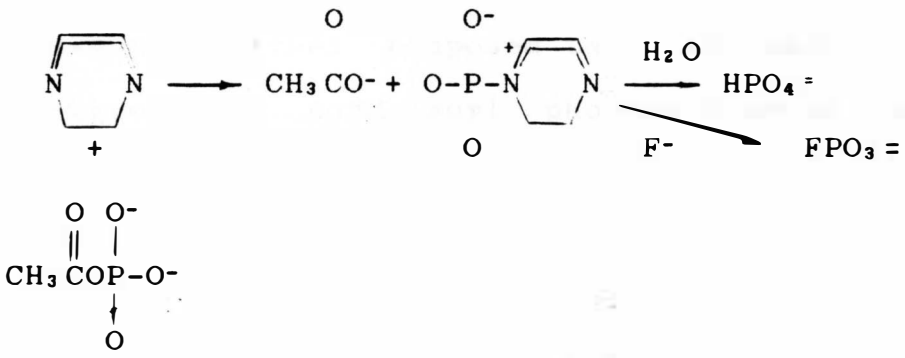
In their study, they carried out the hydrolysis($H_2{}^{18}O$) of acetyl phosphates in the presence of Mg^{2+} and Ca^{2+} over the pH range 7.4 to 8.2. They proposed that the breakdown of a divalent metal ion complexed acetyl phosphate (AcPM) occurred by a dissociative mechanism. They reported that the phosphate underwent P-O cleavage at neutral pH in the presence of Mg^{2+} and C-O breakage under both acidic and basic condition with Ca^{2+} catalysis.

In 1961 DiSabato and Jencks showed that the spontaneous nucleophilic reaction of an acetyl phosphate anion with n-butylamine, ammonia, glycine or hydroxylamine transferred the acyl group from the phosphate to the nucleophile.[3] They reported that the C-O bond cleavage proceeded predominantly through an acid catalyzed pathway while P-O bond breakage occurred under neutral or basic conditions.

Kluger and Wasserstein studied the chemistry of acetyl ethylene phosphate, and discovered that this compound was very reactive towards water.[4] They proposed that a neutral acetyl phosphate was a more reactive acylating agent than an anionic acetyl phosphate. The reactions of acetyl phosphate at neutral pH with nucleophilic agents such as hydroxylamine, aniline, morpholine, N-methylimidazole, glycine or glycylglycine underwent C-O bond cleavage, a cleavage which also proceeded under acidic condition. The

reaction with either pyridine, 4-methylpyridine, or triethylenediamine and probably trimethylamine gave P-O bond cleavage. This was proved by a trapping reaction, Scheme.3.

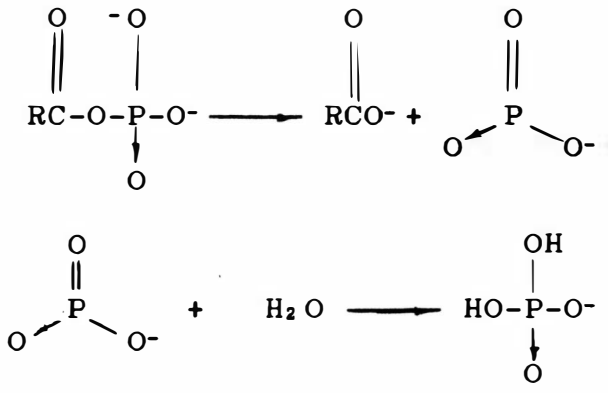
Scheme.3.



In the presence of fluoride, the formation of fluorophosphate suggested that these reactions represent nucleophilic catalysis of phosphoryl transfer.

Klinman and Samuel reported that the P-O cleavage of acetyl phosphate dianion and monoanion was a unimolecular process which involved the formation of a very reactive intermediate, metaphosphate ion, Scheme.4.

Scheme.4.

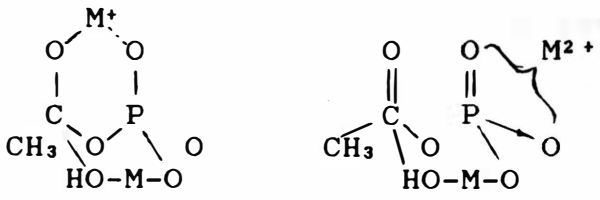


On this assumption they proposed that the metal complexed active intermediate AcPM (acyl phosphate metal complexed) has a cyclic structure, Fig.2.

.2. Fig

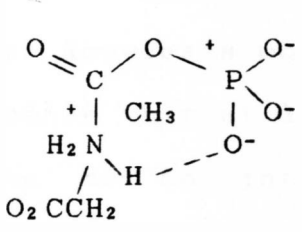
In the case of C-O cleavage, a tetrahedral cyclic species was proposed as an intermediate, Scheme.5.

Scheme.5.



In 1959 Kurz and Gutsche[5] proposed a quasi-six-membered ring transition state for the glycinolysis of acetyl phosphate, Fig.3.. The nucleophilic character of the nitrogen is increased by transfer of a proton to a phosphate oxygen.

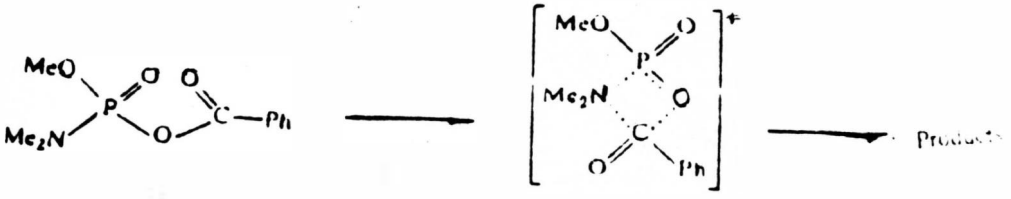
Fig.3.



In 1986 Symes and Modoro proposed an additional

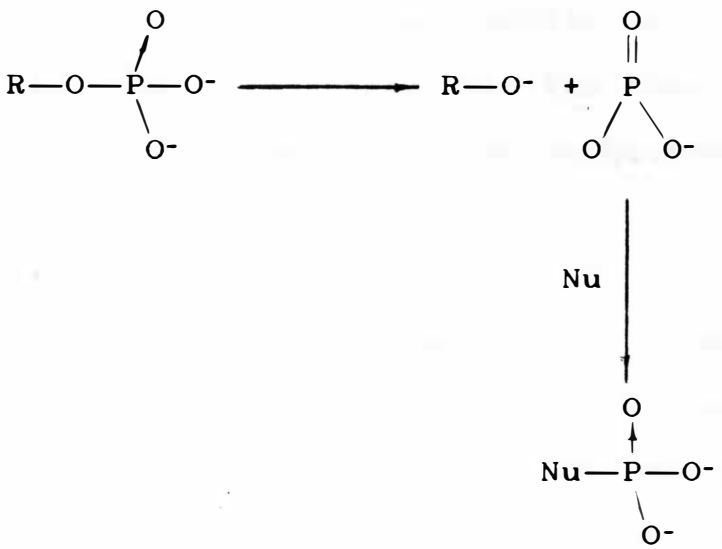
mechanism for the fragmentation of acyl phosphates.[6] The mechanism of the transfer of an acyl group from one nucleophilic center to another was proposed to proceed through a four member ring transition state,. Scheme.6.

Scheme.6.



Recently, chemists have became interested in dissociative processes. Knowles's most recent paper gave an overview and some evidence that at least in certain cases a metaphosphate ion can be an intermediate in solution, Scheme.7.[7]

Scheme.7.



In summary, most of the above proposed mechanisms involve dissociative processes. Even though they may be correct under certain reaction conditions, they are unlikely under biochemical conditions. They don't explain why acyl phosphates are so extremely reactive and how they can function both as acylating and phosphorylating reagents.

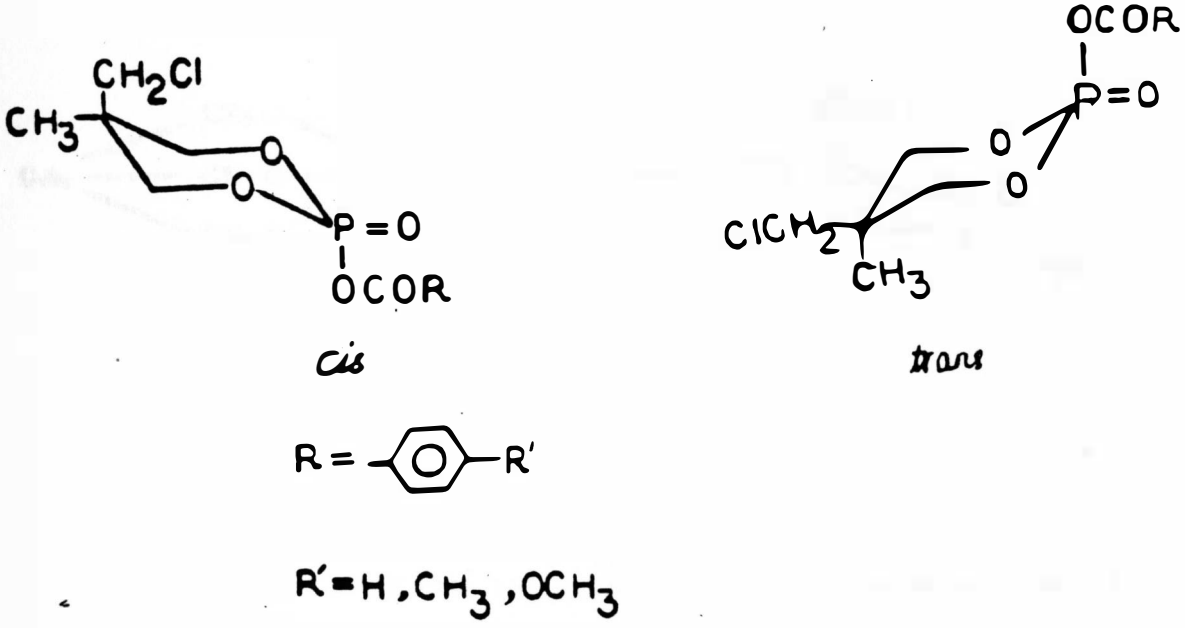
SYNTHESIS AND DISCUSSION

In order to solve the above problem, we studied the reaction of a model acyl phosphate with the hope of being able to propose reasonable reaction mechanisms for this type of compound.

The first problem encountered when attempting to determine a mechanism is in finding a suitable system which will be ideal for a mechanistic study. The most ideal system is one in which the configurations and thus the stereochemistry of the reactants and products can be easily determined. With such a system, upon substitution the stereospecificity of a reaction can be determined.[8]

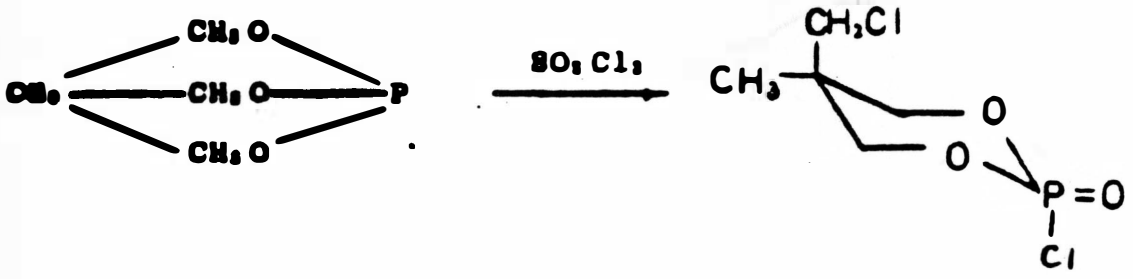
The system employed made use of the 2-substituted-5-chloromethyl-5-methyl-2-oxo-1,3,2-dioxaphosphorinans, which can exist in one of two possible geometrical configurations, Fig.4.

Fig.4.



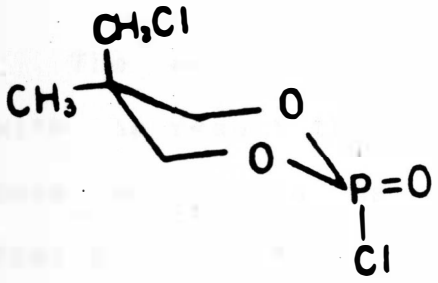
The configuration of the reactants and products can easily be determined by observing the proton NMR chemical shift values of the 5-methyl hydrogens[9]. The initially substituted compound was the cis-2-chloro phosphorinan referred to as the cis chloridate. This compound was prepared by treating methyl bicyclic phosphite with sulfuryl chloride in an Arbuzov type reaction, Scheme.9..

Scheme.9.

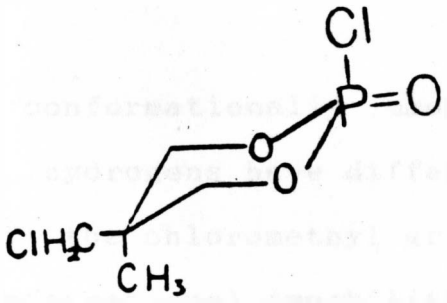


An Arbuzov reaction is typified by the reaction of a trialkyl phosphite with an alkyl halide to form a dialkyl phosphonate[10]. In this case because of the nature of the bicyclic phosphite, a single geometrical isomer is obtained which is the cis-chloridate. The cis configuration has the axial 5-chloromethyl group and the equatorial phosphoryl oxygen on the same side of the ring. In the case of the trans the groups would be both equatorial. Fig.5.

Fig.5.



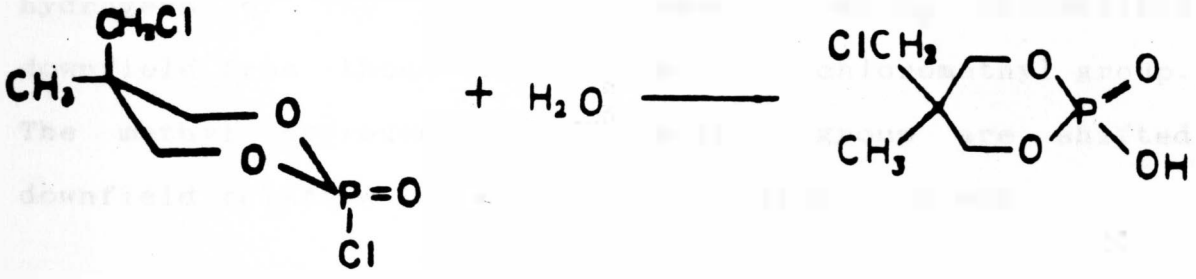
cis

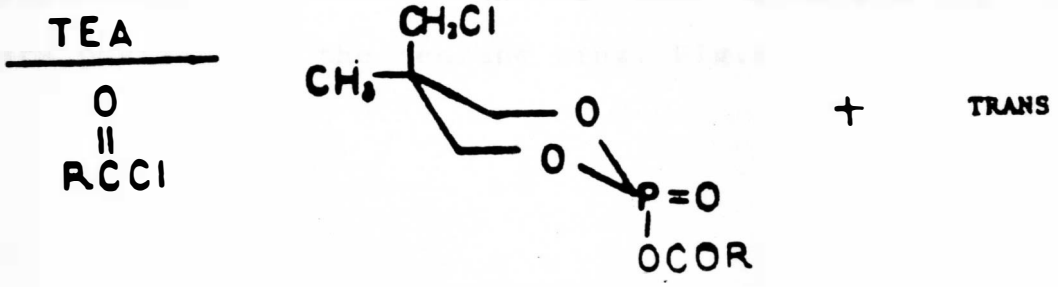


trans

Only the cis isomer is formed which is a consequence of the mechanism of the Arbuzov reaction.

Scheme.11.





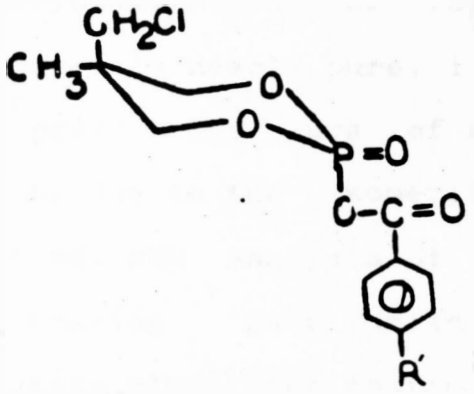
CIS

Treatment of the cis-chloridate with certain nucleophilies yields both cis and trans products, Scheme.11.

The six-mumbered rings are conformationally immobile with the result that the 5-methyl hydrogens have different chemical shifts as do those of the chloromethyl group. That geometrical isomers have conformational immobility is due to the large preference of the phosphoryl oxygen to be equatorial. Thus, the phosphoryl oxygen atom in both the cis and trans anhydrides is in an equatorial position.[11] Edmundson has shown that groups at the 5 position give NMR signals with different chemical shifts depending upon whether they are in an axial or equatorial position.[12] The hydrogens of the axial chloromethyl group are shifted downfield from those of an equatorial chloromethyl group. The methyl hydrogens of an axial group are shifted downfield relative to those of an equatorial group.[8]

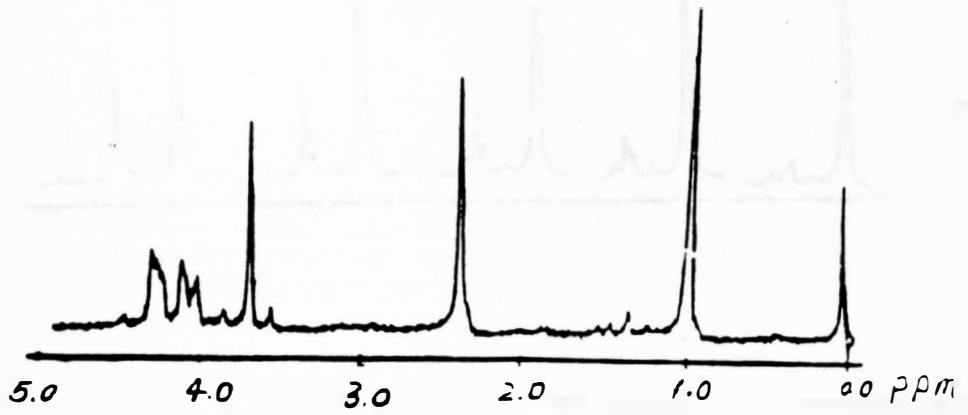
The specific system used in this research was the 2-benzoyloxy-5-chloromethyl-5-methyl-2-oxo-1,3,2-dioxaphosphorinans with and without substitution in the para position of the benzene ring. Fig.6.

Fig.6.



Cis and trans isomers could be separated by simple fractional recrystalization from carbon tetrachloride. Proton NMR gave a clean spectrum, Fig .7.

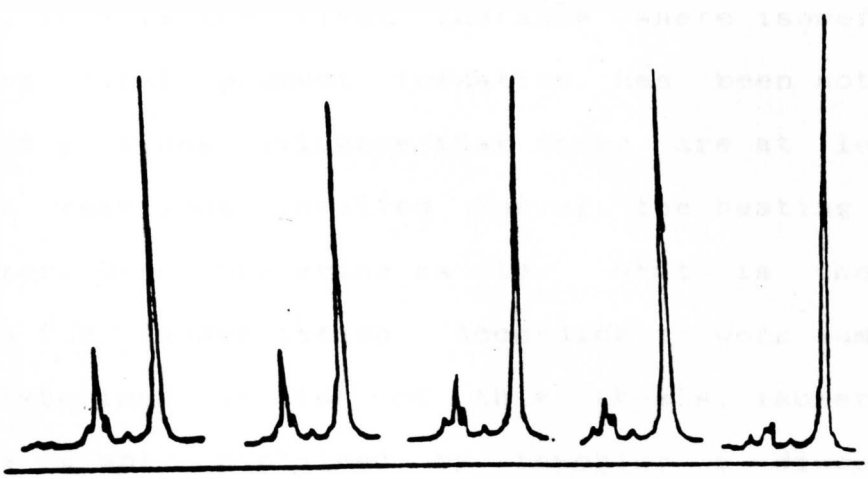
Fig.7.



The Equatorial 5-methyl hydrogens found in the spec[illegible] of

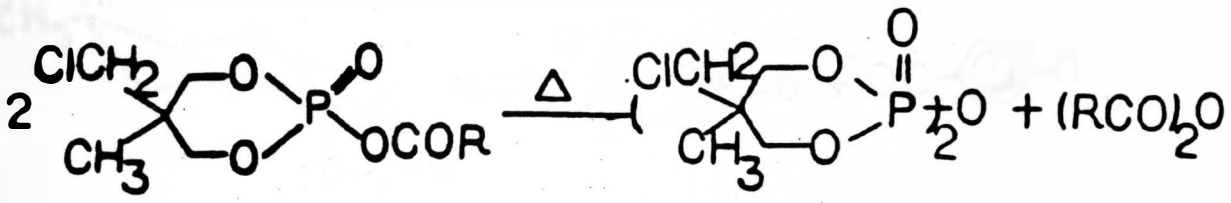
the cis isomer absorb around 1.0 ppm, whereas the axial 5-methyl hydrogens found in the trans isomer absorb around 1.3 ppm.[13] From the spectrum , the ratio of cis to trans isomers after recrystalization was found to be 48:1. Although the cis isomer is nearly pure, it does not give a definite melting point. The lack of a definite melting point was found to be due to the isomerization of starting material upon heating. NMR analysis of a solution of the pure isomer during heating gave definite evidence that isomerization does take place upon heating, Fig.10.

Fig.10.



Upon heating a solid sample, the initial isomerization is followed by formation of pyrophosphate and symmetrical anhydride. Scheme.12.

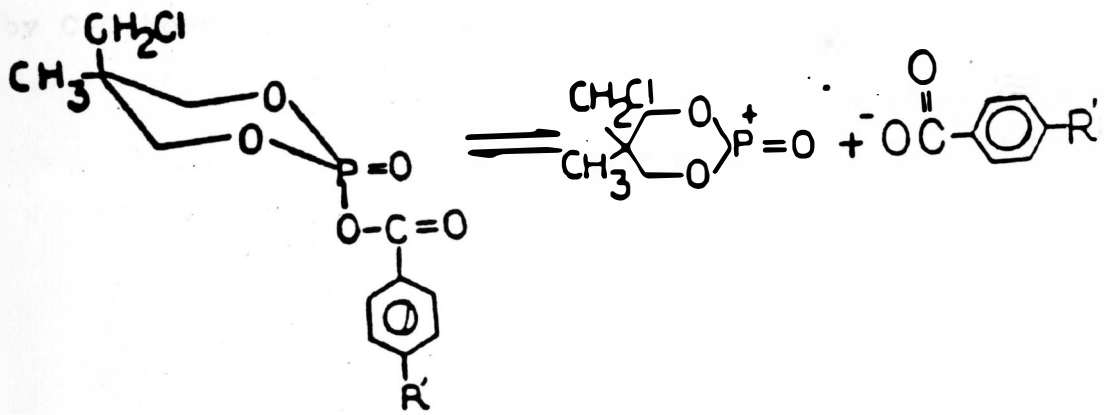
Scheme.12.



Although formation of the latter has been previously reported, this is the first instance where isomerization preceeding final product formation has been noted.This experiment provides evidence that there are at least two different reactions involved during the heating of the pure isomer. Questions arise as to what is the proper mechanism for isomerization. According to work summarized in the historical section of this thesis, isomerization could be simply explained by invoking a dissociation process which could proceed by two different pathways, P-O bond cleavage, or C-O bond cleavage.

Consider P-O bond cleavage first. In this process the reaction could be described as in scheme.13.

Scheme.13.

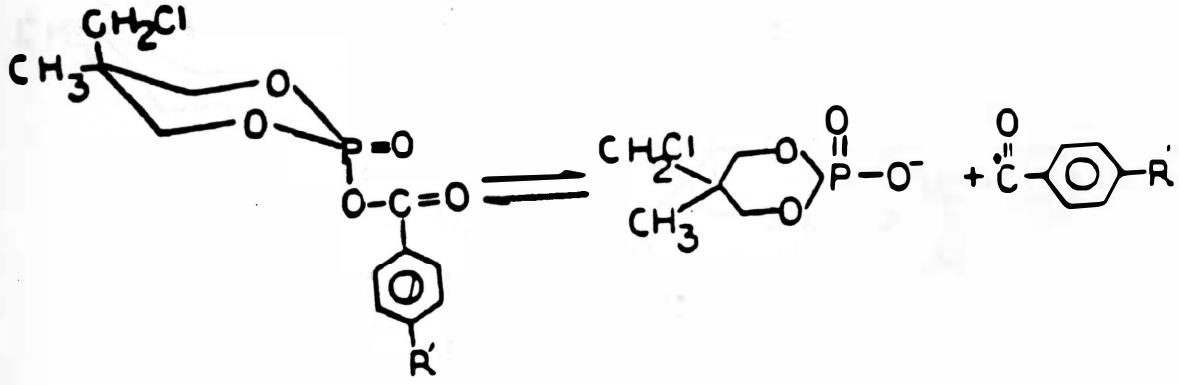


Upon heating, formation of a phosphacylium and carboxylic ions would occur if the isomerization preceeds by this process. The phosphacylium ion which has a planar structure, Scheme.13., could be attacked from either side of the plane by a carboxylic anion to give a mixture of cis and trans isomers. In this case, an electron withdrawing group would stabilize the negative charge on the carboxylic oxygen by resonance and inductive effects thereby lowering the free energy of activation and making the reaction favorable. Phosphacylium ion formation is not improbable for it is similar to metaphosphate (PO_3)(Scheme.1.) which has been proposed as an intermediate in the solvolysis of some highly reactive phosphate ester.[5] The existence of both ions is still questionable. If the phosphacylium ion is formed as an intermediate, it should be capable of being trapped as can other electrophilic intermediates of

this type.

The second possible dissociative process is described by C-O bond cleavage, Scheme.14.

Scheme.14.

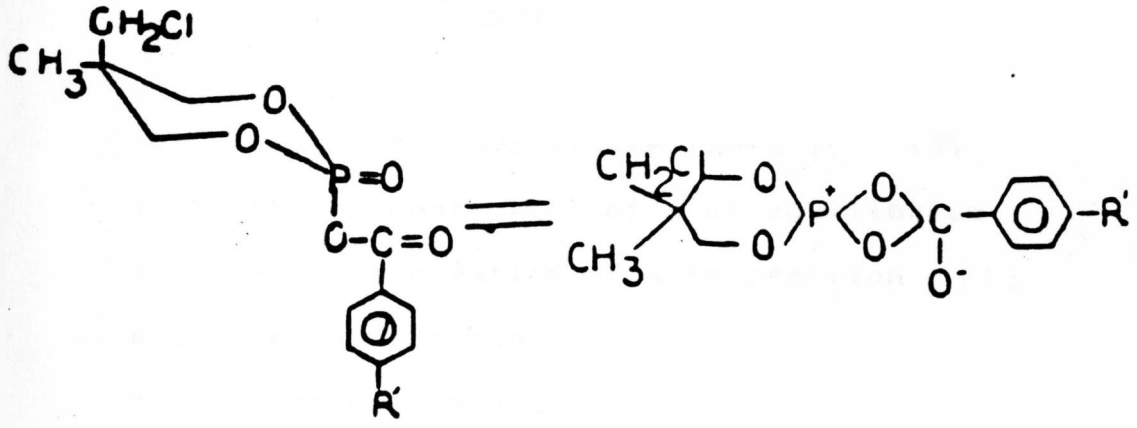


The negative charge is not localized on a simple oxygen atom of the phosphate anion but is, due to resonance, delocalized over both oxygen atoms. Thus, either oxygen atom could attack the acylium ion leading to isomerization. This dissociative process would yield an acylium cation and phosphate anion and should be favored by an electron donating R group, a group which would stabilize the positive charge on carbon by resonance. Also acylium ion if

formed should be capable of being trapped, by an activated aromatic substrate.

Besides the dissociative mechanism described above, an intramolecular rearrangement could also explain the isomerization. Scheme.15.

Scheme.15.



Phosphoryl oxygen could attack the carbonyl carbon to form a four membered ring intermediate. In the intermediate there are two equivalent P-O-C bridges. Isomerization could occur if either of the O-C bonds were broken. The intermediate bears the positive charge at phosphorus. The negative charge is on carbonyl oxygen, and its formation should be favored by an electron withdrawing R' group. The cyclic intermediate is similar to the well-known tetravalent phosphonium ion. Phosphorane cyclic systems are

well described for five membered rings but have not been reported for four membered rings.

In order to help in deciding which of the above mechanisms is correct, rate studies on representative compounds were carried out (Table.1-5). If the isomerizations are first order and reversible, they can be described by the following rate equations.

$$A \rightleftharpoons Z \qquad (1)$$

$$k_{-1} + k = 1/t*\ln(Xe/(Xe-X)) \qquad (2)$$

$$k_1*t = (Xe/a)*\ln(Xe/(Xe-X)) \qquad (3)$$

Xe is the concentration of A at equilibrium

X is the concentration of A in reaction

a is the initial concentration of A

t is reaction time

k_{-1} and k_1 are reaction rate constants of forword and reverse reactions.

We carried out the isomerization of the model compounds in distilled benzene and $CDCl_3$ at 65°C. Table.1.

Table.1. Sample 12-63 (R = CH_3) in $CDCl_3$ at 60°C

Time(hour)	Peak height		Ratio %	$\ln X_e/(X_e X)*10^2$
	Trans	Cis		
.00	.00	46.00	.00	.00
5.39	3.00	46.00	6.12	.28
7.95	7.00	44.00	13.73	.79
11.52	6.00	35.00	14.63	.88
13.67	7.00	35.00	16.67	1.10
18.48	7.50	35.00	17.65	1.22
24.55	7.50	32.00	18.99	2.42
37.05	9.50	29.00	24.68	4.35
45.02	10.00	30.00	25.00	/

Plot $\ln X^e/(X_e-X)$ vs time t gives a decent straight line. Obviously, the isomerization does follow a first order equilibrium reaction law. The kinetic parameter k, the reaction rate constant of the isomerization, can be obtained from the slope of the plot, Fig.11.

Table.2.

Sample	R'	σ	σ^+	k*10²	ln k
12-62	H	.00	.00	23.23	-1.63
12-63	CH_3	-.16	-.31	45.80	-1.34
12-73	OCH_2	-.28	-.78	158.80	-.80

Due to solubility problems, only three compounds were used for this study. Isomerizations were carried out in benzene for with this solvent separate isomer and acid peaks could most easily be observed. In chloroform as solvent the 5-methyl hydrogen signals of any acid impurity are hidden under those of the cis isomer. Rates are not absolute for the acid impurity (dialkyl phosphoric acid) is difficult to completely remove and may act as a catalyst. It was particularly apparent in the case of the p-methoxy anhydride which is most reactive. Due to facile hydrolysis last traces of acid are difficult to remove during the final purification of the starting material. The σ and σ^+ constants are substituent constants used in the Hammett equation.

The Hammett linear free energy equation was used to determine how the p-substituents affect isomerizations. The equation is

$$\log k_X/k_H = \rho\sigma$$

where σ is the substituent constant which can be found in any text on physical organic chemistry, and is the reaction constant obtained by plotting $\log k^X/k_H$ vs . Rho is 1 for the standard reaction, ionization of substituted benzoic acid. When there is direct conjugation of the benzene ring with an electron poor center , another equation, $\log k_X/k_H = \rho\sigma^+$ is used. If a plot of sigma plus (σ^+) values vs $\log k_X/k_H$ gives a straight line with a negative slope, the reaction is aided by electron donating substituents and is indicative of the development of a positive charge at a reactive center. In both cases k_X is the rate constant of the reactant with a para substituent and k_H is the rate constant for the unsubstituented reactant.

Fig.12. Plot of σ vs lnk

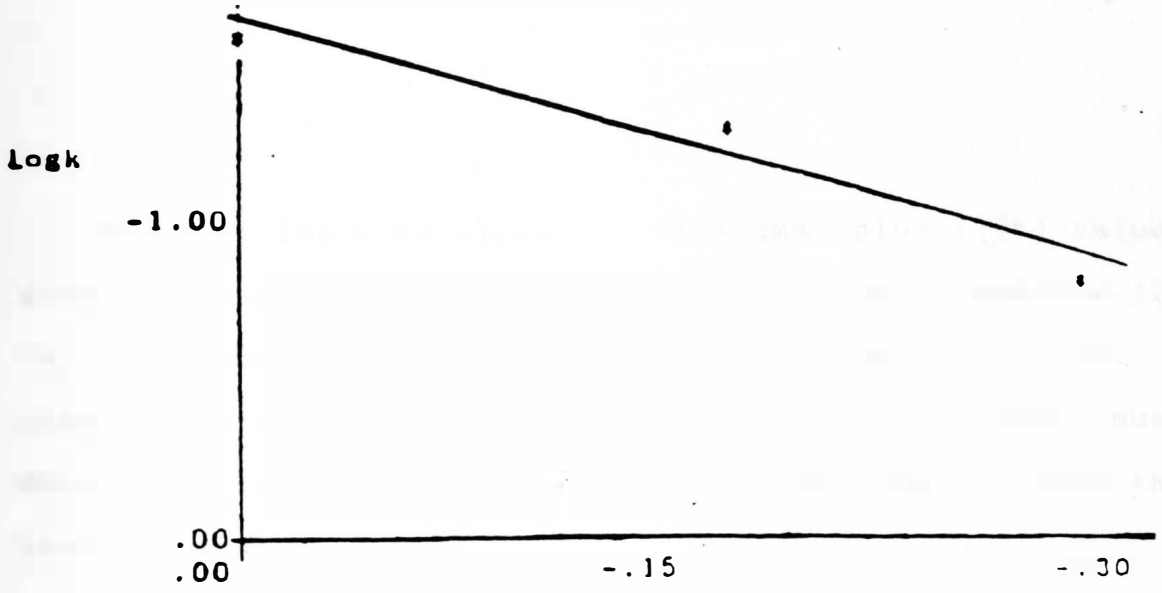
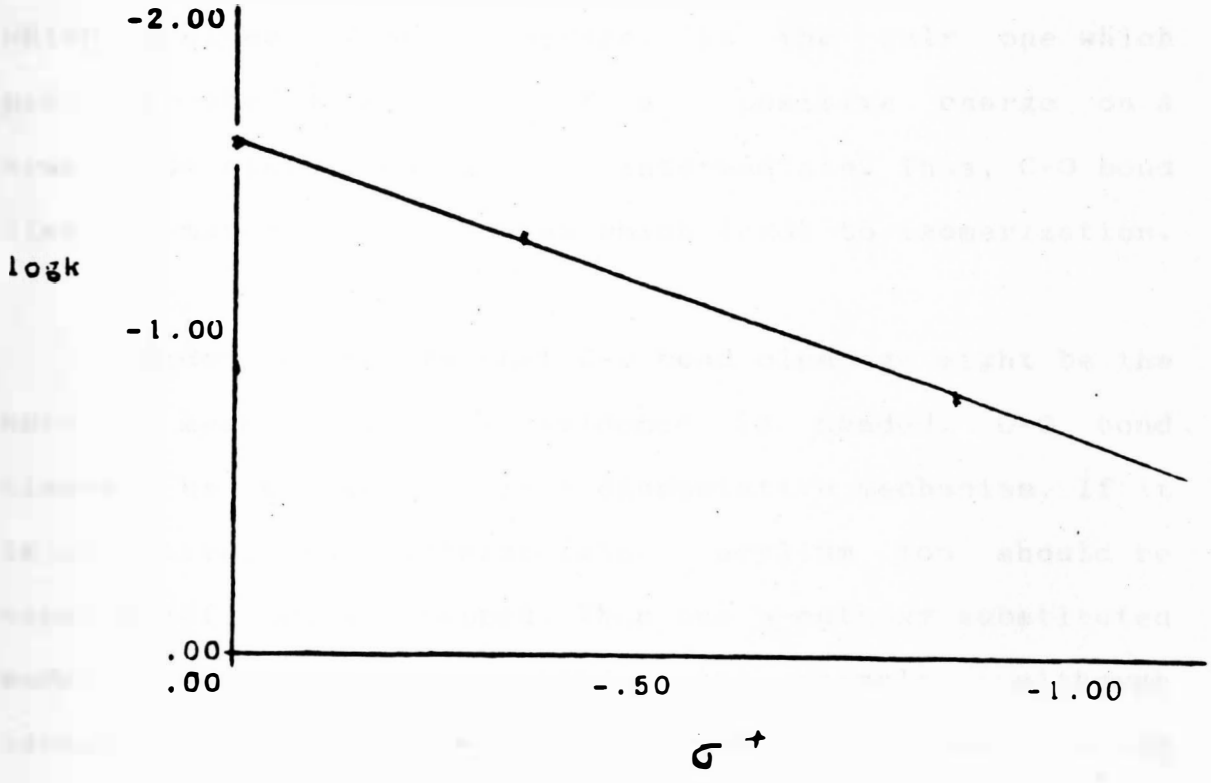


Fig.13. Plot σ^+ vs lnk



Plots of log k vs sigma (σ) or sigma plus (σ^+) values give the best straight line with sigma plus values, Fig 12, 13. The reaction parameter rho is equal to -0.98. A negative slope indicates that a positive charge must develop at the reaction site in direct conjugation with the benzene ring.

From the above experimental data, the proposed mechanism 1, P-O bond cleavage, and 3, intramolecular rearrangement can be eliminated for contrary to these mechanisms, electron withdrawing groups aid reaction in both cases. The other proposed mechanism, mechanism 2, which involves C-O bond cleavage, is the only one which predicts the development of a positive charge on a transition state leading to an intermediate. Thus, C-O bond cleavage may be the mechanism which leads to isomerization.

In order to confirm that C-O bond cleavage might be the correct mechanism, more evidence is needed. C-O bond cleavage by mechanism 2 is a dissociative mechanism. If it is operative, the intermediate acylium ion should be capable of being trapped. When the p-methoxy substituted model compound was heated in anisole, although isomerization proceeded, no aroylated anisol was detected as a final product. Thus, a dissociative process with C-O bond cleavage is highly unlikely. Also, if the dissociative mechanism were correct, the entropy of activation for isomerization would be expected to increase which is contrary to experiment.

The isomerization of a model compound at different temperatures was carried out under identical reaction conditions. Table.3.and .4.

Table.3. Sample 12-63 in Distilled Benzene and Heating

Temp. K	$1/T*10^3$	lnk	ΔH^* kj	ΔS^* eu
333.00	3.00	-4.06	45.14	-34.43
343.00	2.92	-3.65	45.06	-34.66
353.00	2.83	-3.12	44.98	-34.62

Table.4. Sample 12-63 in $CDCl_3$ and Heating

Temp. K	$1/T*10^3$	lnk	ΔH^* kj	ΔS^* eu
336.00	2.98	-3.08	68.74	-15.15
346.00	2.89	-1.81	68.65	-14.98
366.00	2.73	-.70	68.64	-15.51

Only three temperatures were used due to solubility problems at lower temperatures. In the above tables, k is the rate constant which was obtained by using the first order opposing rate law, H^* and S^* are enthalpy and entropy of activation respectively obtained from the Arrhenius equation:

$$\ln k = \text{constant} - \Delta E_a/RT \qquad (6)$$

$$\Delta H^* = \Delta E_a - RT \qquad (7)$$

$$\Delta S^* = R(\ln k - \ln k't/h) + \Delta H^*/T \qquad (8)$$

k is the rate constant for isomerization

R = Gas constant, 8.314Kj/mol[14]

T = reaction temperature

ΔE_a = energy of activation

$\Delta H^{\ddagger}$ = enthalpy of activation

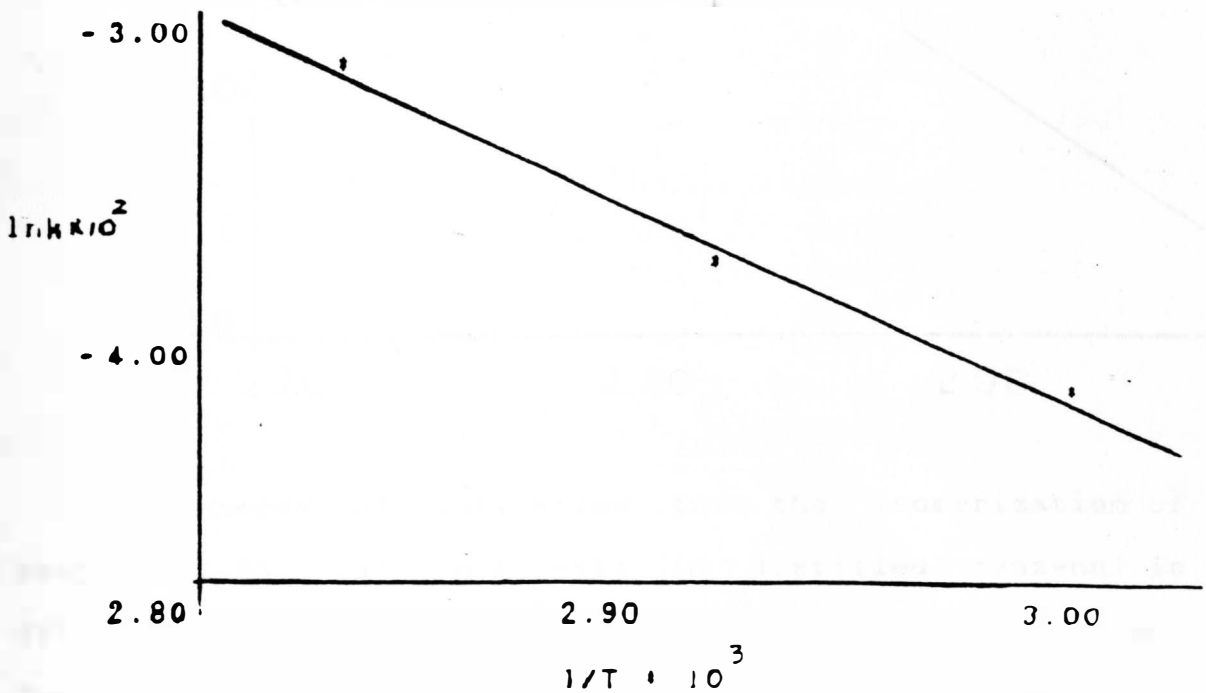
$\Delta S^{\ddagger}$ = entropy of activation

k' = Boltzmann's constant, $1.38*10^{-23}$ j/mol*K[11]

h = planck's constant , $6.626*10^{-34}$ js[11]

The energy of activation is obtained by plotting logk vs 1/T, Fig 15, 16.

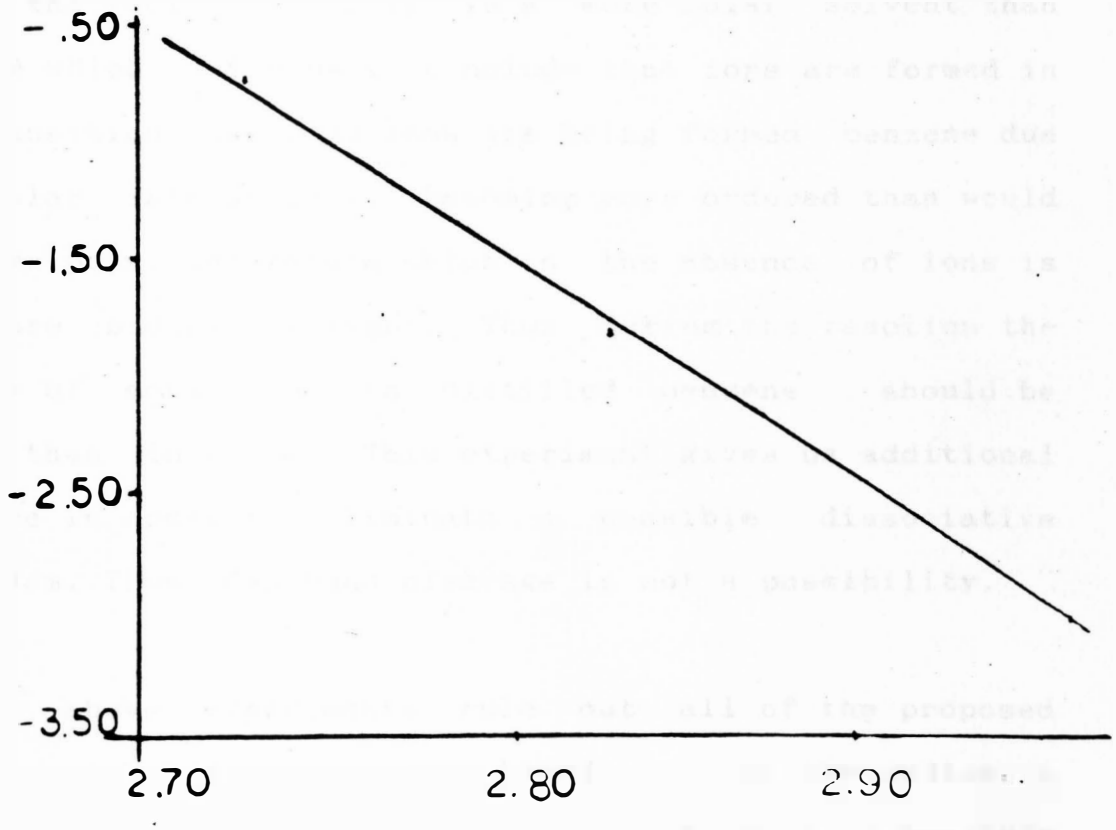
Fig.15. The plot of lnk vs 1/T for Sample 12-63 in Benzene



The plots give straight lines and the energy of activation is obtained from the slope. By meaning of equation 7 and 8

$\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ can be calculated.

Fig.16. The plot of lnk vs 1/T
for Sample 12-63 in $CDCl_3$

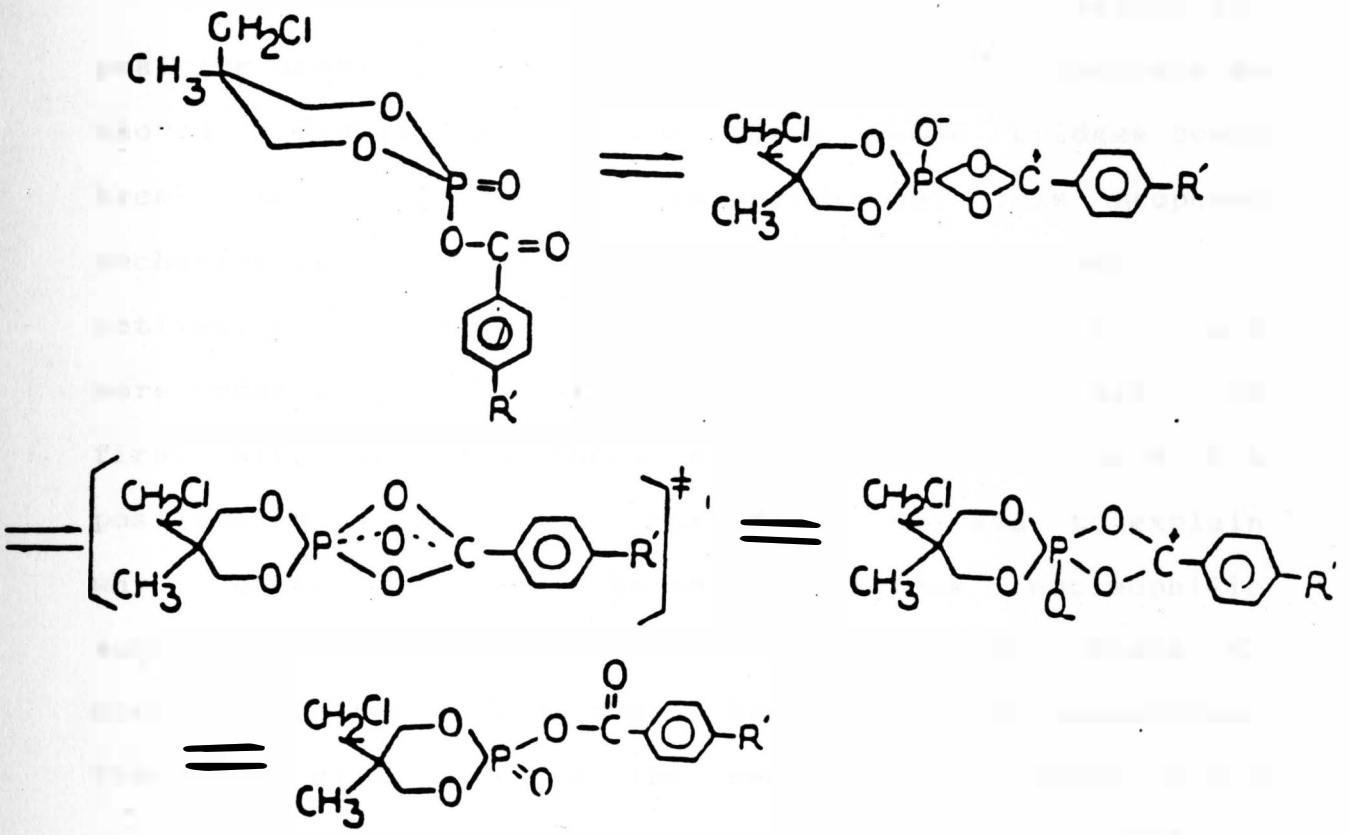


The energy of activation for the isomerization of sample 12-63 (R'= p-methyl) in distilled benzene is 68Kj/mol and the entropy of activation, $\Delta S^{\ddagger}$ is -34.5 eu. The entropy of activation is temperature independent. In contrast to benzene, the entropy of activation for isomerization in $CDCl_3$ is around -15.00 eu and is also

temperature independent. The entropy of activation for isomerization in CDCl3 is,as seen, much smaller than in distilled benzene. The large negative values indicate that the transition state introduces more order than found in the reactant. The differences between the entropy of activation for the same reaction in different solvents is due to the fact that $CDCl_3$ is a more polar solvent than benzene which allow us to conclude that ions are formed in the transition state. As ions are being formed benzene due to dipolar interaction is becoming more ordered than would the more polar chloroform which in the absence of ions is the more ordered solvent. Thus during the reaction the entropy of activation in distilled benzene should be larger than in $CDCl_3$. This experiment gives us additional evidence in order to eliminate a possible dissociative mechanism. Thus, C-O bond cleavage is not a possibility.

The above experiments rule out all of the proposed mechanisms. We do, however, based on our thermodynamic parameters have enough evidence to propose a possible mechanism for isomerization. Since the Hammett relationship shows that electron donating groups aid the reaction and thermodynamic data indicate that there are ions being formed as intermediates, we propose a new mechanism, Fig.16.

Fig.16.



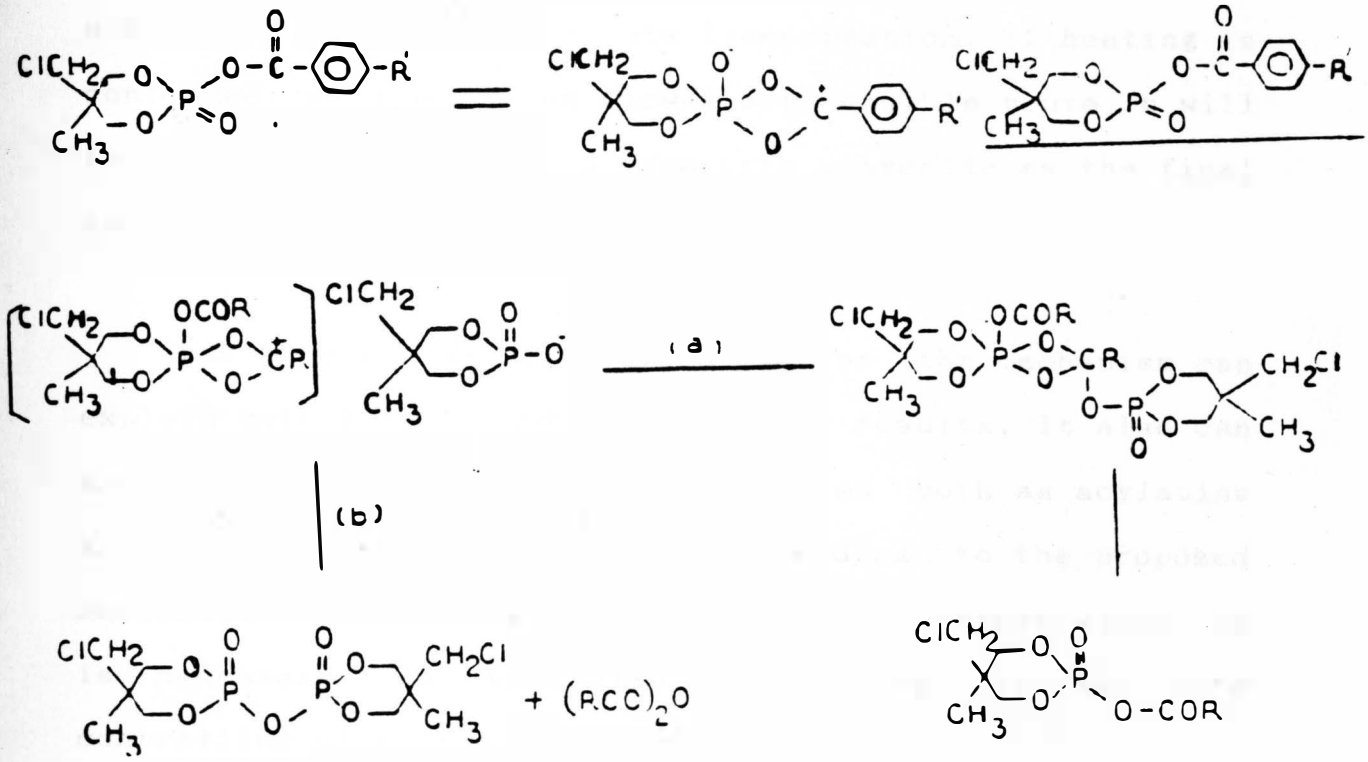
In this mechanism, the carbonyl oxygen attacks the phosphorus atom to give a four membered ring intermediate. A positive charge is formed on the aroyl carbon and as formed electron donating groups would be expected to aid the reaction, also the mechanism accounts for the negative

slope and negative rho value obtained from the Hammett plot.

In the second step, phosphoryl oxygen might attack the positive aroyl carbon to form a tricyclic intermediate as showed in Fig.16.. Any of the three P-O-C bridges could break to complete the isomerization. This proposed mechanism can further explain the large negative entropy of activation for both the first and second step introduce more order with respect to the starting material. Also, the first step which produces a cyclic intermediate with a positive charge on carbonyl carbon may be used to explain why under non-equilibrium conditions nucleophilic substitution always occurs at carbon which leads to cleavage of the carbon oxygen bond and not at phosphorus. The only difficulty is the second step which would represent a highly strained transition state and is not too probable. No comparable tricyclic compounds are known.

A more logical route is that after the first step another molecule of cis isomer is involved. Scheme.14.

Scheme.14.



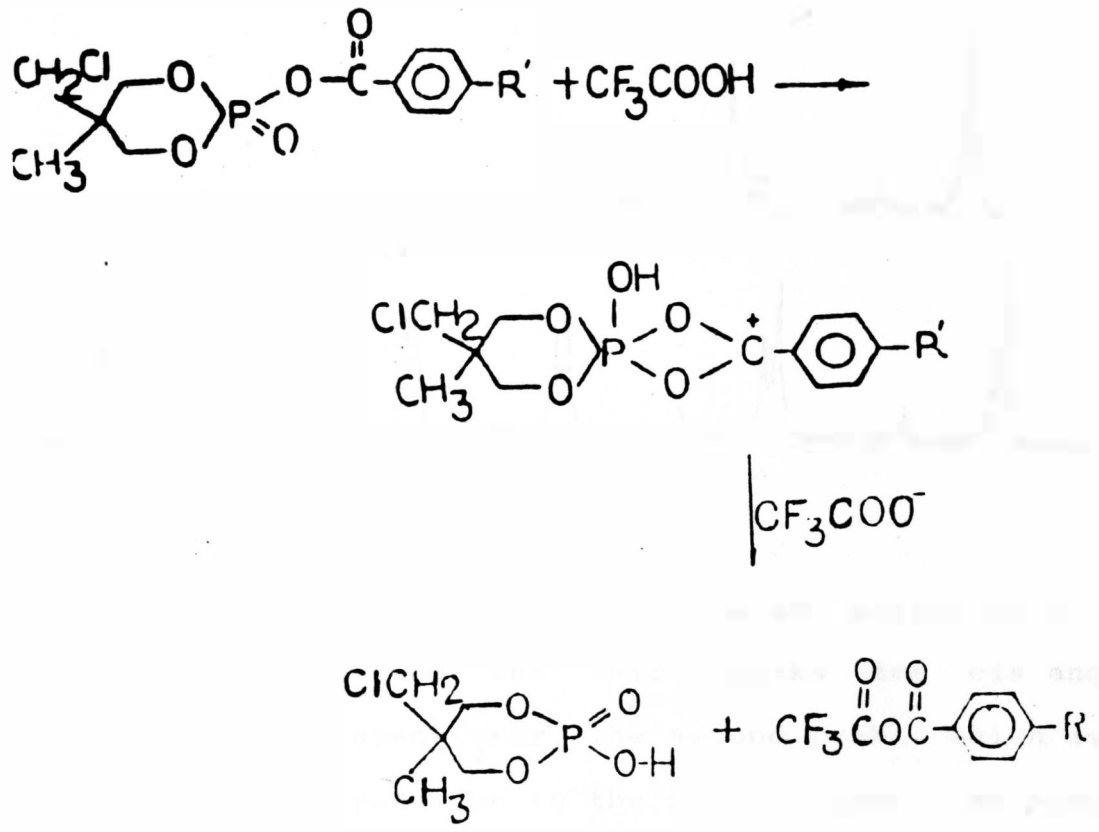
After the first step, another molecule of the starting compound is attacked by the cyclic intermediate. The second molecule of the cis isomer will undergo C-O bond cleavage. Due to attack by the negative phosphoryl oxygen the newly formed monophosphate anion will have its negative charge spread out over both oxygen atoms due to O=P-O- resonance. If the phosphate anion attacks the aroyl carbon, the reaction will undergo route a, Fig14. If it attacks the

phosphorus atom, the reaction will undergo route b to form pyrophosphate and symmetric anhydride. Both route a and b are irreversible. Route a can repeat which due to dispersal of negative charge on the mono phosphate anion eventually leads to complete isomerization. If heating is continued, eventually the slower irreversible route b will lead to pyrophosphate and symmetric anhydride as the final and only products.

This proposal is very reasonable for the mechanism can explain both kinetic and thermodynamic results. It also can explain why acyl phosphates can function both as acylating and phosphorylation reagents. According to the proposed mechanism, the reaction takes place by multiple steps. It is necessary to determine which step is the rate controlling step, i.e. the slowest step.

One indication as to which is the slowest step is furnished by reaction of a cis mixed anhydride with various carboxylic acids having different pKas, Scheme 15.

Scheme.15. The reaction of cis isomer with F_3CCOOH



Surprisingly, CF3COOH reacts instantaneously even though CF3COO- is a very weak nucleophile. It takes only a few minutes for the reaction to yield 100% dialkyl phosphoric acid and mixed carboxylic anhydride.

Spectra.3. The Reaction of 12-63 with Acetic Acid

In the above spectra in which the weak acid, acetic acid , was employed the first and the third peaks are cis and trans isomer peaks respectively. The second peak , which is new, is the 5-methyl peak due to the acid product. As seen from the spectra there is almost no isomerization. Comparable reactions were carried out, with other acids having variable strengths, Table 5.

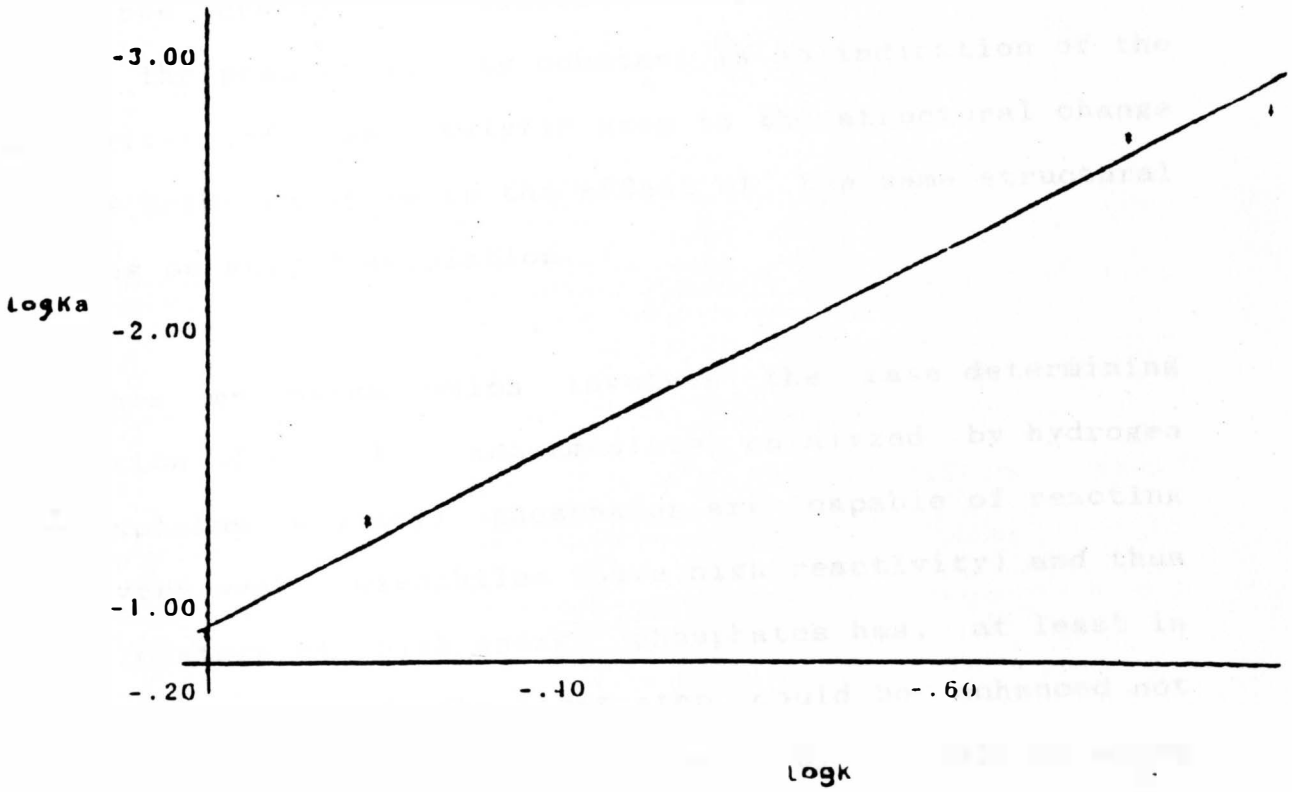
Table.5. Reactions of 12-63 with Carboxylic Acid

Acids	logKa	k	logk
Cl_3CCOOH	-.90	.63	-.20
$Cl_2CHCOOH$	-1.30	.55	-.26
$BrCH_2COOH$	-2.69	.21	-.68
$ClCH_2COOH$	-2.80	.18	-.75
F_3CCOOH	strong acid	/	/

The table shows that reaction rates of the model compound with acids is dependent upon the acidity of the acids but independent of the nucleophility of the conjugate base of the acid. The reaction rates are proportional to the pKa of the acids. Thus, the first step, cyclization to the cyclic intermediate, has to be rate controlling. If the second step were rate controlling strong nucleophlies, the conjugate base of weak acids, would speed up the reaction and the weaker acids would react faster which is contrary to the results of the experiment. The reason that the rate of the reactions is proportional to the pKa of acids can be explained as due to the protonation of phosphoryl oxygen which would lower the energy of activation of the slow, rate determining first step and speed up the reaction.

Fig.17. is a plot of the acidity of the acids vs the rate of reaction.

Fig.17.



It was found that the reaction of the model compounds with acids follows a general acid catalysis law, the Bronsted catalysis law:

$$R + HA \xrightarrow{\text{slow}} RH^+ + A^-$$

$$RH^+ + A^- \xrightarrow{\text{fast}} \text{Product} + HA$$

$$\log k_{cat} = \log K_a + b$$

This equation shows that the free energies of activation of a catalytic step for a series of acids is proportional to the free energy of dissociation for the same series of acids. The proportionality constant is an indication of the sensitivity of the catalytic step to the structural change of the acids relative to the effect of the same structural changes on acid dissociation.

This mechanism which involves the rate determining formation of a cyclic intermediate catalyzed by hydrogen ion explains why acyl phosphates are capable of reacting with very weak nucleophiles (have high reactivity) and thus the mystery of high energy phosphates has, at least in part, been revealed. The first step could be enhanced not only by protons but also perhaps by metals to which negative oxygen could complex,(i.e. Mg^{2+} ,Ca^{2+} etc). Our results explain why cell metabolism varies with pH and perhaps why metabolic rates vary with certain metal cation concentrations.

SUMMARY

By means of nuclear magnetic resonance (NMR) spectroscopy, we have studied the isomerization of the model system, the mixed anhydride of 2-substituted-5(chloromethy)-5-methyl-2-oxo-1,3,2-dioxaphosphoran and p-substituted benzoic acid.

This study has allowed us to determine how acyl phosphates function both as acylating and phosphorylating agents and to propose a reasonable mechanism for acylation and phosphorylation by acyl phosphates. The result may be applicable to other high energy compounds. Our mechanism accounts for the extremely high reactivity of the acyl phosphates and their ability to react with the weakest of nucleophilies.

We have accounted for the large increase in rate of reaction, both acylation and phosphorylation, with an increase in acidity.

From a Hammett plot of the isomerization of the model compounds, we obtained thermodynamic parameters for the isomerization. Plots of log k vs sigma or sigma plus values , gave the best straight line with sigma plus values and a

negative parameter (rho=-0.98). Further calculations gave the energy of activation, Ea = Δ68 kj/mol, and a negative entropy of activation (ΔS*= -15 eu in benzene and ΔS* =-34.5eu in chloroform). The entropy of activation is dependent upon solvent but not temperature.

EXPERIMENTAL

Proton nuclear Magnetic Resonance (H-NMR) spectra were obtained on a Perkin Elmer R 12B spectrophotometer at 60 MHz. Tetramethyl silane (TMS) in deuterated chloroform was used as an internal standard. Cis and trans ratios were determined by peak integration of the 5-methyl hydrogens. All melting point are in degrees centigrade and are uncorrected. Values were determined on a Thomas Hoover capillary melting point apparatus.

Preparation of Methyl Bicyclic phospite

A mixture of 60.0 grams (0.50 moles) of 1,1,1-trihydroxy methyl ethane and 62.0 grams (0.50 moles) of trimethyl phosphite in 100mL toluene was slowly distilled for 24 hours, or until methanol formation was completed. The temperature of the reaction mixture never climbed above 120 degrees centigrade. Toluene was stripped from the product under reduced pressure. The white crystalline product was distilled under 1 mm pressure and collected over an ice bath. It was not necessary to purify the product further.

Preparation of Cis-2-chloro-5-chloromethyl-5-methyl-2-oxo-1,3,2-dioxaphosphorinan, (chloridate)

With ice-bath cooling and stirring, a solution of methyl bicyclic phosphite in 100 ml of carbon tetrachloride was added dropwise to a solution of sulfuryl chloride, 67.5 grams (0.50 moles) dissolved in 100mL of carbon tetrachloride. After the exothermic addition the solution was stirred for 1 hour, then stripped under reduced pressure. The liquid residue crystallized on standing. Carbon tetrachloride was used to recrystallize the product. The chloridate was dried and stored using a C_aCl_2 drying tube. The approximate yield was 85%. The product is a white crystal with 69-71 degrees centigrade of melting point.

Preparation of the 2-Benzoyloxy Phosphorinan

To a solution of 4.0 grams (0.02 moles) of chloridate and 2.02 grams (0.02 moles) of triethylamine in 50 ml of acetonitrile was added a solution of 2.8 grams (0.02 moles) of benzoyl chloride in 10 ml of acetonitrile with stirring at 5-10 degrees centigrade. Stirring was continued for 1 hour after addition. The solution was allowed to stand overnight at room temperature. The reaction mixture was suction filtered and the filtrate stripped under reduced pressure. The viscous semi-solid residue was taken up in excess water and the mixture allowed to stand over-night.

During the standing time the semi-solid residue became crystalline. The mixture was suction filtered and the crystalline product dried. NMR(CDCL3) analysis showed a three to one ratio of geometrical isomers. The product was recrystallized from a large amount of carbon tetrachloride to give the nearly pure cis isomer. The melting point, around 110 degrees centigrade, was not percise. This is sample 12-62.

The Preparation of the 2-p-toluoyloxy Phosphorinan.

To a solution of 4.0 grams (0.02 moles) of cis-chloridate and 2.02 grams (0.02 moles) of triethylamine in 100 ml of acetonitrile was added a 3.08 grams (0.02 moles) of p-methyl benzoyl chloride with stirring at 5 to 10 degrees centigrade. A precipitate appeared immediately. The mixture was stirred over night at room temperature, gravity filtered and the precipitate washed well with acetonitrile. The acetonitrile filtrate was stripped under reduced pressure to give a crystalline solid. The product was recrystallized from a large amount of carbon tetrachloride gave a white crystalline product with a ratio of cis and trans isomers at 5.7 to 1. This is sample 12-63.

The Preparation of the 2-p-methoxybenzoyloxy Phosphorinan.

To a solution of 4.0 grams (0.02 moles) of chloridate and 2.02 grams (0.02 moles) of triethylamine in 100 ml of

chloride in 10 ml of acetonitrile. A precipitate appeared upon mixing. The mixture was allowed to stir overnight at room temperature, gravity filtered and solvent removed under reduced pressure. The residue was taken up in excess water and the mixture allowed to stand until the residue became crystalline. The mixture was suction filtered and the precipitate washed well with water. The precipitate was dried under reduced pressure, and recrystallized from carbon tetrachloride. NMR(CDCL3) showed the ratio of the two isomers to be three to one, yield 59.7%. Product can be recrystallized from chloroform but the yield is fairly low due to the high reactivity and consequently rapid hydrolysis.

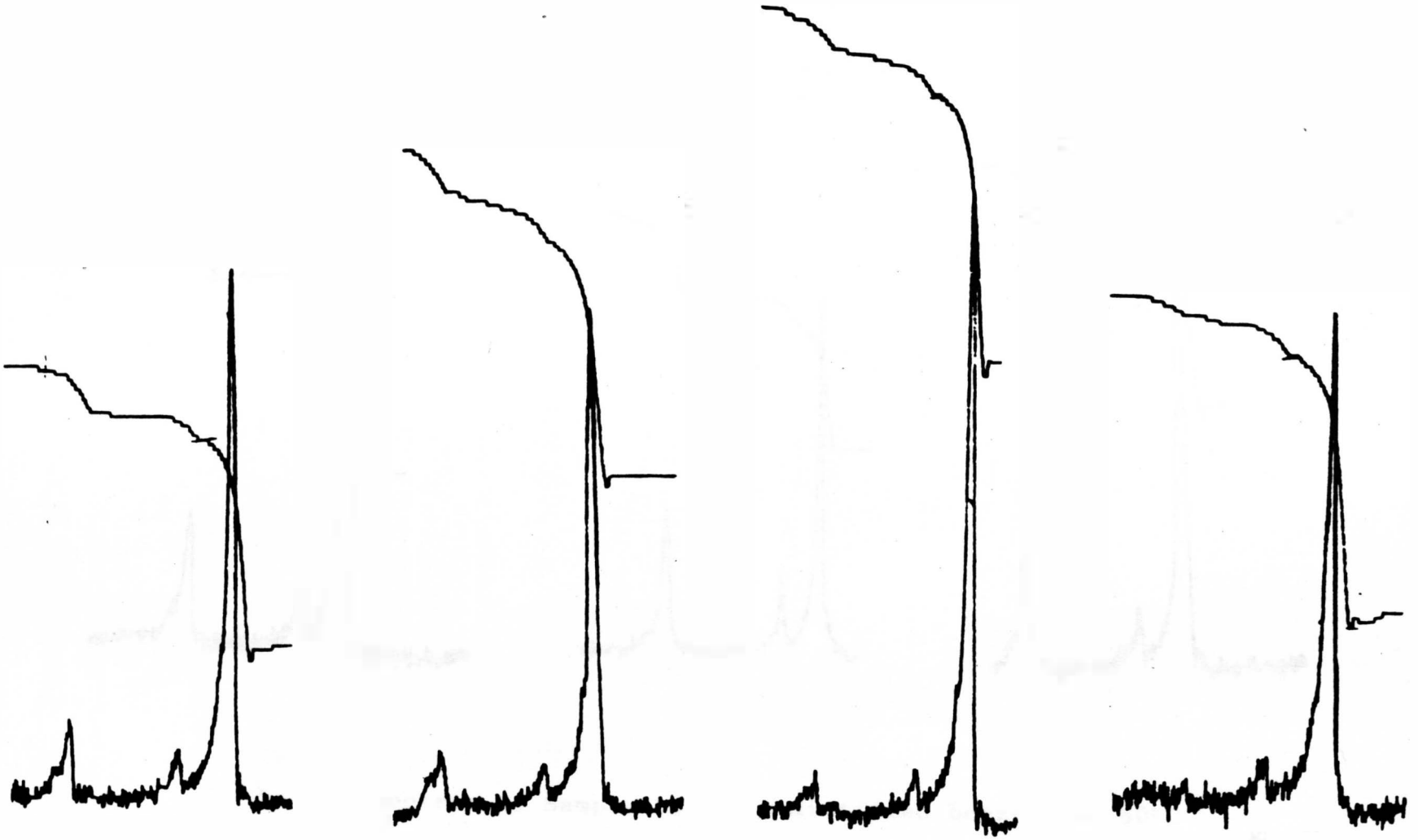
Isomerization of the Phosphorinans in NMR solvent:

Benzene and chloroform solutions of substituted phosphorinans were heated at constant temperatures. Heating was conducted in sealed NMR tubes in an oil bath. Oil bath temperature were controlled by a Therm-o-Watch. The temperatures selected were 60°C, 70°C, 80°C and 90°C respectively for each sample. NMR spectra were taken every hour until there were no changes in the cis/trans isomer ratio. NMR spectra showed isomerization of the phosphorinans to take place under the above reaction conditions. Isomerization rates were followed by measuring the changes of peak heights with time. The spectra are

recorded in the Appendix.

The Reaction of 2-p-Toluoyloxy Phosphorinan with carboxylic Acids in Distilled Benzene;

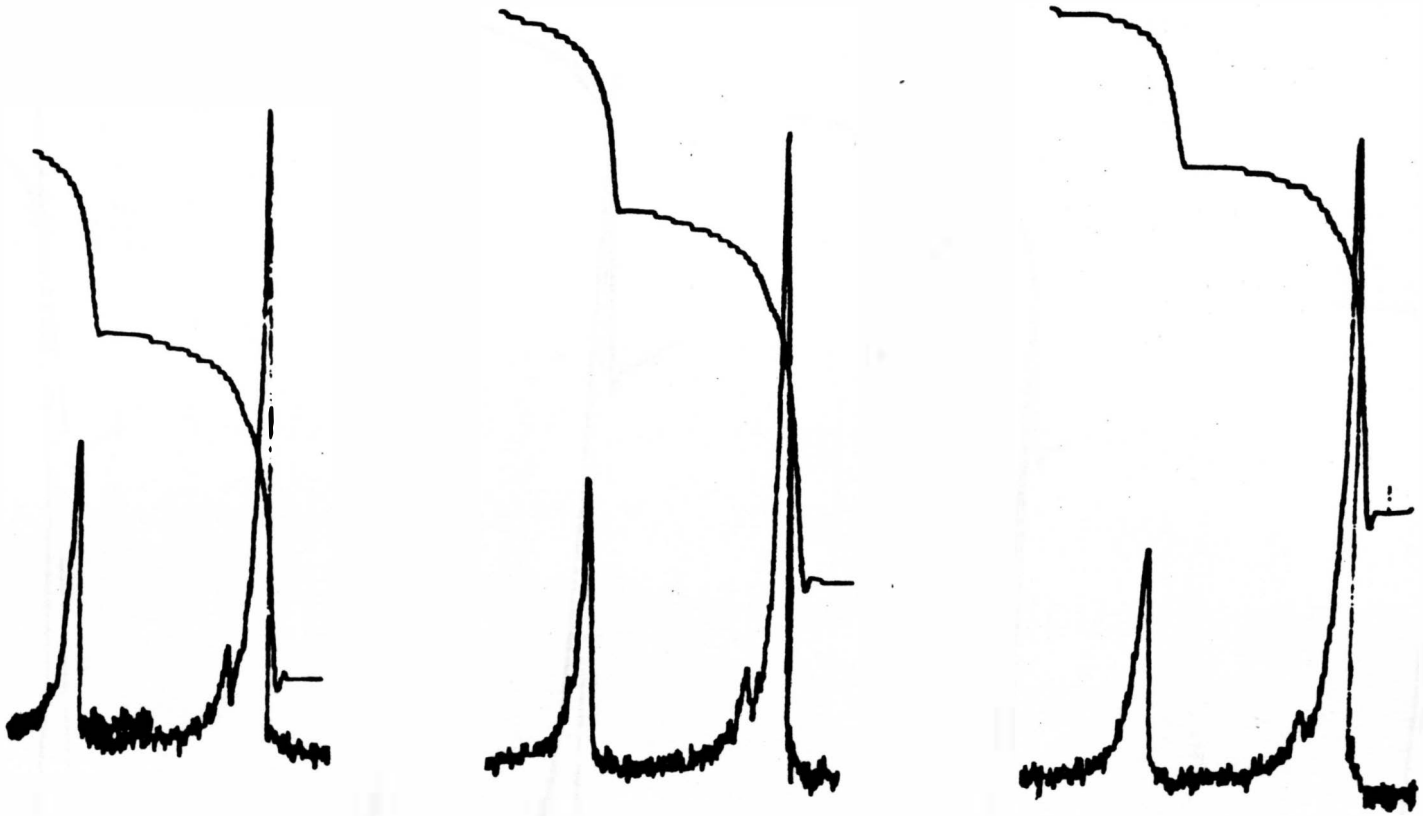
To separate solutions of 2-p-toluoyloxy phosphorinan in distilled benzene was added a molar equivalent of trifloroacetic acid, trichloroacetic acid, dichloroacetic acid, chloroacetic acid and bromoacetic acid in distilled benzene. NMR spectra of the solutions were taken immediately after the two solutions were mixed and at intervals of 15 minutes all at room temperature. Equilibrium was reached at that point when no further changes in peak heights are observed. NMR spectra showed the growth of a new 5-methyl peak besides those attributed to trans and cis isomers. The new peak is due to phosphoric acid formation for it coincides with that of an authentic sample. As indicted by NMR spectra taken at intervals, isomerization does not occur prior to or during cleavage by the carboxylic acids.



1. NMR Spectrum of Sample 12-63 in distilled benzene at 60°C

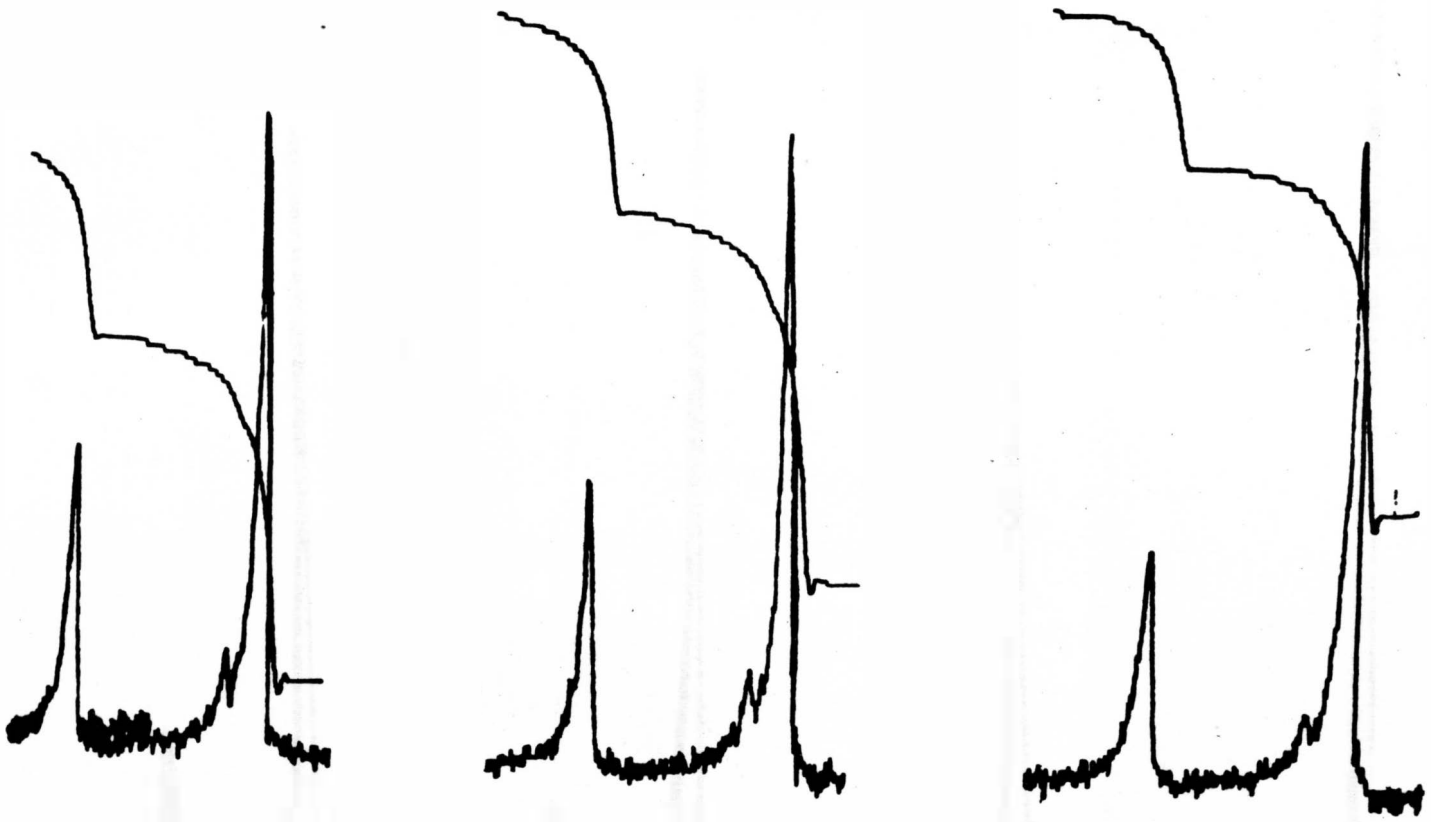
2. NMR Spectrum of Sample 12-63 in distilled benzene at 60°C

3. NMR Spectrum of Sample 12-63 in distilled benzene at 70°C

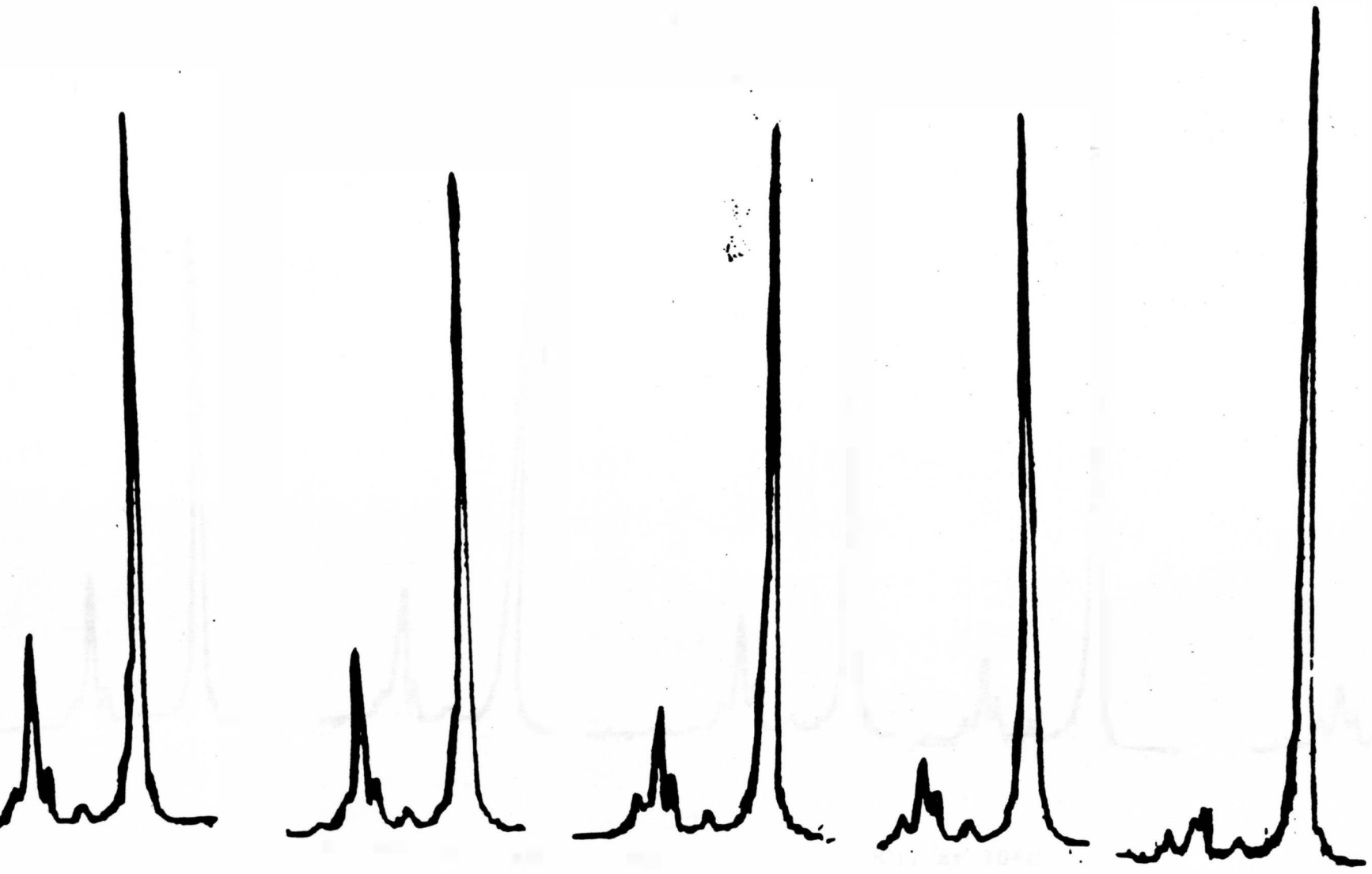


4. NMR Spectrum of Sample 12-63 in Distilled benzene at 70°C

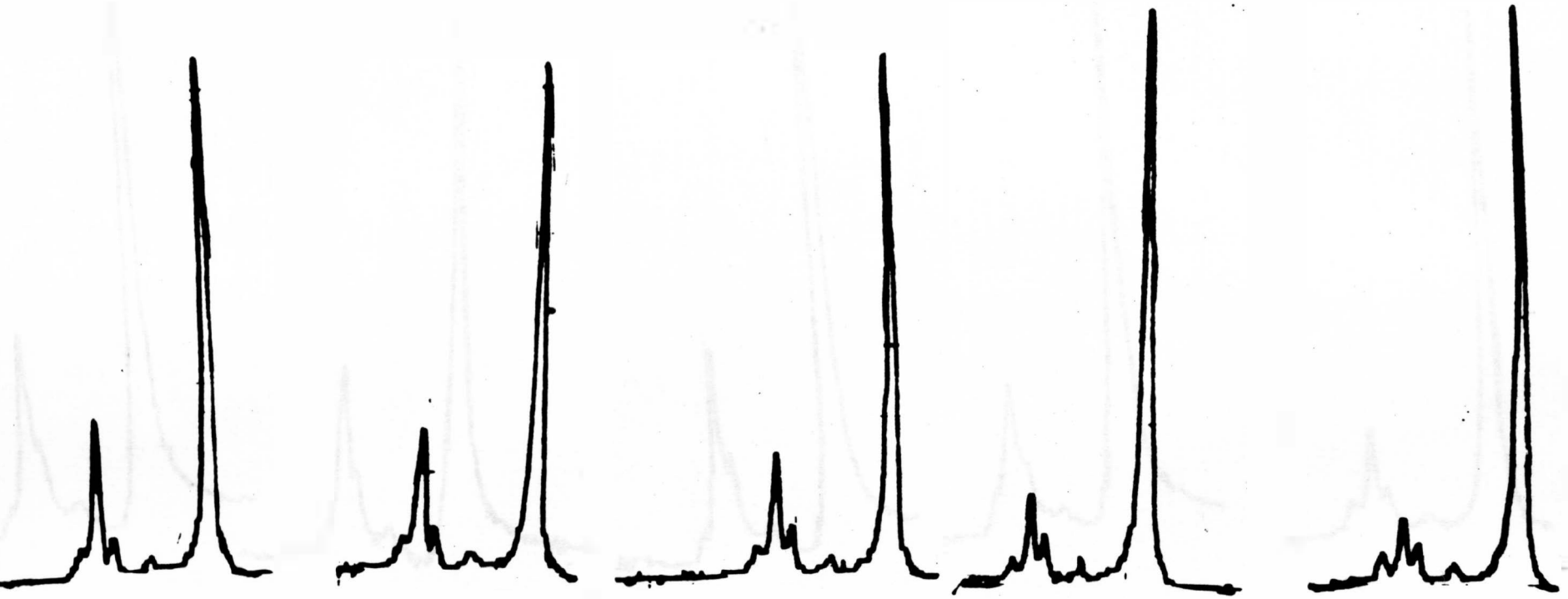
5. NMR Spectrum of Sample 12-63 in distilled benzene at 80°C



6. NMR Spectrum of Sample 12-63 in distilled benzene at 80°C

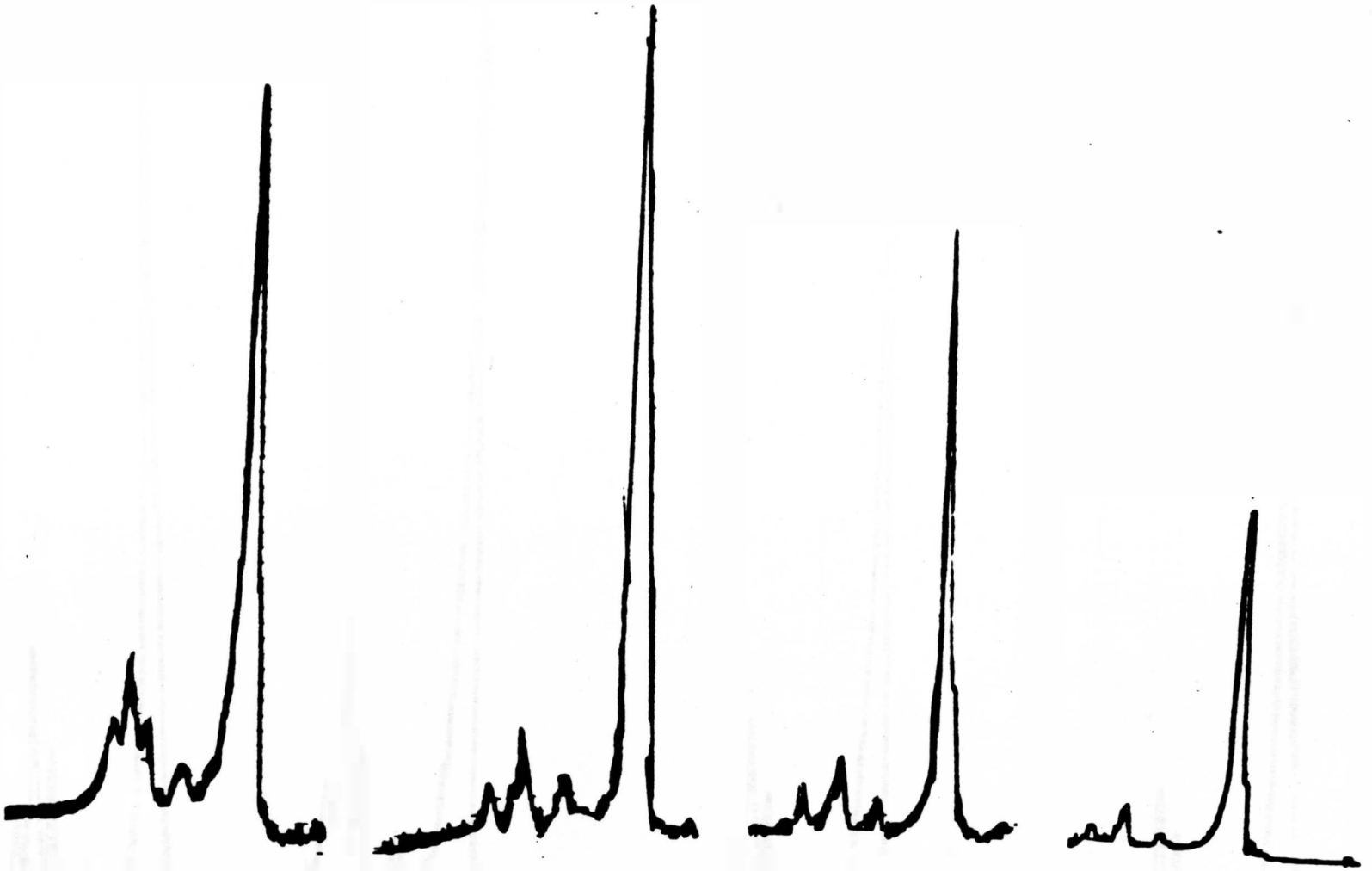


7. NMR Spectrum of Sample 12-62 in $CDCl_3$ at 60°C

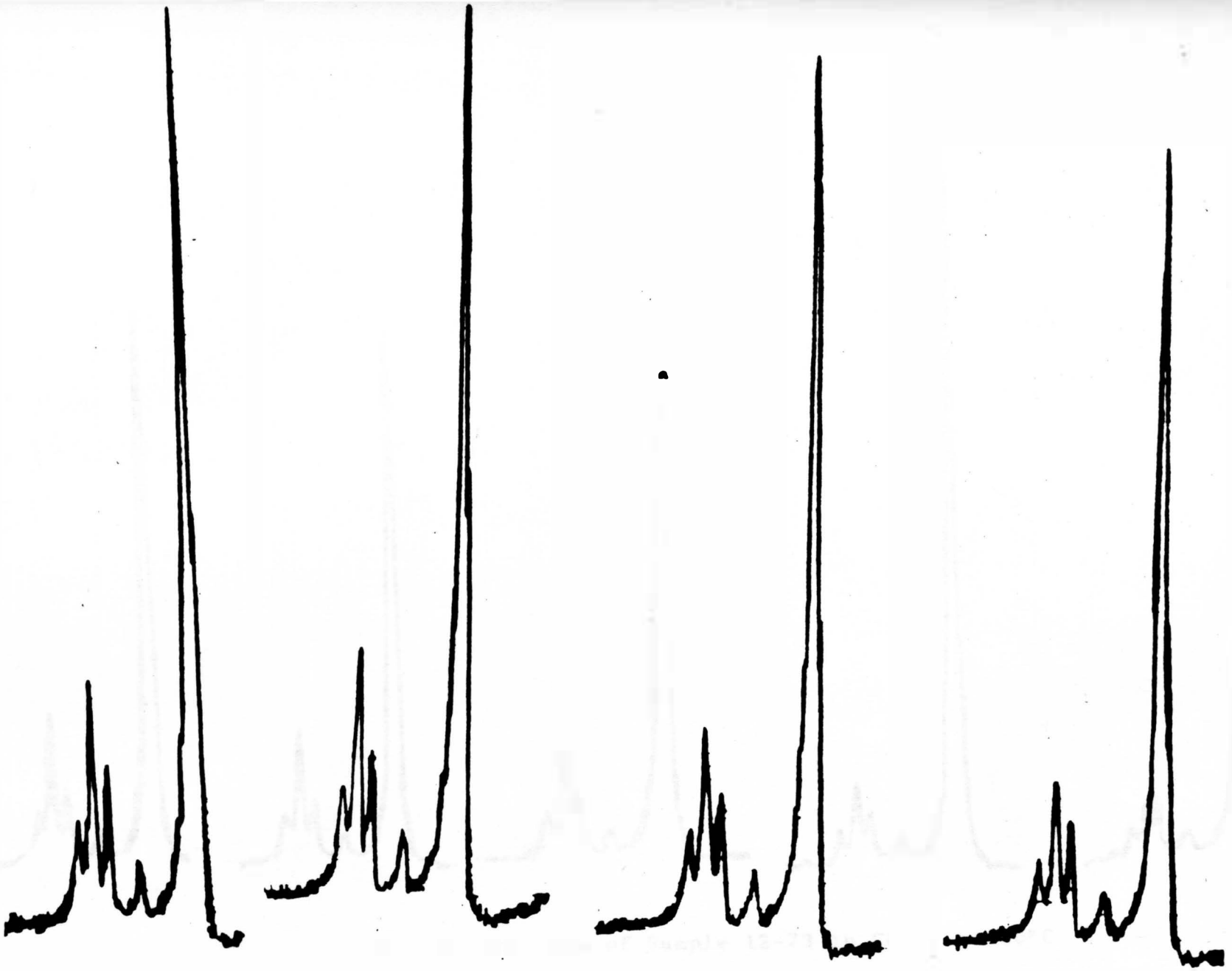


8. NMR Spectrum of Sample 12-63 in $CDCl_3$ at 70°C

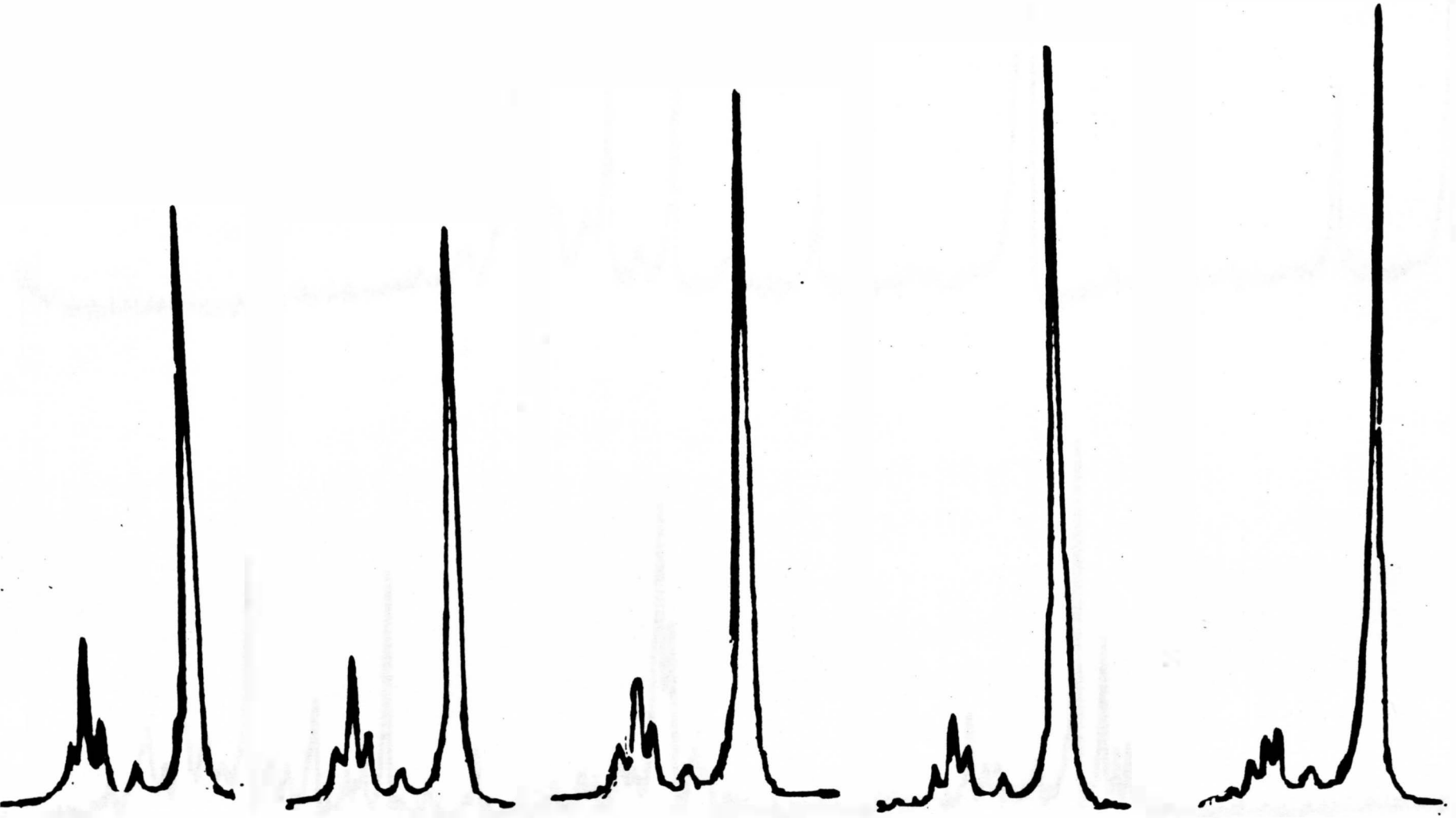
9. NMR Spectrum of Sample 12-63 in $CDCl_3$ at 90°C



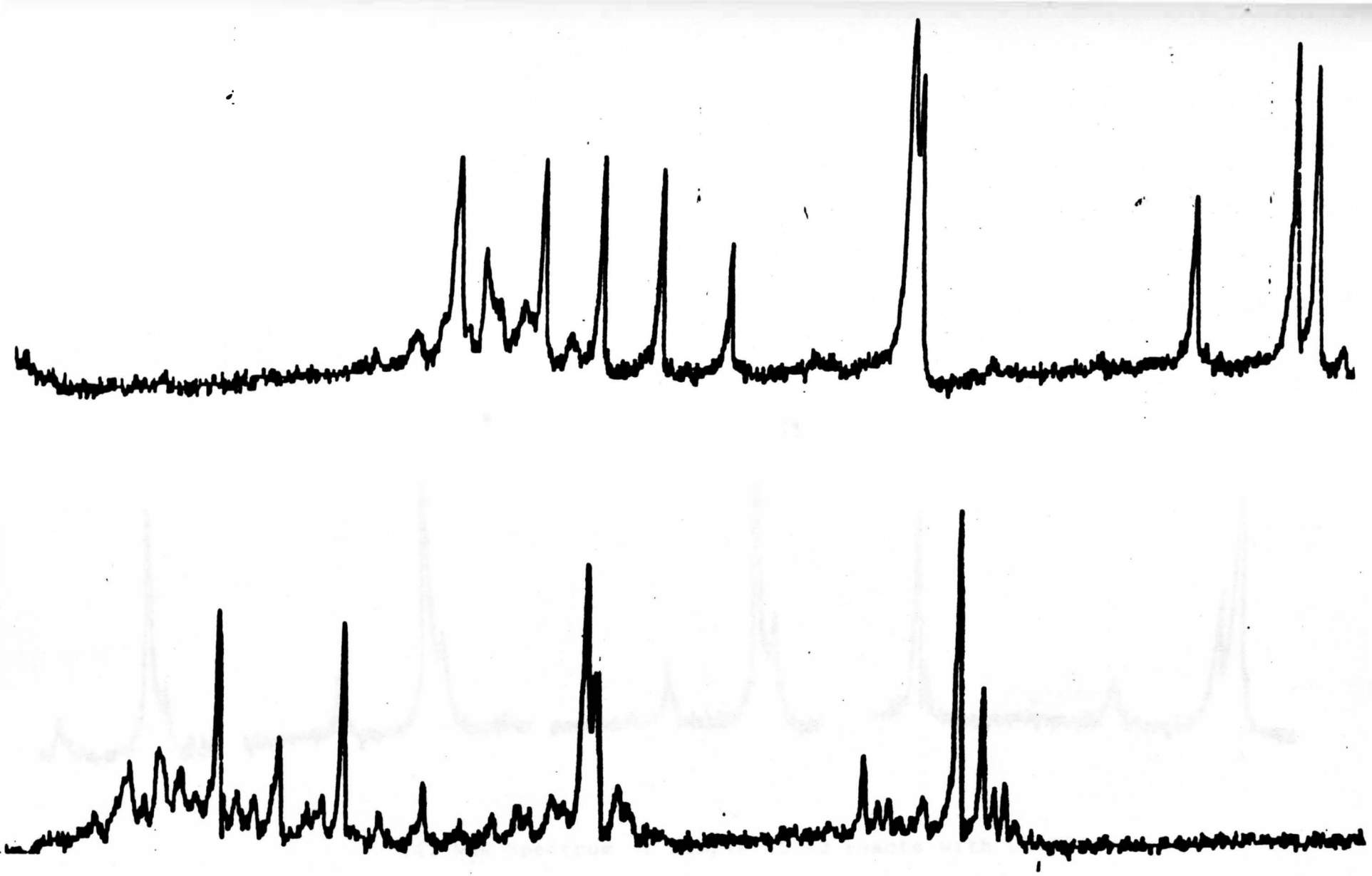
10. NMR Spectrum of Sample 12-62 in $CDCl_3$ at 60°C



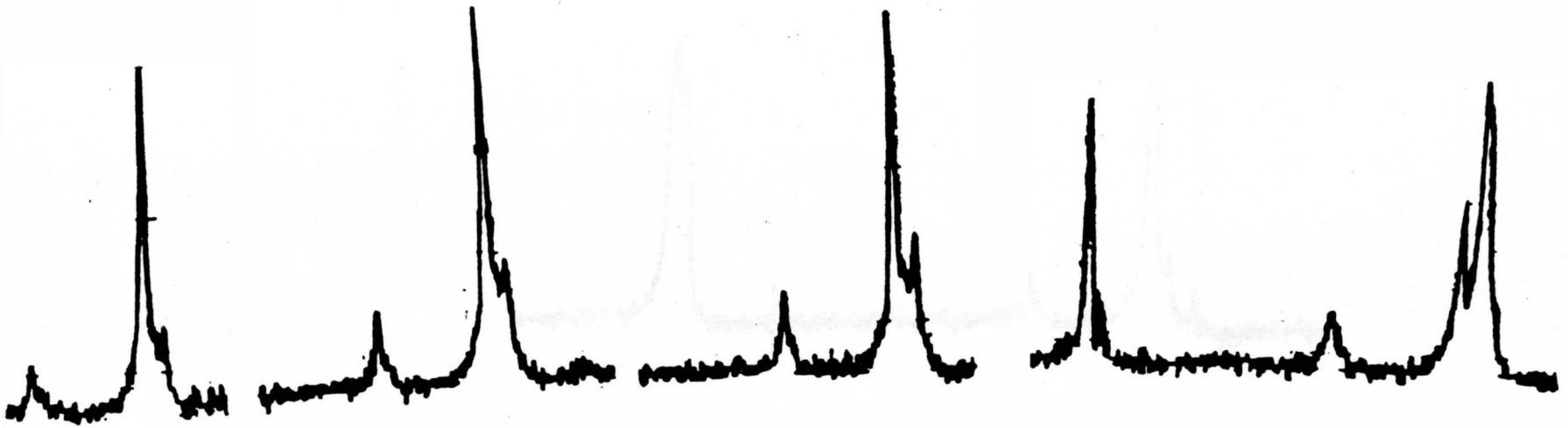
11. NMR Spectrum of Sample 12-62 in $CDCl_3$ at 60°C



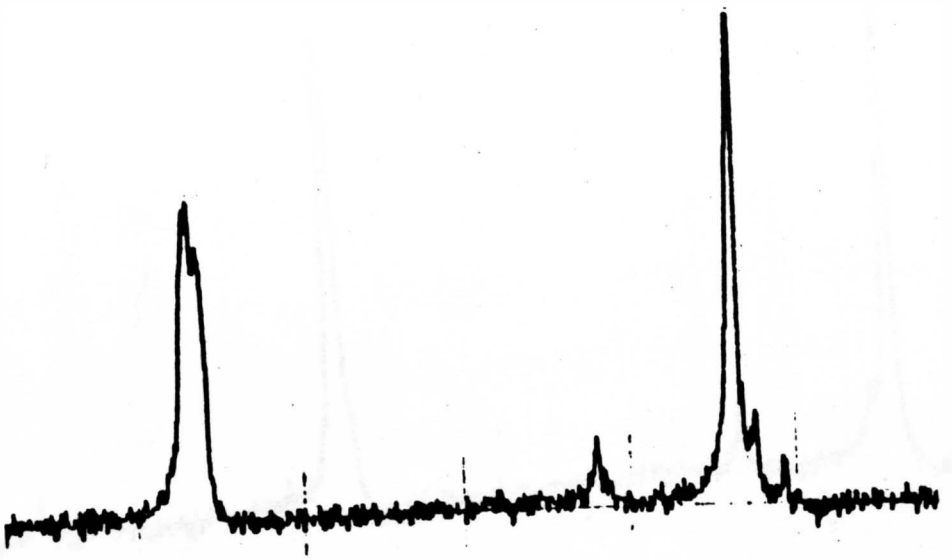
12. NMR Spectrum of Sample 12-73 in $CDCl_3$ at 60°C



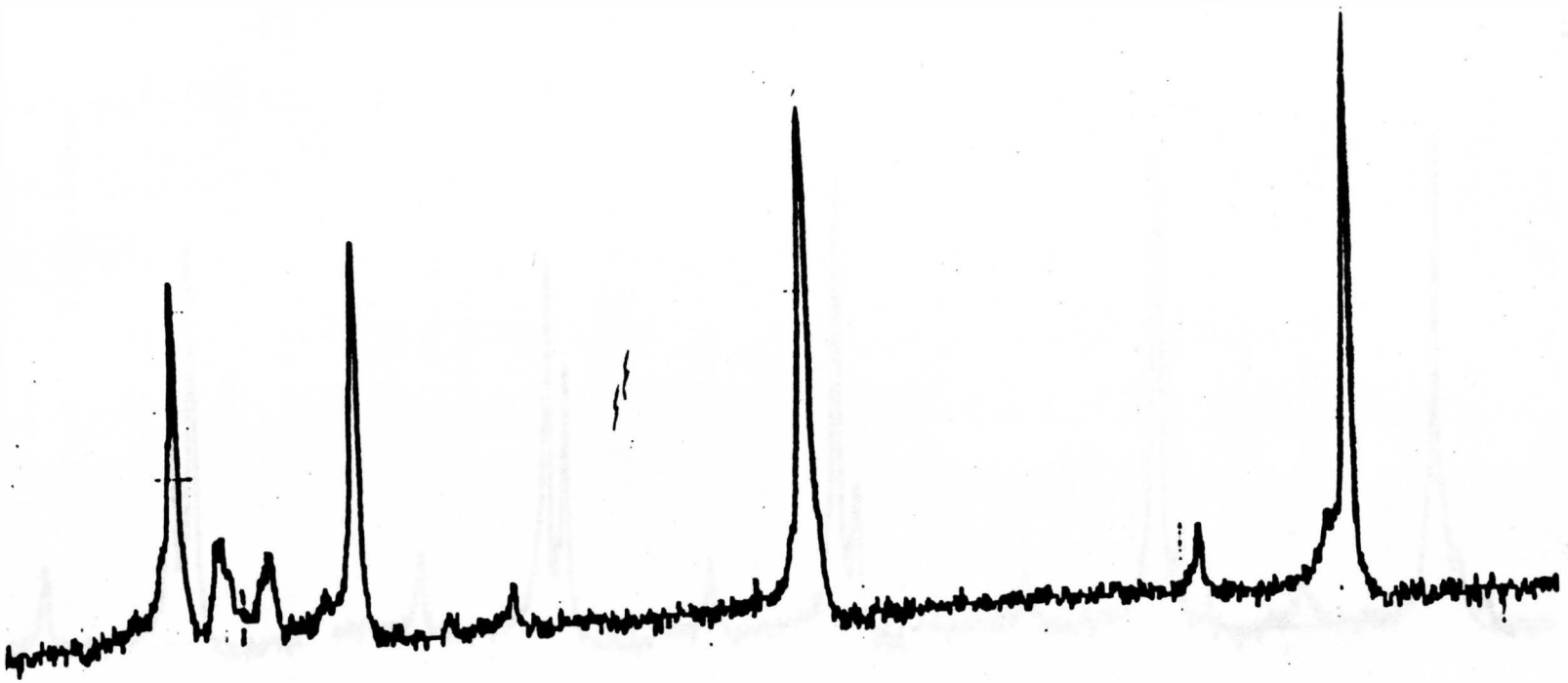
13. NMR Spectrum of Sample 12-63 reacts with F_3CCOOH



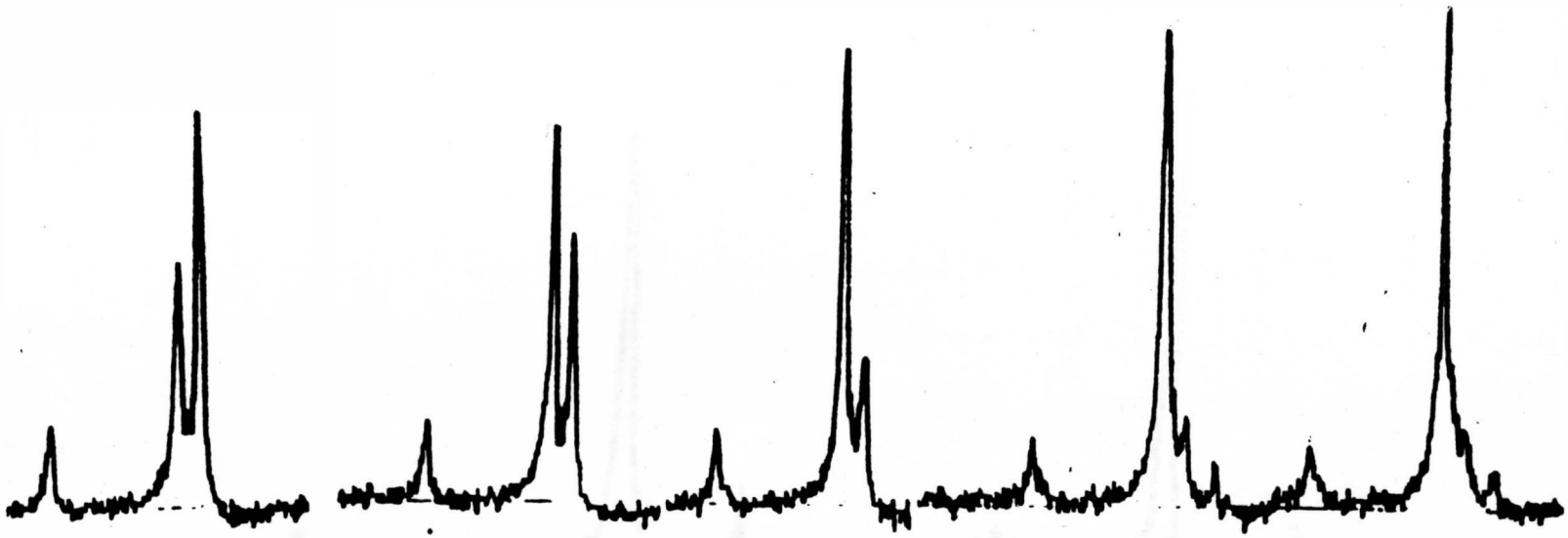
14. NMR Spectrum of Sample 12-63 reacts with Cl_3CCOOH



15. NMR Spectrum of Sample 12-63 reacts with Cl_3CCOOH

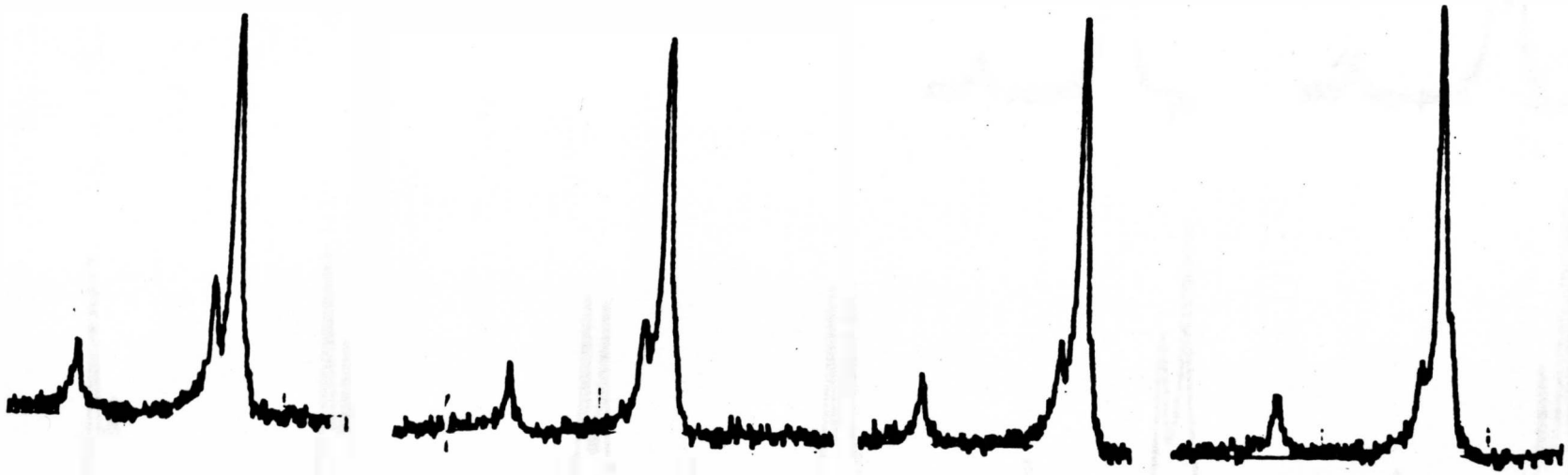


16. NMR Spectrum of Sample 12-63 reacts with $Cl_2CHCOOH$



17. NMR Spectrum of Sample 12-63 reacts with $Cl_2CHCOOH$

18. NMR Spectrum of Sample 12-63 reacts with $ClCH_2COOH$



19. NMR spectrum of Sample 12-63 reacts with $ClCH_2COOH$

20. NMR Spectrum of Sample 12-63 reacts with $BrCH_2COOH$

Table.1. Sample 12-63 in benzene at 60°C

Time(hour)	Peak height		Ratio %	$\ln Xe/(Xe-X)*10^{+2}$
	Trans	Cis		
.00	.00	46.00	.00	.00
5.39	3.00	46.00	6.12	.28
7.95	7.00	44.00	13.73	.79
11.52	6.00	35.00	14.63	.88
13.67	7.00	35.00	16.67	1.10
18.48	7.50	35.00	17.65	1.22
24.55	7.50	32.00	18.99	1.42
37.05	9.50	29.00	24.68	4.36
45.02	10.00	30.00	25.00	/

Table.2. Sample 12-63 in Benzene at 70°C

Time (hour)	Peak height		Ratio %	$\ln Xe/(Xe-X)*10^{2}$
	Trans	Cis		
.00	.00	39.00	.00	.00
2.50	2.00	32.00	5.88	.19
6.10	3.00	26.00	9.10	.32
9.78	3.00	31.00	10.30	.37
19.98	7.00	31.00	18.40	.80
22.63	11.00	25.00	26.20	1.54
33.90	11.50	31.00	31.50	2.90
42.08	15.00	24.00	32.60	3.82

Table.3. Sample 12-63 in benzene at 800C

Time (hour)	Peak Height		Ratio%	$\ln Xe/(Xe-X)*10^2$
	Trans	Cis		
.00	.00	39.00	.00	.00
1.18	3.00	39.00	7.10	.20
1.80	3.50	35.00	9.10	.26
2.48	4.50	35.00	11.40	.34
4.40	7.50	40.50	15.60	.49
4.90	7.50	36.00	17.24	.56
5.37	8.00	36.00	18.18	.61
7.59	9.50	33.50	22.10	.75
8.22	12.00	32.00	27.30	1.15
9.74	18.50	39.00	32.20	1.64
10.59	19.00	35.50	34.80	2.04
13.69	22.50	34.00	39.80	5.30
17.40	24.00	36.00	40.00	/

Table.4. Sample 12-63 in $CDCl_3$ at 60°C

Time (hour)	Peak Height		Ratio %	$\ln Xe/(Xe-X)*10^2$
	Trans	Cis		
.00	.00	150.00	.00	.00
1.00	8.00	148.50	5.35	.29
2.00	10.00	128.00	7.25	.41
3.72	15.00	117.00	11.35	.75
4.73	17.00	122.50	12.19	.84
5.78	21.00	119.00	15.00	1.20
6.78	22.50	117.50	16.07	1.38
7.82	22.50	106.00	17.51	1.68
9.25	25.00	102.00	19.69	2.48
22.47	28.00	109.00	20.44	3.00
30.70	28.50	106.50	21.11	/
33.57	30.00	110.00	21.43	/
42.87	27.50	101.00	21.40	/
44.41	27.50	100.00	21.57	/

Table.5. Sample 12-63 in $CDCl_3$ at 70°C

Time (hour)	Peak Height		Ratio%	$\ln Xe/(Xe-X)*10^2$
	Trans	Cis		
.00	10.00	88.50	10.15	.58
.83	14.00	88.50	13.66	.90
1.60	17.00	87.50	16.27	1.23
2.27	18.50	80.00	18.78	1.70
3.83	21.00	79.50	20.90	2.39
4.37	21.50	78.00	21.61	2.81
9.98	23.00	80.00	22.33	3.54
11.88	25.00	82.00	23.36	/
12.93	24.00	79.00	23.30	/
13.43	24.00	80.00	23.08	

Table.6. Sample 12-63 in $CDCl_3$ at 90°C

Time(Hour)	Peak Height		Ratio%	$\ln Xe/(Xe-X)*10^2$
	Trans	Cis		
.00	16.50	78.50	17.37	1.19
.58	22.00	80.00	21.16	1.87
1.58	24.00	80.00	23.44	2.77
3.02	29.00	95.00	23.39	2.74
3.92	31.00	95.00	24.60	4.13
5.52	32.00	93.50	25.43	/
6.03	30.00	93.00	24.39	/

Table.7. Sample 12-62 in $CDCl_3$ at 60°C

Time(hour)	Peak Height		Ratio%	lnXe/(Xe-X)*10²
	Trans	Cis		
.00	10.00	100.50	9.95	.76
.75	11.50	90.00	11.33	.93
1.75	14.50	108.00	11.84	1.00
3.25	17.00	124.00	12.06	1.03
3.82	18.00	128.00	12.33	1.07
4.77	18.00	125.00	12.59	1.11
5.20	18.00	119.00	13.14	1.17
16.08	25.00	111.50	18.32	3.77
18.37	22.00	95.50	18.72	6.44
22.77	31.00	138.50	18.29	/
24.33	35.00	151.00	18.82	/
27.15	36.00	156.00	18.75	/

Table.8. Sample 12-73 in $CDCl_3$ at 60°C

Time(hour)	Peak Height		Ratio%	$\ln Xe/(Xe-X)*10^2$
	Trans	Cis		
.00	.00	125.00	.00	.00
.20	10.00	127.00	7.30	.38
.40	13.00	123.50	9.52	.53
.60	16.00	122.50	11.55	.69
.80	16.50	116.00	12.45	.77
1.30	20.50	112.50	15.41	1.09
2.60	16.50	74.50	18.13	1.51
3.50	23.50	92.00	20.35	2.08
4.50	26.00	95.00	21.49	2.58
5.10	24.00	80.00	22.22	3.11
6.00	24.50	81.00	23.22	6.65
7.70	23.00	74.50	23.59	/
8.30	21.50	71.50	23.12	/

Table.9. Sample 12-63 reacts with $ClCH_3COOH$

Time(hour)	Peak Height			Ratio%	$lnXe/(Xe-X)*10^2$
	Trans	Cis	Acid		
.00	9.00	67.50	.00	.00	.00
.50	9.00	71.50	9.00	11.18	.17
1.00	9.00	65.50	10.00	13.25	.20
1.42	9.00	61.00	13.50	18.12	.28
1.92	9.50	58.50	14.50	19.86	.31
2.42	9.50	54.00	15.00	21.74	.35
3.00	10.50	54.50	19.00	25.85	.43
4.38	10.50	48.50	25.00	34.01	.61
5.55	10.00	42.50	27.00	39.29	.77
6.33	8.50	35.00	27.50	43.55	.90
7.71	9.50	34.00	33.50	49.26	1.12
8.14	9.50	32.50	33.50	50.76	1.18
9.99	8.00	28.00	37.50	57.25	1.52
10.58	8.00	27.50	39.50	59.00	1.64
12.24	8.50	21.50	40.50	65.32	2.22
13.16	7.00	21.00	44.50	67.94	2.54
14.32	7.00	18.50	43.50	70.16	3.16
15.16	6.50	15.50	42.50	73.27	/

Table.10. Sample 12-63 reacts with $BrCH_2COOH$

Time(hour)	Peak Height			Ratio%	$\ln Xe/(Xe-X)*10^2$
	Trans	Cis	Acid		
.00	5.00	72.50	.00	.00	.00
1.00	11.00	87.00	11.00	7.94	.10
2.00	11.50	82.00	12.00	12.70	.16
2.42	10.00	82.50	18.50	22.56	.31
6.90	12.50	63.50	28.00	32.18	.48
11.12	12.00	59.00	33.50	38.29	.61
12.95	15.00	54.00	35.50	39.44	.63
14.15	14.50	54.50	41.00	43.85	.74
22.65	15.50	52.50	68.00	58.12	1.28
23.80	19.50	49.00	71.50	61.11	1.30
25.87	12.50	43.00	71.00	63.96	1.43
27.55	12.50	40.00	72.50	66.21	1.55
29.85	13.50	34.50	79.50	69.13	1.73
30.92	13.50	35.50	82.00	71.00	1.97
33.27	11.50	31.00	85.00	72.32	2.41
35.27	11.50	30.50	94.00	73.60	2.82
38.34	12.00	29.00	99.50	78.96	4.49
47.55	8.00	26.50	100.50	83.06	/
48.82	9.50	20.50	98.00	83.69	/
49.84	7.50	19.00	93.00	83.03	/
50.95	9.50	19.00	97.50	83.69	/

Table.11. Sample 12-63 reacts with $Cl_2CHCOOH$

Time(hour)	Peak Height			Ratio%	$\ln Xe/(Xe-X)*10^2$
	Trans	Cis	Acid		
.17	9.50	78.00	10.50	11.86	.15
1.00	11.00	52.00	32.00	38.09	.58
1.67	10.50	35.00	49.50	58.58	1.13
2.17	11.00	28.00	60.20	68.25	1.57
2.67	10.00	19.50	60.00	75.47	2.07
3.67	9.00	19.00	64.00	77.11	2.23
3.75	10.00	18.00	65.50	78.44	2.38
6.08	9.00	12.00	63.00	84.00	3.58
9.07	8.00	10.50	66.50	86.34	6.76
11.33	7.50	10.00	63.00	86.30	/

Table.12. Sample 12-63 reacts with Cl_3CCOOH

Time(hour)	Peak Height			Ratio%	$lnXe/(Xe-X)*10^2$
	Trans	Cis	acid		
.08	9.50	42.50	25.50	37.50	.63
.62	12.00	20.00	52.50	72.41	2.36
.88	11.50	17.50	55.00	75.86	2.96
1.06	10.50	17.00	54.50	76.22	3.05
1.30	10.50	15.50	57.40	78.76	4.17
1.72	10.00	16.00	57.40	78.23	3.81
2.00	10.50	14.50	55.00	79.14	4.53
2.35	10.00	14.00	55.50	79.86	6.35
2.82	10.00	14.50	55.00	79.14	/

REFERENECES

1. Lehninger, "Principles of Biochemistry" Worth Publishers, inc. 1986, pp. 408

2.Klinman, Judith Pollock; and Samuel, David Biochemistry, Vol. 10, No.11 1971 pp. 2126-2130

3. Sabato, Giovanni Di; Jenck, William (1961) J. Amer. Chem. Soic. Vol.83, pp. 4393-4400

4. Kluger, Ronald; Wasserstein, Philip Biochemistry, Vol 11, No. 8, 1972, pp. 1544-1546

5. Kurz, Joseph L.; Gutsche, C. David: J. Amer. Chem. Soic. 1960, pp. 2175-2181

6. Symes, Jill; Modoro, Tomasz A. Can. J. Chem. Vol. 64 1986, pp. 1702-1708

7. Friedman, Jonathan M.; Freeman, Sally; Knowles Jeremy R. J. Amer. Chem. Soic. 1988, PP. 1268-1275

8. Wadsworth, W. S.,Jr; Horten, H.L., J. Amer. Chem. Soic., 1970, 92, pp. 3785

9. W.S. Wadsworth, Jr.; S. Larson and H.L. Horton, J. Org. Chem., 1973, 38, pp. 256.

10. A. L. Ternay, Jr., "Contemporary Organic Chemistry," W.B. Saunders Co., Philadelphia, 1976, Chap. 23.

11. Wager, R. E.; Jensen, W.; Wadsworth, W. S. Jr.; Johnson, Q.; Presented at the 8th Midwest Regional Meeting of the American Chemical Society. 1985

12. Edmundson, R.S.; Mitchell, E. W. J. Chem. Soic. (C), 1968, pp. 3033

13. Wadsworth, W. S. Jr; ACS Symposium Series No. 171 Phosphorus Chemistry 1981, 113, pp. 547.

14. Pauling, Linus and Pauling, Peter; Chemistry, Freeman 67, pp. 104.