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MOLECULAR CATALYSIS AND INTERACT-
IONS IN AQUEOUS SOLUTIONS
Final Report
Grant No. DA-CML-18-108-G-39

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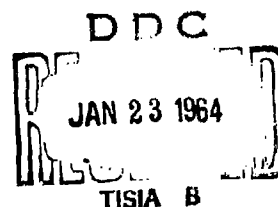
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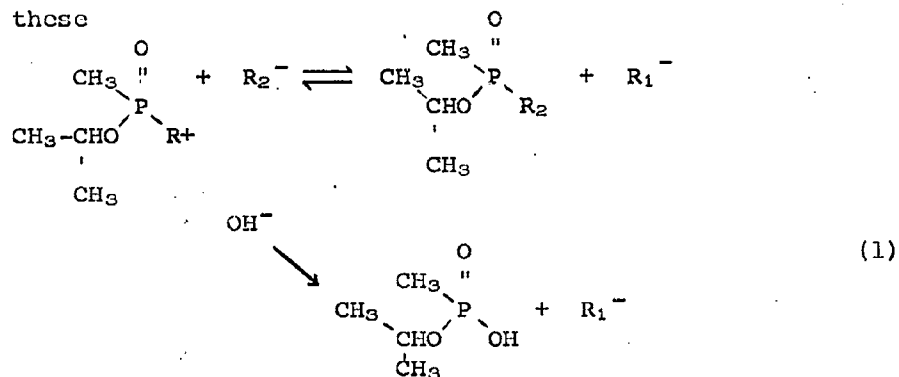
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INTRODUCTION

Results of research investigation under CML-18-108-G-39 and earlier background studies on several mechanisms leading to eventual hydrolysis of certain phosphonate esters are presented. These projects covering the period up to September 30, 1962 were concerned in general with nucleophilic exchange reactions involving these esters, as shown in equation 1, and more particularly with the replacement reactions of isopropyl p-nitrophenyl methyl phosphonate (1370) and with the hydrolytic rates of certain of these



esters. Evidence is presented which indicates that some of these compounds undergo rather unexpected and facile hydrolytic cleavage strongly suggestive of some form of intramolecular catalysis. The relative influence of various nuclear substituents on the rates of hydrolysis of phenolate phosphonates have also been evaluated.

The chemical behavior of these compounds are of particular significance because of increasing recognition of their biochemical, commercial, and industrial importance. Although they have been

known since the middle of the last century it is only in recent years that the organophosphorous compounds have received much attention. Most of the published papers are in reference to the phosphates of sugar and related compounds. The interest in these compounds has grown since they are found in living organisms and also play an important role in the metabolism of carbohydrates and in the synthesis of nucleotides. Simpler phosphoric esters have also achieved some importance in industry as solvents, plasticisers, and fuel additives, while the partially ionic esters of higher alcohols were used to some extent as surface active agents. Discovery that many of these compounds were extremely toxic led to their development as potential war gases and in agriculture as insecticides.

Reactions of some of these esters, moreover, appear to be mediated by polyfunctional interactions somewhat analogous to enzymatic reactions. More than one grouping in both the ester and the attacking reactant seems to participate in bringing about certain of these reactions. For this reason it was hoped that the results of these studies might shed some light on the more complex biological reactions.

PAST WORK

Organophosphorous Compounds Reactions

Because of the importance of the organophosphorous compounds in medicine and as potential chemical warfare agents, a great deal of work has been performed in elucidating the mechanism by which these compounds act in the body. It has been shown that these compounds act as irreversible inhibitors of acetylcholinesterase and thus exert their toxic effects due to the accumulation of acetylcholine. Therefore these organophosphorous compounds act as parasympathomimetic agents, which means that they cause symptoms associated with the stimulation of the parasympathetic nervous system. The symptoms of poisoning with these organophosphorous compounds includes miosis, salivation, sweating, muscular weakness, loss of muscular coordination, gasping, and finally cessation of respiration due to bronchial constriction. One of the most common antidotes used in poisonings is atropine, a parasympatholytic agent.

Since the organophosphorous compounds react with acetylcholinesterase, in the body, a great many studies have been undertaken to determine the mechanism by which acetylcholinesterase reacts with acetylcholine and these organophosphorous compounds. In the acetylcholinesterase hydrolysis of acetylcholine, Wilson (1-3) has proposed that acetylcholinesterase has two active sites,

an anionic and an esteratic site. The esteratic site has both a nucleophilic and an electrophilic group. The anionic site has a negative group which binds with the positive quaternary nitrogen of the ester thus allowing the nucleophilic group in the esteratic site to exert its catalytic activity. The function of the electrophilic group has not yet been explained although it may participate in a push-pull mechanism. The organophosphorous compounds are proposed to act by attaching themselves to the esteratic site and thus preventing the normal reaction from occurring. Considering the structure of the esteratic site and the fact that these organophosphorous compounds are esters or halides we can envision a potentially negative center on the enzyme and a closely located center containing a reactive hydrogen (OH or NH groups). The formation of a hydrogen bond is pictured followed by the attachment of the partially positive phosphorous atom to the negative site and the elimination of the fluoride ion. It is not wise to assert that the initiating reaction is usually hydrogen bonding between the fluorine of the phosphorous compound and the active hydrogen, but it should be noted that the phosphochloridic esters are not toxic and here hydrogen bonding is not possible. By attaching themselves thusly the organophosphorous compounds prevent the normal reaction with acetylcholine from occurring.

The hydrolysis of the organophosphorous compounds has been

shown to be catalyzed in a bifunctional manner by many types of functional groups. The alkaline hydrolysis of sarin and some of its analogs has been investigated and has been shown to proceed by an S_N2 mechanism (4).

In a search for compounds which could react with these toxic organophosphorous compounds under physiological conditions the reactions of these agents with hydroxylamine and its N substituted derivatives have received much attention. Several workers (5,6) have studied the reactions of sarin with oximes. The rates were found to be proportional to the concentration of the oximate ion and the most effective compounds were 1-2 dione monoximes. The rate determining step was proposed to be the nucleophilic attack of the oximate ion on the phosphorous compound. Hackley et al and other workers (7-13) have found that the reaction of sarin with hydroxamic acids involves a nucleophilic attack by the hydroximate ion as the rate determining step. The tautomeric form of the ion was proposed to be the reactive species in order to explain the very high activity of these compounds.

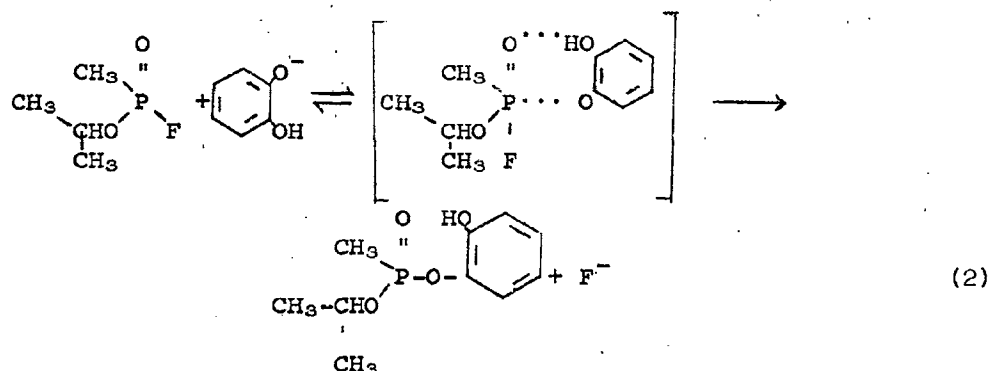
The catalytic effect of a number of metal chelates on the hydrolysis of sarin have been studied (14-16). Cu(II) chelates were the most effective but chelates of $UO_2(VI)$, $ZrO(IV)$, $Th(IV)$, and $MoO_2(VI)$ also catalyzed the reaction. The Cu(II) chelates of dipyridyl and tetramethylenediamine were the most effective. It has been proposed that the electrophilic metal ion probably increases the polarization of the P-O and P-F bonds by which

nucleophilic attack of the hydroxyl ion on the phosphorous is facilitated. Another possibility based on the pH dependence of the reaction rate, proposes that $\text{Cu}(\text{OH})(\text{H}_2\text{O})_3^+$ is the active species and a "push-pull" bifunctional mechanism is responsible for the high rate of reaction of the relatively weak bases.

From an investigation of the reaction of sarin with hydrogen peroxide, Larsson (17) proposed an intermediate bifunctionally attached complex to explain the greater reactivity of the perhydroxyl ion as compared to the hydroxyl ion although the latter is much more basic.

Since the catalytic activity of acetylcholinesterase was probably associated with amino acid side chains, Berry et al (18) studied the ability of a number of amino acids to protect cholinesterase in vitro. Of all of the amino acids tested only DOPA (3-4-dihydroxyphenylalanine) was effective. Further studies showed that the protective action was a property of the catechol portion present.

Epstein et al (19) have studied the reaction of sarin with certain catechol derivatives and have proposed the following mechanism:



These investigators found marked differences in the reactivities of phenols and catechols (of comparable basicity) which strongly suggested the participation of the undissociated hydroxyl group. It was pointed out that the unionized hydroxyl group may facilitate the catechol reactions by its ability to hydrogen bond with the phosphoryl oxygen of the sarin thus favoring the nucleophilic attack by the ionized hydroxyl on the partially positive phosphorous of sarin. This proposed mechanism indicates that a two point attack by the monocatecholate ion on the sarin is responsible for the difference in the reaction rate between the catechols and the phenols. Recent work by Larsson (20) has shown, by infrared techniques, that there was hydrogen bonding between the phosphoryl oxygen and the phenolic hydrogens of catechol in aprotic solvents which tends to substantiate the proposed mechanism. The above mechanism is further supported by the fact that:

- a) the reaction rate is dependent on the concentration of sarin and the monocatecholate ion.

- b) catalytic activity of the catechols is much greater than of phenols of comparable basicity.
- c) resorcinol and hydroquinone are no more active than phenol while the dicatecholate ion is less active than the mono-catecholate ion.

Intramolecular Catalysis

The study of enzymes is a subject which has special interest because it lies on the borderline where the biological and physical sciences meet. Enzymes are of supreme importance since life depends upon the complex network of chemical reactions brought about by specific enzymes and also they are receiving increasing attention in attempts to elucidate their mechanisms of action, as catalysts.

A characteristic property of enzymes is their power to catalyze certain definite reactions. An idea of their effectiveness as catalysts is sometimes given by expressing their turnover numbers, which are the number of molecules of the substrate which are decomposed per minute by one mole of catalytic site. Some of these turnover numbers show the great efficiency of these enzymes, catalase has a turnover number of 5×10^8 for the decomposition of hydrogen peroxide. Most workers are inclined to believe that this remarkable efficiency is not due to any special kind of chemistry but probably due to combinations of ordinary mechanisms.

Most enzyme reactions are considered intramolecular in their mechanisms. This can easily be visualized since enzymatic reactions appear to proceed through the formation of an absorptive complex between the enzyme and the substrate. The catalytic process thus occurs while the substrate is held locked together with the enzyme as a single unit. Such a mechanism likens enzymatic action to intramolecular catalysis. Thus like many intramolecular reactions in organic chemistry, enzymatic catalysis should proceed at a greater rate than the corresponding intermolecular process.

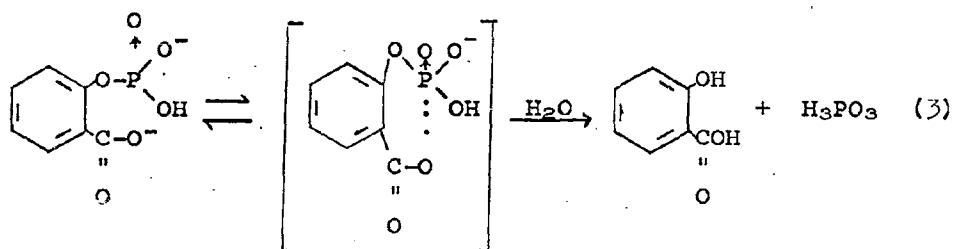
It has been shown that imidazole, which occurs in histidine and is considered the active site of chymotrypsin, is much less active than chymotrypsin itself in hydrolyzing esters. Bruice and Sturtevant (21) used γ -(4-imidazolyl) butyric acid esters as enzyme models in which imidazole acts as an intramolecular catalyst in the hydrolysis of the ester grouping. This compound gave a rate comparable to that of the enzymes in the ester hydrolysis. This would indicate that although imidazole is the catalytic factor its activity is enhanced by its incorporation into the substrate molecule.

Also working on the premise that intramolecular catalysis may be likened to enzymatic catalysis, since the substrate is held in a position close to the catalytic group, Bender (22-24) has investigated the hydrolysis of phthalamic acid and methyl

hydrogen phthalate as model compounds. The hydrolysis of the phthalamic acid was found to be 10^5 times faster than the hydrolysis of benzamide at comparable hydrogen ion concentration. The large rate of enhancement was due to the fact that the carboxylic group catalyzes the amide hydrolysis by a direct intramolecular catalysis. The authors have suggested that this intramolecular process is a general acid base catalyzed reaction and that the function of the carboxylic acid is to attack the carbonyl carbon atom of the amide while simultaneously donating a proton to the departing ammonia molecule.

Further examples of intramolecular catalysis have been proposed by Garrett (25) in the hydrolysis of aspirin, by Morawetz and Oreskes (26) in the hydrolysis of succinyl salicylic acid, and by Leach and Lindley (27) in the amide hydrolysis of certain amino acids. Winstein and co-workers (28-30) have also given many examples which demonstrates that a neighboring group may not only participate in the removal of the neighbor, but by such participation accelerate its removal.

Chanley and co-workers (31-33) have shown that salicyl phosphate and other phosphate esters are subject to intramolecular catalysis. These studies led to the conclusion that it was the ionized carboxyl group which brought about the catalysis, by its attack on the phosphorous atom. A proposed factor which



might explain this type of attack is the ease with which the phosphorous is capable of expanding its valence shell, thus becoming more susceptible to attack by a nucleophilic reagent.

Kinetic comparisons between a number of corresponding cases of intermolecular and intramolecular catalysis of hydrolytic reactions (34) indicates the powerful nature of intramolecular catalysis and further suggests that in a rigidly held enzyme substrate complex nucleophilic and/or electrophilic catalysis is responsible for the powerful action of enzymes in hydrolytic reactions.

HYDROLYTIC AND TRANSESTERIFICATION REACTIONS OF ISOPROPYL
P-NITROPHENYL METHYL PHOSPHONATE

The relative rates of these reactions were investigated in an attempt to determine the respective roles of such factors as nucleophilicity, basicity, steric, and complexing tendency on the speed of attack on these esters by nucleophiles. Possible participation of more than one grouping in polyfunctional reactants such as polyhydroxy phenols and oximes have been studied. These systems were selected in the belief that they may represent a very much simplified version of the type of interaction responsible for the very high specific activities of enzymes.

Since enzymes are responsible for all biological processes occurring in living organisms a great deal of work has been done in trying to elucidate the nature of enzyme catalysis. A better understanding of their mechanisms of action should lead to a more rational basis for developing drugs and other substances of interest. Since most enzymes have very complicated structures most workers in recent years have turned their attention to model systems in an attempt to better understand enzyme action.

Relatively little work has been done on model enzyme systems for investigating the nature of the interactions involved in forming enzyme substrate complexes. There appears to be little

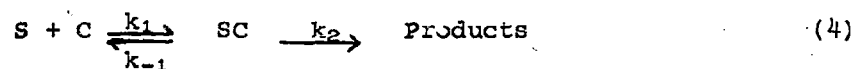
doubt that in many cases the binding between the enzyme and the substrate takes place at more than one site. Therefore, it seems quite likely that a system in which polyfunctional catalysis between two species has been observed (as in the catechol sarin interaction) should provide an ideal model for a study of the type of interaction occurring in the formation of enzyme substrate complexes. This analogy is quite natural since the reaction of acetylcholinesterase with the organophosphorous compounds also appears to occur with a polyfunctional attack of a nucleophilic group on the phosphorous and an electrophilic attack on the fluorine. Thus, the reaction of the catechols with the organophosphorous compounds can be considered as a model of the reaction between acetylcholinesterases and the organophosphorous compounds.

The proposed mechanism for the reaction of sarin with catechols are reminiscent of the Michaelis-Menten mechanisms of enzyme catalysis. This assumes the formation of a strongly bonded complex between the enzyme and the substrate followed by breakdown of the complex into products with the regeneration of the enzyme.



The mathematical treatment of this mechanism applies the steady state approximation for ES and leads to the rate law $V = \frac{V_{\max}}{K/S+1}$

where V is the reaction rate and $V_{\max}$ is $k_2 E$. Since many of the reactions of sarin appear to proceed through formation of an intermediate complex, they should lend themselves quite readily to a similar type of mathematical treatment. Consider therefore, the rapid reversible reaction of a phosphonate ester, S , with a reactant, C , to form a complex, SC , which subsequently decomposes into products, P . This designated as equation 4:



If $k_2 \ll k_{-1}$ the initial concentration of the intermediate complex may be determined from the equilibrium:

$$(SC) = K (S) (C) \quad (5)$$

where $K = k_1/k_{-1}$

also initially $(S) = (S)_0 - (SC)$

$$(C) = (C)_0 - (SC) \quad (6)$$

where $(S)_0$ is initial phosphonate ester concentration and $(C)_0$ is the initial reactant concentration.

If $(C)_0 \gg (S)_0$ the $(C) \cong (C)_0$ and

$$(SC) = K (C)_0 \left\{ (S)_0 - (SC) \right\} \quad (7)$$

$$(SC) = \frac{K (C)_0 (S)_0}{1 + K (C)_0} \quad (8)$$

$$\text{Rate} = k_2 (SC) = \frac{k_2 K (C)_0 (S)_0}{1 + K (C)_0} \quad (9)$$

Since the C is normally in excess, the observed reaction will be pseudo first order. Using the initial rate:

$$-\frac{d(S)_0}{dt} = k'_1(S)_0 \quad (10)$$

where k'_1 is the observed first order rate constant. Equating equations 9 and 10 one gets:

$$k'_1 = \frac{k_2 K (C)_0}{1 + K (C)_0} \quad (11)$$

From equation 11 it can be seen that at a low $(C)_0$ the reaction will be first order with respect to (C) , but at sufficiently high $(C)_0$ the rate will be zero order with respect to (C) . The latter case corresponds to the situation where essentially all of (S) is in the form of (SC) and addition of more (C) will not increase the rate.

Taking reciprocals of both sides of equation 11:

$$\frac{1}{k'_1} = \frac{1}{k_2 K (C)_0} + \frac{1}{k_2} \quad (12)$$

A plot of $1/k'_1$ as a function of $1/(C)_0$ should give a straight line with a slope of $1/Kk_2$ and an intercept of $1/k_2$. By this means both K and k_2 may be evaluated. The K values thus obtained for the interactions of a substrate with a series of reactants should be a measure of the relative affinities of these compounds for the particular substrate.

Previous work carried out in this laboratory (35) utilized sarin (isopropyl methyl phosphonofluoridate) as the organo-phosphorous compound. In this present work a different compound isopropyl p-nitrophenyl methyl phosphonate (1370) has been studied. The 1370 should lend itself more effective in this type of study

than sarin, because of its slower rate of hydrolysis will allow greater concentrations of the reactant to be used, as well as the fact that the nitro group on the molecule may cause interactions with the positive centers on the attacking group.

This part of the study was mainly concerned with the effect on the strength of binding between various possible reactants and 1370 brought about by changes in the structure of the former.

Experimental

Apparatus

Sargent "Thermonitor" temperature control.

Ultra buret, Model 200, Scientific Industries, Inc.

Klett Summerson Photoelectric Colorimeter, Model 900-3

with a No. 42 filter.

Beckman Model G pH meter with glass and calomel electrodes.

Circulating pumps.

"Time it" second timer, Precision Scientific Co.

Magnetic stirrer, with teflon coated stirring bar.

Reagents

Isopropyl p-nitrophenyl methyl phosphonate (1370),

as supplied by U.S. Army Chemical Corps.

Potassium nitrate, reagent grade.

Potassium biphthalate, reagent grade.

Nitrogen gas, high purity.

p-Nitrophenol, recrystallized from water.

1-2-3-Cyclohexanetrione trioxime, Eastman Kodak Co.

5-Methyl 1-2-3-cyclohexanetrione trioxime, Eastman
Kodak Co.

5-Methyl 1-2-3-cyclohexanetrione 1-3-dioxime, Eastman
Kodak Co.

Pyrogallol, Eastman Kodak Co.

Gallic acid, reagent grade.

Propyl gallate, reagent grade.

Formaldehyde, 40% aqueous solution.

Dimethylamine, 25% aqueous solution.

Procedure for kinetic study

The reaction assembly used in this work is shown in Figure 1. The reaction vessel consisted of a jacketed cell fitted with a polystyrene cover with inlets for the electrodes from the pH meter, nitrogen gas, and the ultraburet. There was also an opening for the removal of samples. Water at a constant temperature was circulated through the cell jacket and the reaction mixture was stirred continuously with a magnetic stirrer.

Exactly 100ml. of a 0.1 molar potassium nitrate solution was transferred to a cell by pipette and an accurately weighed quantity of reactant was added. The solution was then brought to the desired pH by the addition of sodium hydroxide solution

and a sample was taken and analyzed as a blank. The 1370 solution was then added, the timing begun, and the samples were taken at appropriate intervals. Throughout the run the pH was maintained at the initial value by the addition of small amounts of alkali to compensate for any acid produced during the reaction.

Assay procedure

The rate of hydrolysis of 1370 was measured by the rate of appearance of p-nitrophenol.

a) With reactants which imparted no color to the solutions the assay was carried out in the following manner: Samples were adjusted to pH 8.8 with a borate buffer solution and the intensity of the yellow color due to the p-nitrophenol was measured with the Klett Summerson colorimeter. The total amount of p-nitrophenol present after complete hydrolysis of the 1370 with sodium hydroxide was determined and in this manner the initial amount of 1370 was determined by the use of a calibration curve relating the amount of p-nitrophenol to the 1370 concentration.

b) For reactants which imparted color to the solutions the assay was modified as follows:
Samples were taken and adjusted to pH 4 by the addition of phthalate buffer. Then 10 ml. of xylene was added with shaking

and 5 ml. of the xylene solution was taken. To the xylene solution 3 ml. of pH 8.8 borate buffer was added with shaking and the aqueous solution was separated and read on the Klett Summerson colorimeter. By this method the p-nitrophenol in its acid form was extracted by the xylene and then re-extracted out by the aqueous alkaline solution which was assayed.

Determination of the acid dissociation constants

The acid dissociation constants of the compounds used as reactants were determined by potentiometric titration or obtained from the literature, where available. When determined potentiometrically the pKa was taken as the pH at the point of half neutralization.

Preparation of reactants.

Salicylaldoxime- 0.1 moles of salicylaldehyde and 0.1 moles of hydroxylamine hydrochloride were dissolved in 110 ml. of 2 N sodium hydroxide solution and the resulting mixture was warmed and allowed to react. After thirty minutes the mixture was acidified with acetic acid and cooled. Upon cooling salicylaldoxime separated out as an oil. This oil was purified by recrystallizing first from benzene and then from petroleum ether. Melting point 56 - 57°C, literature value 57°C.

1-2-Cyclohexane dioxime- was prepared by the method of Belcher et al (36). Melting point 186 - 188°C, literature value 186-188°C.

Methyl gallate- 0.25 moles of gallic acid, 0.25 moles of methanol, and 4 ml. of sulfuric acid were mixed and allowed to reflux for eight hours. The remaining methanol was distilled off under reduced pressure. Fifty ml. of cold water was added to the residue and the resulting suspension was filtered and then washed with more cold water. The remaining methyl gallate was recrystallized from water. Melting point 200°C , literature value 202°C .

3-6-Bis(dimethylaminomethyl) catechol dihydrochloride (CDMA)- 0.25 moles of catechol was dissolved in a 25% aqueous solution of dimethylamine representing 0.5 moles of dimethylamine. The solution was placed in a three-necked flask fitted with a stirrer, condenser, and a dropping funnel. Formaldehyde, 0.5 mole, as a 40% aqueous solution was added dropwise with stirring, the stirring being continued for two hours after all of the formaldehyde had been added. The mixture was then extracted with ether and the ether solution evaporated to dryness under nitrogen to remove any residual dimethylamine. The residue was redissolved in ether, the ether solution dried and anhydrous hydrochloric acid gas admitted to saturation. The gummy precipitate was filtered off and recrystallized from methanol. Melting point $227-228^{\circ}\text{C}$ with decomposition. The equivalent weight determined by potentiometric titration was 297, the calculated equivalent weight was 297.3.

Propyl ester of 2-diethylaminomethyl gallic acid

dihydrochloride (GME)- Propyl gallate, 0.25 mole, was dissolved in methanol and 0.25 mole of diethylamine, as a 25% aqueous solution, was added to a three-necked flask. With stirring 0.25 moles of formaldehyde was added dropwise. A precipitate formed which was washed twice with water and then dissolved in 20% hydrochloric acid solution. The solution was then evaporated to dryness and the residue was recrystallized from isopropanol. Melting point 155-156°C. The equivalent weight determined by potentiometric titration was 325, the calculated equivalent weight was 333.

Propyl ester of 2-6(bis diethylaminomethyl) gallic acid

dihydrochloride (GDE)- Propyl gallate, 0.25 moles, was dissolved in methanol and 0.50 moles of diethylamine was added in a three-necked flask. Formaldehyde, 0.50 moles, as an aqueous solution was added dropwise with stirring, the stirring being continued for one hour after the last formaldehyde was added. The reaction mixture was then refluxed for three hours and the solution was then evaporated to dryness leaving a thick red oil. This oil was taken up in ether and washed with water five times. The ether solution was then dried and anhydrous hydrochloric acid gas was admitted until saturation. A gummy precipitate was formed which was recrystallized from isopropanol. Melting point 88-90°C. The equivalent weight determined by potentiometric titration was 454, the calculated equivalent weight was 455.5.

Results and Discussion

pH Dependence of the hydrolysis of isopropyl p-nitrophenyl methyl phosphonate (1370)

The dependence of the rate of hydrolysis of 1370 on the hydroxyl ion concentration was determined at 25°C and at 35°C. When the logarithm of the pseudo first order rate constants thus obtained were plotted against pOH, straight lines as shown in Figure 2 resulted. The equations for these lines were calculated to be:

$$\log k_1 = 1.357 - 0.984 \text{ pOH at } 35^\circ\text{C}$$

$$\log k_1 = 1.021 - 0.977 \text{ pOH at } 25^\circ\text{C}$$

where k_1 is the observed pseudo first order constant.

The heat of activation calculated from these data by means of the Arrhenius equation was found to be 13.9 kcal/ mole after the subtraction of the 12 kcal/ mole due to the heat of ionization of water, was in good agreement with the reported 13.8 kcal/mole (37).

Kinetics of reactions of isopropyl p-nitrophenyl methyl phosphonate (1370) with several nucleophiles

As discussed in the earlier sections one of the objectives of these studies was to establish whether reactions as represented by equation 1 were mediated in part by formation of a complex according to equation 4. For this purpose rates of reaction of 1370 with various concentrations of several catechols, oximes,

and mannich bases were determined at 35°C. The resulting data, corrected for the hydroxyl reaction, were plotted reciprocal wise against the reciprocal concentration of the attacking species (eg. catecholate or oximate). Resulting plots, a few of which are shown in Figures 3-6 can be interpreted in terms of equation 12 to yield K and k_2 values shown in equation 4.

Although extrapolation of these plots is associated with a considerable degree of uncertainty, the plotted data seems to yield in these instances significantly positive, non-zero intercepts corresponding to $1/k_2$. The relative magnitudes of the stability constants of the indicated complexes are evident in Table I which lists all of the observed values obtained from the reciprocal plots. The probable error in determination of the equilibrium constants as judged from the precision of the measurements is estimated very approximately to be of the order of ± 4 units.

The range in the stability constants obtained kinetically as shown in the table is in accord with the values determined by other techniques in these laboratories for the interactions of organic solutes in water (38). The data suggests that the gallates in particular are good binders. Some differences in binding between the reactants may be explained by the fact that the extra hydroxyl group, in the case of the gallates, or the extra oxime group increases the probability of attachment if only by statistical considerations.

TABLE I

<u>Compound</u>	<u>Code name</u>	<u>pKa</u>	<u>K (35°C)⁺</u> <u>(l/mole)</u>	<u>Second order</u> <u>rate constant</u> <u>(min⁻¹)</u>
Salicylaldoxime		8.93	8	6.24
1-2-Cyclohexanedione dioxime	DO	9.73	12	2.28
1-2-3-Cyclohexanetrione trioxime	TO	7.98	21	3.38
5-Methyl 1-2-3-cyclo- hexanetrione trioxime	MTO	7.99	8	3.12
5-Methyl 1-2-3-cyclo- hexanetrione 1-3- dioxime	MDO	8.60	10	8.40
Catechol		9.26	5	3.15
3-6-bis(dimethylamino- methyl) catechol dihydrochloride	CDMA	6.35	30	0.78
Pyrogallol		9.15	9	10.40
Gallic acid		8.55	35	5.30
Methyl gallate		7.90	31	1.90
Propyl gallate		8.05	18	1.94
Propyl ester of 2-di- ethylaminomethyl gal- lic acid dihydrochloride	GME	6.78	27	0.95
Propyl ester of 2-6-bis- (diethylaminomethyl) gal- lic acid dihydrochloride	GDE	5.45	9	0.32

⁺ Estimated probable error $\pm$ 4 units.

Despite the fact that these plots yield numerical values, one must exercise considerable caution in interpreting the results entirely in terms of equation 12. It must be realized that all binding configurations between the two molecules do not necessarily lead to displacement of the p-nitrophenolate group by the attacking nucleophile. Therefore, although the equilibrium constant is a measure of the degree of binding between the 1370 and the reactant it was not necessarily a measure of the hydrolysis of the 1370. There was a strong possibility that "unreactive" binding may occur which will not lead to hydrolysis but simply prevents the substrate from reacting. This may possibly explain certain anomalies found in the results between the size of the equilibrium constant and the size of the second order rate constant.

In summarizing, it seems as if the results obtained in this study do not appear to lead to unambiguous relationships among the several factors involved at the present stage of this investigation. Any attempts to evolve a direct correlation with the observed over-all rate is impractical, since it is impossible to determine which properties of molecular complexes possess the necessary steric orientation to facilitate the reaction.

ROLE OF INTRAMOLECULAR CATALYSIS IN THE HYDROLYSIS OF ISOPROPYL
O-HYDROXYPHENYL METHYL PHOSPHONATE

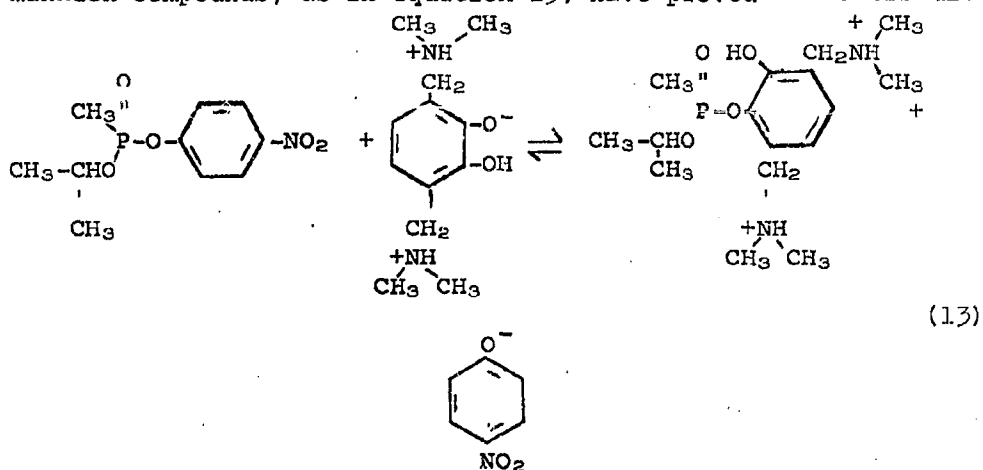
Epstein et al (19) have found marked differences in the reactivities of phenols and catechols (of comparable basicity) which strongly suggested the participation of the undissociated hydroxyl group. It was pointed out that the unionized hydroxyl group may facilitate the catechol reactions by its ability to hydrogen bond with the phosphoryl oxygen of the sarin thus favoring the nucleophilic attack by the ionized hydroxyl on the partially positive phosphorous of sarin.

The effect of the unionized phenolic hydroxyl on the facilitation of the catechol-sarin reaction may be either of truly catalytic nature or may be elicited through some pronounced energetic changes in which the group participates. If the facilitation is catalytic the unionized phenolic hydroxyl must act by lowering the energy barrier between the reactants and the end products without affecting the eventual equilibrium. If the facilitation is of more general energetic nature the unionized phenolic hydroxyl may produce a lowering in the energy levels of both the activated complex and the end product. If the reaction between sarin and catechol was truly catalytically facilitated by the action of the free phenolic hydroxyl on the phosphoryl oxygen, then the rate of the hydrolysis of the sarin-catechol

reaction product may be expected to be equally facilitated.

Previous work has shown that the fluoride of sarin can be replaced by the p-nitrophenolate ion and 1370 (isopropyl p-nitrophenyl methyl phosphonate) can have its p-nitrophenyl replaced by the phenolate ion. This evidence leads to the possibility that an equilibrium could occur between two different phenolates on the parent isopropyl methyl phosphonate nucleus. Studies on equilibriums between catechols and phenols should lead to some indication of the effect of the ortho hydroxyl group in these reactions, especially in the reverse reaction.

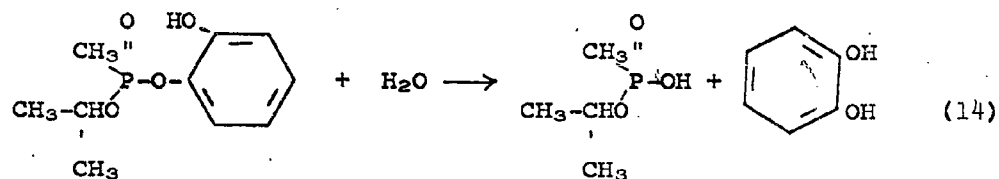
Attempts made as part of the present study to determine the rates of an exchange reaction between 1370 and various catechol mannich compounds, as in equation 13, have proved unsuccessful.



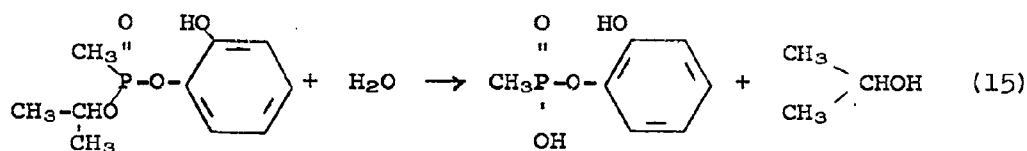
If an equilibrium between these compounds existed one would suspect the equilibrium to be greatly in favor of the catecholate compound possibly because of the greater stability brought about by the two point attachment of the catechol mannich compound on the parent isopropyl methyl phosphonate nucleus. The results obtained, however, show that although the p-nitrophenolate ion is replaced by the mannich compound the reverse reaction apparently does not occur significantly. Even when great amounts of p-nitrophenol were added to the reaction mixture, to shift the equilibrium to the left, the reverse reaction was still not observed. Since p-nitrophenol has been found to replace other phenols a possibility that a further reaction was occurring with the mannich reaction product preventing the exchange reaction from occurring with the p-nitrophenol was suggested.

Since the equilibrium between 1370 and the catechol mannich compound did not occur it was considered of interest to determine if another reaction was occurring with the catecholate reaction product which prevented the expected reaction. Results of an investigation into this possibility are presented in this part of the study.

The end product of the catechol sarin reaction, isopropyl o-hydroxyphenyl methyl phosphonate, may hydrolyze in two ways:

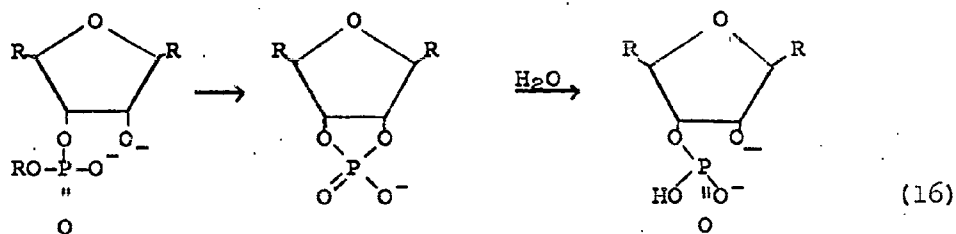


or



In the first, there is catechol cleavage with the liberation of catechol while in the second there is cleavage of the isopropoxy group with the catechol remaining on the molecule. If a reaction such as that in equation 15 was occurring it would explain the apparent non reversibility of the reaction between 1370 and the catechol mannich compound as the isopropyl group was eliminated rather than the catechol.

Because of the greater nucleophilicity of the isopropoxide ion in contrast to the catecholate group, one may expect the latter to be preferentially ejected during hydrolysis as shown in equation 14. On the other hand the second reaction may be favored through the formation of the cyclic o-phenylene methyl phosphonate intermediate. This mechanism is quite analogous to the reactions catalyzed by ribonuclease (39-44) in which a sugar hydroxyl residue reacts to form a cyclic phosphate which is then attacked by water to yield an alkyl phosphate as shown below:



The object of this part of the study was to prepare and investigate the kinetics of the hydrolysis of isopropyl o-hydroxyphenyl methyl phosphonate.

Experimental

Apparatus

Beckman Model G pH meter with glass and calomel electrodes.

Cary No. 11 Recording Spectrophotometer.

Beckman IR5 Infrared Spectrophotometer.

Constant Temperature Baths.

Reagents

Sarin, about 98% pure, supplied by U.S. Army Chemical Corps.

Methyl phosphonic dichloride, supplied by U.S. Army Chemical Corps.

Pyrocatechol, resublimed under reduced pressure.

Carbon tetrachloride, reagent grade.

Sodium hydroxide, reagent grade.

Sodium dihydrogen phosphate, reagent grade.

Trisodium phosphate, reagent grade.

Sodium chloride, reagent grade.

Boric acid, reagent grade.

Sodium borate, reagent grade.

Sodium Citrate, reagent grade.

Sodium acetate, reagent grade.

Procedure for kinetic study

An aqueous stock solution of isopropyl o-hydroxyphenyl methyl phosphonate was prepared and proper aliquots were placed into phosphate and acetate buffers, kept at constant ion strength by the addition of sodium chloride. The pH of the finished solutions was then measured and 8 ml. samples were placed in 10 ml. ampules. The ampules were then sealed under nitrogen and immersed in a constant temperature bath at the desired temperature. The solutions were allowed to equilibrate to bath temperature before removal of the sample corresponding to zero time. The ampules were removed at specified intervals, the reaction quenched by immersion into an ice bath, and assayed for residual amounts of the compound being studied as described below. In all of the runs eight samples were taken over a period of time equivalent to approximately two half lives.

Assay procedure

The withdrawn samples were assayed for residual catechol content by measuring the ultraviolet absorption spectrum of the compound being studied after removal of the degradation

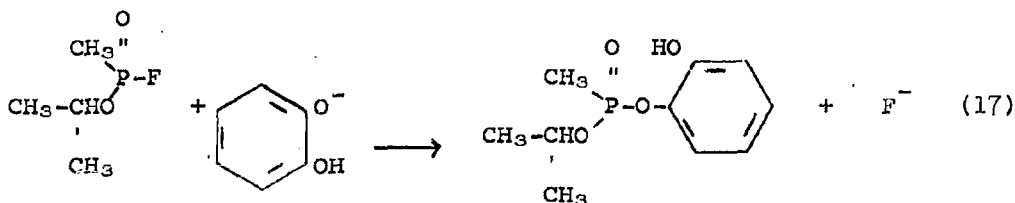
products. In all cases the absorbance was found to be proportional to the concentration of the phosphonate present.

From each ampule a 5 ml. aliquot was taken. This sample was acidified with 1 ml. of dilute hydrochloric acid solution and extracted with two 5 ml. portions of carbon tetrachloride. The carbon tetrachloride solution was then extracted with two 5 ml. portions of pH 12 borate buffer. The absorbance of the aqueous alkaline solution was then determined at 288 mu using the Cary No. 11 recording spectrophotometer.

Preparation of organophosphorous compounds

Isopropyl o-hydroxyphenyl methyl phosphonate (CS):

Sarin (isopropyl methyl phosphonofluoride) was allowed to react with an excess of catechol in an aqueous solution maintained at pH 7.5 under a nitrogen atmosphere until no more acid was being produced.



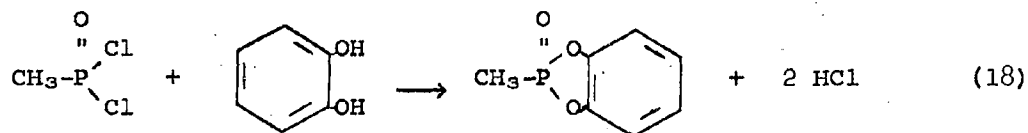
The reaction mixture was then made acidic by the addition of dilute hydrochloric acid and extracted with carbon tetrachloride. The carbon tetrachloride solution was extracted with pH 12 borate buffer, the aqueous solution then being reacidified and extracted with fresh carbon tetrachloride. The entire transfer procedure was repeated three times to remove any residual sarin. The final

carbon tetrachloride solution containing the product was allowed to evaporate slowly at room temperature.

The resulting product was a yellowish oil with an ultra-violet absorption spectrum showing a bathochromic shift in alkaline solution (peak at 270 mu in acidic solution and 288 mu in alkaline solution) typical of phenols. The infra red spectrum shows the presence of a hydrogen bonded hydroxyl as well as phosphoryl and aromatic bands. The pKa was found to be 8.89.

o-Phenylene methyl phosphonate (PP):

Equimolar quantities of catechol and methyl phosphoric dichloride were allowed to react for two hours at 90°C in a round-bottomed flask.



The reaction was accompanied by the evolution of hydrochloric acid gas and was considered completed when the evolution of gas ceased. The reaction mixture was then distilled under reduced pressure, the desired product distilling over at 133°C under 1 mm of mercury pressure.

The resulting material was a white crystalline solid which was very hygroscopic in air, liquifying within minutes. It was soluble in water, isopropanol, alcohol, chloroform, carbon tetrachloride, and acetone but insoluble in petroleum ether.

o-Hydroxyphenyl methyl phosphonic acid (CSA):

A solution of CS was made alkaline to pH 8, by the addition of concentrated sodium hydroxide solution, and allowed to react at 45°C for 10 hours in a closed container. The reaction mixture was then reacidified and extracted with carbon tetrachloride to remove any unreacted CS which remained. The acidified aqueous solution was next extracted with ether to remove any catechol which may have been present. CSA was not extractable from aqueous solution with any organic solvents, even in the presence of strong acid.

This material had an ultraviolet spectrum exactly like CS showing the presence of an aromatic phenol. The infrared spectrum obtained by evaporating the aqueous solution showed the presence of a free hydroxyl group as well as aromatic and phosphoryl bands. The pKa's were found to be 2.28 for the first hydrogen and 10.3 for the phenolic hydrogen.

Appendix 1 shows the structure of these organophosphorous compounds.

Determination of acid dissociation constants

The acid dissociation constants of CS and CSA were determined spectrophotometrically by the method of Hammett et al. (45). This method consists of determining the absorption in a solution where the compound is completely unionized, in a solution where

the compound is completely ionized, and at some known pH where both forms exist. The pKa is then determined from the following equation:

$$pK_a = pH - \log \frac{A_{BH^+} - A}{A - A_{B^-}} \quad (19)$$

In this equation A_{BH^+} is the absorbance of the completely unionized form, A_{B^-} is the absorbance of the completely ionized form, and A is the absorbance of a solution at a pH containing both forms.

Results and Discussion

Nature of the reaction

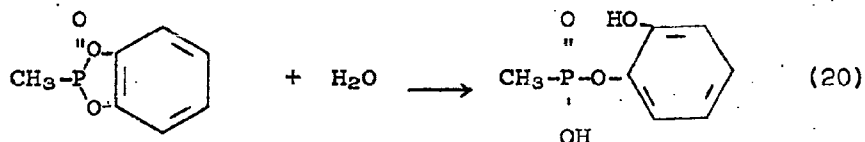
Due to the fact that one of the major aims of this phase of the present investigation was to determine the mechanism of the hydrolysis of isopropyl o-hydroxyphenyl methyl phosphonate it was of great importance to determine the products of the reaction. As previously discussed CS may hydrolyze in two ways as shown in equations 14 and 15 respectively. The experimental results obtained in this investigation show that CS probably is cleaved in aqueous solution by the latter route.

When a sample of CS was hydrolyzed in alkaline aqueous solution no catechol could be recovered from the reaction mixture. The end product of the hydrolysis, however, had an ultraviolet spectrum very similar to that of CS but could not be extracted into any organic solvents even in the presence of strong acid. Since only the aromatic moiety was capable of absorbing in the

ultraviolet region and the catechol was not eliminated during hydrolysis it was assumed that the catechol remained on the end product. The complete water solubility of the end product indicated that a strongly acidic group was present in the molecule. This would be expected if the isopropoxy group was replaced by a hydroxyl group, since phosphonic acids are very strong acids. The pK_a of the first hydrogen determined potentiometrically, was found to be 2.28 indicating that the end product was a strong acid. This explains the water solubility of the end product by showing its strongly acidic character. The infrared spectrum substantiated these assumptions by showing the presence of aromatic and phosphoryl groups as well as the presence of a free hydroxyl group. It, therefore, appeared that the end product of the hydrolysis of CS may be o-hydroxyphenyl methyl phosphonic acid (CSA).

To confirm the identity of the catechol containing end product, a sample of o-hydroxyphenyl methyl phosphonic acid was prepared by another route. o-phenylene methyl phosphonate, prepared from methyl phosphonic dichloride and catechol, when hydrolyzed in water to yield presumably CSA gave a product identical in all ways to that obtained through CS. The ultraviolet spectrum of an aqueous solution containing the hydrolysis product of PP was identical to that of CSA and the second pK_a was found to be 10.2 which was in good agreement with that of CSA which was 10.3. The solubility and the infrared spectrum of

this material was also identical to that of CSA. Therefore, it can be assumed that the following reaction has occurred between PP and water:



All of the results obtained seem to substantiate the fact that the hydrolysis of CS proceeds according to the scheme presented in equation 15 with the formation of CSA and not with the expected catechol cleavage.

Order of reaction

The reactions were all observed to be first order with respect to CS. Figure 7 shows the typical dependence of the logarithm of absorbance of CS as a function of time at various pH's. Since the ultraviolet absorption of the reaction solution, after removal of the degradation products, was directly proportional to the concentration of CS, the rate constants were calculated directly from a plot of logarithm of absorbance against time. All of these runs were made keeping the ionic strength constant at 0.6 by the addition of sodium chloride.

Dependence of reaction rate of pH

Buffer effect: since these compounds undergo base catalysis the degradation of CS was studied in a series of solutions containing varying amounts of phosphate and acetate buffers at

constant pH to determine the extent of the buffer effect.

Figure 8 shows the effect of varying phosphate buffer concentrations on the rate at constant pH. These results seem to indicate that HPO_4^- may be acting as a general base catalyst on the unionized CS. A similar investigation indicated that acetate buffers also act as catalysts in the deterioration of unionized CS. The results show that the catalytic effect is due to the acetate ion acting as a general base.

pH profile: although it was impossible to determine directly the rate of reaction at any pH in the absence of buffers because of the acidic nature of the reaction products, the corresponding rate was obtainable by extrapolation to zero buffer concentration a series of results determined in the presence of buffers of varying concentration. Thus, Figure 9 shows the dependence of the reaction rate on pH at zero buffer concentration. This curve shows a pH independent region, a rise at about pH 8.5, another plateau and then an ascending portion with a slope of one in the high pH range.

If the rate of hydrolysis only depends upon the hydroxyl ion and CS activity and the reaction of the water molecules can be neglected then the rate of loss of CS is:

$$-d(\text{CS})/dt = k_1(\text{CS}) + k_2(\text{CS}^-) + k_3(\text{CS}^-)(\text{OH}^-) \quad (21)$$

In the pH range of 4-7 the last two terms appear to be negligible and equation 21 becomes:

$$-d(CS)/dt = k_1 (CS) \quad (22)$$

In the pH range of 9.5 - 11 the first and last terms are apparently negligible and the rate becomes:

$$-d(CS)/dt = k_2 (CS^-) \quad (23)$$

In the upslope region the first term appears to be negligible and equation 21 becomes:

$$-d(CS)/dt = k_2 (CS^-) + k_3 (CS^-) (OH^-) \quad (24)$$

This formulation is further substantiated by the different heats of activation exhibited over these definite phases. The reaction effectively represented by k_1 has a heat of activation of 17.3 Kcal/mole, while that of k_2 is 21.8 Kcal/mole, and that of k_3 is 6 Kcal/mole.

The rate constants calculated from the points on the curve were then used to plot a theoretical curve the points of which coincide rather well with the experimental points. The equation used determining the theoretical curve was derived as follows from equation 21. Since the amount of CS in the unionized form was equal to the total amount of CS times the fraction ionized $(CS)_t (H^+)/K_a + (H^+)$, and the amount of CS in the ionized form was equal to the total CS times the fraction ionized, $(CS)_t K_a / K_a + (H^+)$, then equation 21 becomes:

$$\begin{aligned}
 -d(\text{CS})_t/dt = & k_1(\text{CS})_t(\text{H}^+)/K_a + (\text{H}^+) + k_2(\text{CS})_t K_a/K_a \\
 & + (\text{H}^+) + k_3(\text{CS})_t K_a(\text{OH}^-)/K_a + (\text{H}^+)
 \end{aligned}
 \quad (25)$$

where $(\text{CS})_t$ was the total amount of CS present and K_a was the ionization constant for CS. Dividing both sides of equation 25 by $(\text{CS})_t$ one obtains:

$$\begin{aligned}
 -d(\text{CS})_t/(\text{CS})_t dt = & k_1(\text{H}^+)/K_a + (\text{H}^+) + k_2 K_a/K_a + \\
 & (\text{H}^+) + k_3 K_a(\text{OH}^-)/K_a + (\text{H}^+)
 \end{aligned}
 \quad (26)$$

which then becomes:

$$\begin{aligned}
 -d \ln(\text{CS})_t/dt = k_{\text{obs}} = & k_1(\text{H}^+)/K_a + (\text{H}^+) + k_2 K_a/K_a + \\
 & (\text{H}^+) + k_3 K_a(\text{OH}^-)/K_a + (\text{H}^+)
 \end{aligned}
 \quad (27)$$

where k_{obs} is the observed rate constant.

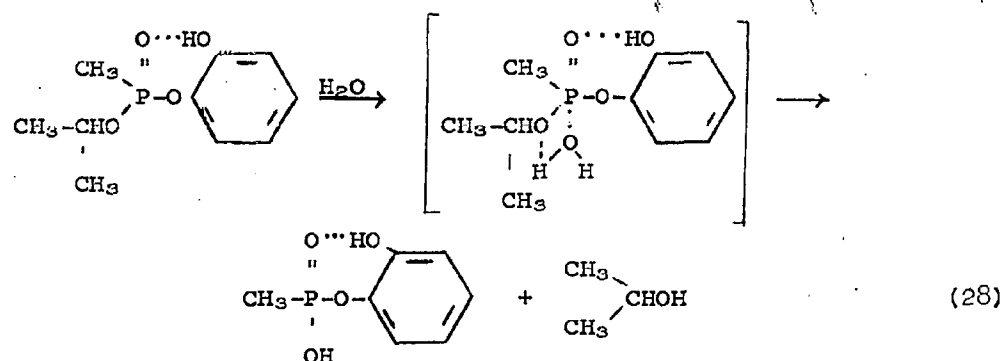
The smooth curve of Figure 9 which apparently fits the experimental points was not drawn through the experimental points but represents equation 27 where $k_1 = 0.08 \text{ hr}^{-1}$, $k_2 = 0.231 \text{ hr}^{-1}$, and $k_3 = 3.32 \text{ hr}^{-1}$. The values of k_1 , k_2 , and k_3 were obtained by substituting into equation 27 respectively the extreme experimental values shown in the curve.

Reaction mechanism of CS

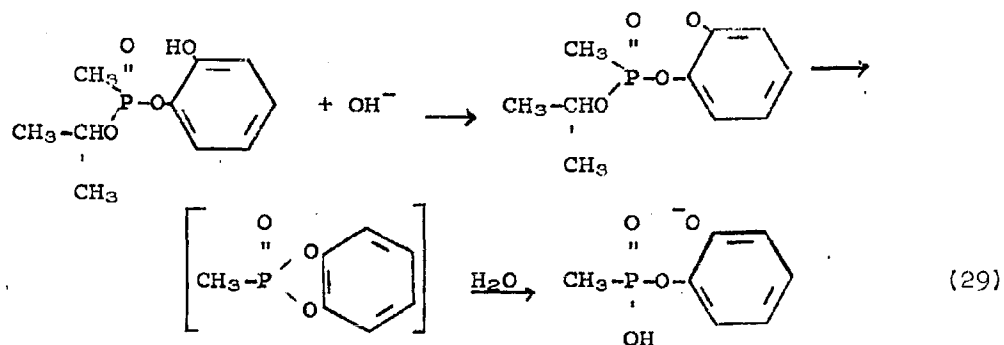
Isolation of the end product of the degradation of CS at both low and high hydroxyl ion concentrations showed that the end product had the properties of CSA as previously mentioned.

A possible mechanism for the hydrolysis of CS at very low hydroxyl ion concentration where the CS was completely unionized may be through a water attack on the phosphorous atom made partially

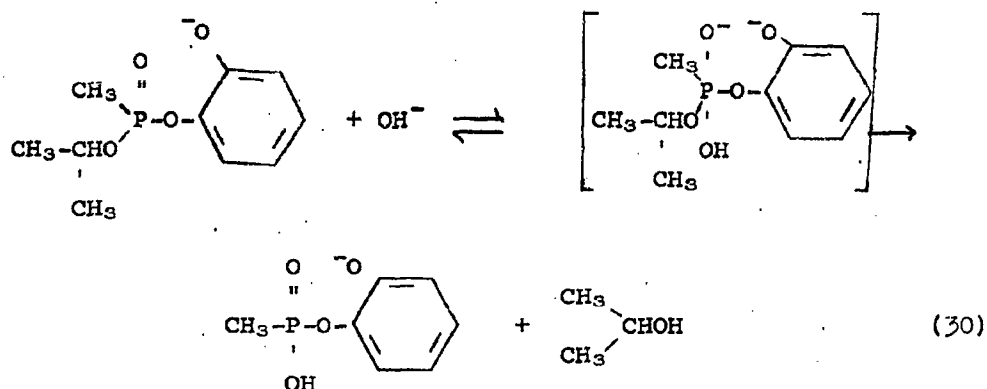
positive by the hydrogen bonding between the o-hydroxy group of the phenol and the phosphoryl oxygen. The water molecule is also capable of simultaneously hydrogen bonding with the isopropyl oxygen, which is then followed by the subsequent elimination of the isopropyl group. This sequence can be shown in the following equation.



In the region where CS exists as the ionized form a first order dependence on the hydroxyl ion was found and the possible mechanism involves the formation of the cyclic structure through some form of intramolecular catalysis, followed by immediate hydrolysis.



At a relatively high pH values the mechanism of hydrolysis may be through direct attack of the hydroxyl ion on the ionized CS as follows:



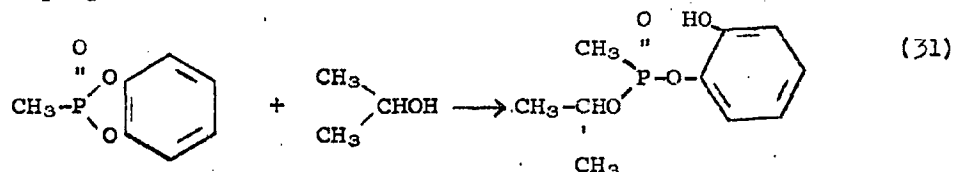
All of the proposed mechanisms discussed would be consistent with the experimentally obtained kinetic results.

The highly negative entropy obtained for the third mechanism can perhaps be explained by the fact that when the hydroxyl ion attaches itself to the ionized species the resulting activated complex is more highly charged. Thus, the activated complex is markedly solvated, reducing the freedom of the solvent and decreasing the entropy of activation. The more positive entropy of the second mechanism indicates that the activated complex results without too much trouble, which might be the case if some form of intramolecular catalysis was occurring.

Reactions of o-phenylene methyl phosphonate

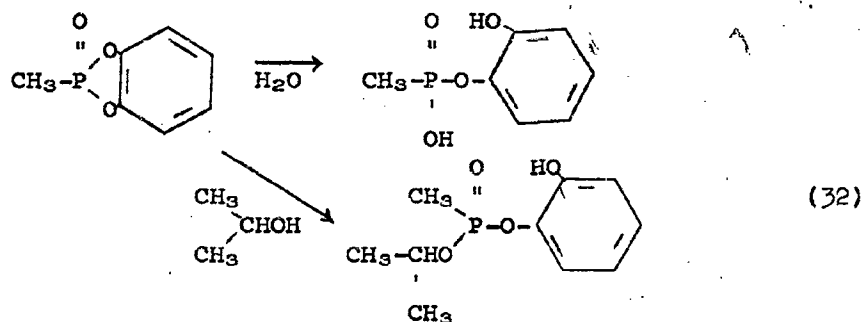
The white needles of PP when dissolved in isopropanol formed CS. The yellowish oil, separating after isopropanol was evaporated

and any CSA present was removed by extraction, gave the same infrared spectrum as CS. When this oil was dissolved in water the ultraviolet spectrum was identical to that of CS. The pKa determined for this material was 9.0 which was in good agreement with the pKa (8.9) of CS prepared from catechol and sarin. This material also has the same solubilities as CS and it can be assumed that the following reaction has occurred between PP and isopropanol:



The previously discussed reaction between PP and water to form CSA was found to occur very rapidly at room temperature. No trace of PP could be found once the crystals were dissolved in water.

When PP was dissolved in isopropanol containing very small amounts of water both CS and CSA were formed. The reciprocal of K (the ratio of CS to CSA formed at equilibrium) was found to be a linear function of the percentage of water present, as shown in Figure 10. The reaction mechanism probably consists of two parallel reactions as follows:



The values of K show that the reaction with water is much preferred since much more CSA was formed even when the amount of water present was very small. This can be explained by the fact that the water ionizes much more easily than isopropanol or by the fact that the hydroxyl ion is less sterically hindered than the isopropoxide ion.

HYDROLYSIS OF PHENOLATE PHOSPHONATES

It was considered of interest to study the hydrolysis of the phenol sarin reaction product especially since the catechol sarin addition product hydrolyzed with the unexpected isopropoxide cleavage. This study was intended to give some insight into the effect of the ortho hydroxyl on the hydrolysis of the sarin addition products.

Further studies considered the relative influence of various nuclear substituents on the ease of nucleophilic attack during the hydrolysis of the phenolate group. Since the ease of nucleophilic attack on these phosphonates depends on the electron density of the phosphorous, the addition of an electron withdrawing group should increase the ease of hydrolysis while the addition of an inductive group should hinder the ease of hydrolysis significantly.

Experimental

Apparatus

The apparatus used in this portion of the work was similar to that used in the previous section.

Reagents

Phenol, distilled.

p-Chlorophenol, reagent grade.

p-Methoxyphenol, reagent grade.

All other reagents were similar to those used in the previous section.

Procedure for kinetic studies

The procedure used for these kinetic studies were identical to those used in the previous section.

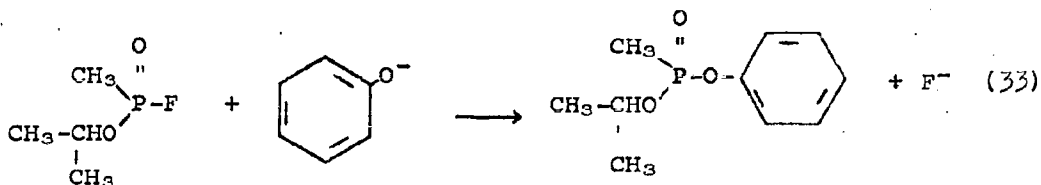
Assay procedure

The withdrawn samples were assayed for residual phenolate content by measuring the ultraviolet absorption spectrum of the compound being studied after removal of the degradation products. From each ampule a 5 ml aliquot was taken and made basic by the addition of 1 ml of 0.4 N sodium hydroxide solution. This solution was then extracted with two 5 ml portions of carbon tetrachloride and the ultraviolet absorption of the carbon tetrachloride solution was measured using the Cary No. 11 recording spectrophotometer.

Preparation of organophosphorous compounds

Isopropyl phenyl methyl phosphonate (PS):

Sarin was allowed to react with excess phenol in aqueous solution maintained at pH 9.5, by the addition of sodium hydroxide solution.



The mixture was allowed to react until no more acid was produced.

The reaction mixture was then made strongly basic with 0.4 N

sodium hydroxide and extracted with carbon tetrachloride. The carbon tetrachloride solution was extracted several times with pH 12 buffer and then allowed to evaporate at room temperature. After the evaporation of the carbon tetrachloride PS remained.

This material had an ultraviolet absorption spectrum in aqueous solution with peaks at 260 and 265 mu. These peaks did not shift in acid or alkaline solution showing the absence of a free hydroxyl group and the presence of the aromatic and phosphoryl groups.

Isopropyl p-chlorophenyl methyl phosphonate (CPS):

This compound was prepared and isolated using the same procedure as for PS except that p-chlorophenol was used instead of phenol.

The isolated material had an ultraviolet absorption spectrum with peaks in aqueous solution at 270 and 273 mu. These peaks also did not shift in either acid or alkaline solution. The infrared spectrum showed the absence of a free hydroxyl group and the presence of phosphoryl and aromatic groups.

Isopropyl p-methoxyphenyl methyl phosphonate (MPS):

The procedure used in preparing and isolating MPS was similar to that used for PS and CPS.

The isolated material was a yellowish oil which had an ultra-violet absorption spectrum with a peak in aqueous solution at 275 mμ. This peak does not shift in alkaline or acidic solution indicating the absence of a free phenolic group.

Paper chromatographic procedure

The compounds to be chromatographed were dissolved in water and placed with micro-pipettes on strips of Whatman No. 1 filter paper. Descending techniques were used, using acidic and basic solvents similar to those used by Larsson (4). The composition of the acid solvent was n-butanol, ethanol, acetic acid, and water (8:2:1:3), and that of the alkaline solvent was tert-butanol, isopropanol, and 2 M ammonium hydroxide (2:2:1).

The development of the spots was carried out by spraying an acid solution of ammonium molybdate, heating for 8 minutes at 85°C and then treating the dried paper with hydrogen sulfide (46).

Results and Discussion

Determination of the reaction products

The hydrolysis of PS in aqueous alkaline solution was accompanied by the liberation of phenol. Similar results were obtained with CPS and MPS. These reactions are similar to those obtained for the hydrolysis of 1370 where p-nitrophenol was liberated with the formation of isopropyl methyl phosphonic acid.

The reaction for the hydrolysis of the phenolate compound is therefore significantly different from that of CS since in the former there is cleavage of the phenoxy group while in the

latter the cleavage is of the isopropoxy group. This was expected since there was no o-hydroxy group present to cause any intramolecular catalysis which might cause the ejection of the isopropoxy group.

Order of the reaction

The reactions were observed to be first order with respect to the phenolate compound being studied. Plots similar to Figure 7 were obtained for PS, CPS, and MPS. Since the ultraviolet absorption was directly proportional to the concentration of phenolate present, after the removal of the degradation products, the rate constants were calculated directly from a plot of logarithm of absorbance against time.

Dependence of reaction rate on pH

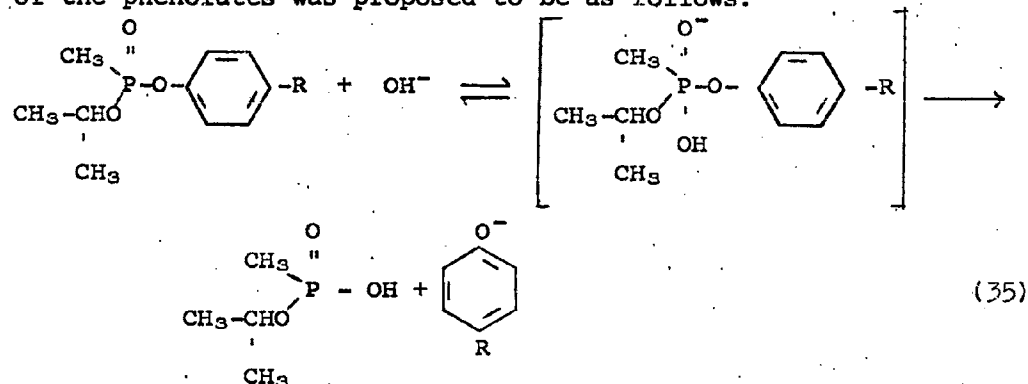
Figure 11 shows a plot of logarithm of k against pH for PS. Similar plots were obtained for MPS and CPS. These plots have a slope of one showing a first order dependence of the hydrolysis of the phenolate on the hydroxyl ion concentration. Thus, if the action of the water molecules on the rate of hydrolysis can be neglected then the rate of loss of the phenolate compound can be expressed as:

$$-d(\text{Phenolate})/dt = k_1 (\text{Phenolate}) (\text{OH}^-) \quad (34)$$

Reaction mechanism of the phenolates

Considering the fact that phenol was one of the end products of the hydrolysis and that there was a first order dependence on

the hydroxyl ion concentration the mechanism for the hydrolysis of the phenolates was proposed to be as follows:



Dependence of various substituents on the rate of phenolate hydrolysis

The logarithm of the second order rate constant for the hydrolysis of the various phenolates when plotted against the pKa of the departing phenol shows a straight line dependence as shown in Figure 12. The phenolates studied were those of p-nitrophenol, p-chlorophenol, p-methoxyphenol, and phenol. The data shows the less basic the leaving phenolate the faster the rate of alkaline induced hydrolysis. This was as would be expected since the rate of reaction depends upon the electron distribution of the substituents. The addition of a stronger electron withdrawing group, as with the nitro group, will tend to make the phosphorous more positive and thus more prone to attack by the hydroxyl ion. Where the substituent has an inductive effect the rate of hydrolysis will be slower since the phosphorous will

not be as electron deficient and thus not as positive. This was found to be the case where the methoxy group was the added substituent and the rate of hydrolysis was decreased compared to CPS.

Temperature dependence

The activation energy for the basic catalyzed hydrolysis of the phosphonates studied have been determined from the slopes of Arrhenius type plots similar to those as shown in Figures 13 and 14. The slope of the line multiplied by $-2.303 R$ was equal to the activation energy. Table II includes the activation energy ΔE_a , and the entropy of activation ΔS for the base catalyzed hydrolysis of the various phosphonates studied. These values were calculated from the following equation:

$$k = \frac{RT}{Nh} e^{\frac{\Delta S}{R}} e^{\frac{-\Delta E_a}{RT}} \quad (36)$$

A possible explanation of the low apparent energy of activation obtained with the phenolate compounds, after the subtraction of the 12 Kcal/mole due to the heat of ionization of water, may be due to the formation of an intermediate compound by means of an equilibrium step. If, as was shown in Figure 15, there is an intermediate formed and there were two activated complexes present, then the true activation energy would be the energy between the intermediate compound and the activated intermediate. The apparent activation energy, however, would be

the difference in energy between the starting products and the activated intermediate. Therefore, depending on the energy of the activated intermediate the apparent activation energy could vary, becoming even negative if the activated intermediate were of higher energy than the reaction products. Thus, although the true activation energy would be of a reasonable value the apparent activation energy, which represents the difference in the energy levels of the starting products and the activated intermediate, may be considerably lower.

Comparative properties of compounds studied as indicated by

Rf values

Table III gives the Rf values of the various phosphonate compounds studied using the acid and alkaline solvents previously described. These results seem to indicate that all of the phenolate phosphonates have similar properties and that CSA, a strong acid, has properties which are strikingly different from all of the rest.

TABLE II

Compound	Apparent Energy of Activation E _a (Kcal/mole)	Entropy of Activation S (E.U.)
Isopropyl phenyl methyl phosphonate (PS)	3.1 ⁺	- 56.4
Isopropyl p-chlorophenyl methyl phosphonate (CPS)	5.1 ⁺	- 48.1
Isopropyl p-methoxyphenyl methyl phosphonate (MPS)	5.5 ⁺	- 48.9
Isopropyl methyl phosphono- fluoridate (sarin)	9.1 ⁺	- 21.8
Isopropyl p-nitrophenyl methyl phosphonate (1370)	13.9 ⁺	- 15.1
Isopropyl o-hydroxyphenyl methyl phosphonate (CS)		
First mechanism	17.3	- 28.9
Second mechanism	21.8	- 13.6
Third mechanism	6.0 ⁺	- 52.8

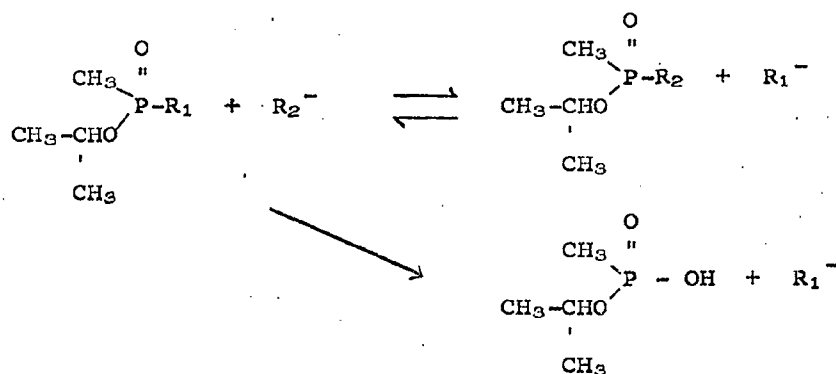
⁺ After subtraction of 12 Kcal/mole due to the heat of ionization of water.

TABLE III

Compound	Rf value	
	acidic	basic
Isopropyl phenyl methyl phosphonate (PS)	0.913	0.869
Isopropyl p-chlorophenyl methyl phosphonate (CPS)	0.900	0.849
Isopropyl p-methoxyphenyl methyl phosphonate (MPS)	0.920	0.886
Isopropyl o-hydroxyphenyl methyl phosphonate (CS)	0.885	0.847
o-Hydroxyphenyl methyl phosphonic acid (CSA)	0.482	0.508

SUMMARY

Results of an investigation of some of the mechanisms leading to the eventual hydrolysis of certain phosphonate esters are presented. These studies were concerned in general with nucleophilic exchange reactions involving these esters, and more particularly with the replacement reactions of isopropyl p-nitrophenyl methyl phosphonate (1370) and with the hydrolytic rates of certain other derivatives. $\rightarrow$ to



These systems were selected for study as possible models of enzyme reactions. Some data were obtained which suggests that the reaction between the ester 1370 and certain gallates, for example, possibly involve intermediate complex formation. Other experiments show conclusively that a free phenolic function in ortho position on the R grouping changes the entire course of

hydrolytic cleavage indicating possible intramolecular catalytic participation of the hydroxyl group. Rates of hydrolysis measurements on a series of other esters differing only in the R group have also been carried out.

Based on the premise that 1370 reacts through the formation of an intermediate complex, a mathematical treatment similar to those of enzyme reactions was derived which led to the rate law

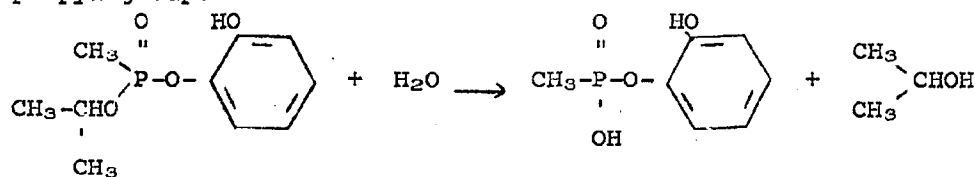
$$1/k_1' = 1/k_2 + 1/k_2K (C)_0$$

where k_1' is the observed pseudo first order rate constant when (C) , the concentration of the reactant, is in excess of (S) , the concentration of 1370, and the subscript zero refers to the initial concentration. From plots of $1/k_1'$ as a function of $1/(C)_0$ the equilibrium constants, for the formation of the complex (K) , and the second order rate constants, for the reaction, have been determined.

The non zero intercepts on the reciprocal plots of the kinetic data have provided evidence that these reactions proceed through the formation of an intermediate complex. The equilibrium constants determined varied in value from 5 to 35, with the gallates being particularly good binders. However, it seems as if the results obtained in this study do not lead to unambiguous relationships among the several factors involved at the present stage of the investigation. Any attempts to evolve a direct

correlation of the equilibrium constants with the over-all rate is impractical, since it is impossible to determine which properties of molecular complexes possess the necessary steric orientation to facilitate the reaction.

~~Results of studies on the kinetics~~ ^{p. 55} of hydrolysis of isopropyl o-hydroxyphenyl methyl phosphonate show that this compound hydrolyzes by three kinetically different mechanisms: (1) water attack on the unionized molecule; (2) hydrolysis of the ionized molecule by probable intramolecular catalysis; and (3) hydroxyl attack on the ionized molecule, with the subsequent formation of o-hydroxyphenyl methyl phosphonic acid and the elimination of the isopropyl group.

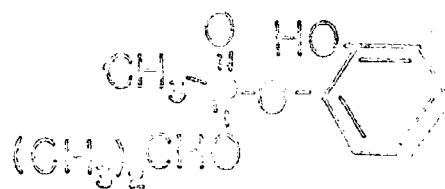


This unexpected method of hydrolysis strongly indicates the presence of some form of intramolecular catalysis through the possible formation of the cyclic o-phenylene methyl phosphonate intermediate. In attempts to determine if this cyclic compound was a possible intermediate, o-phenylene methyl phosphonate was prepared and shown to give the same end products as those obtained in the hydrolysis of isopropyl o-hydroxyphenyl methyl phosphonate. Studies on isopropyl phenyl methyl phosphonate, which does not have the

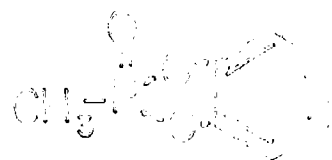
ortho hydroxyl group, show that the hydrolysis proceeds with the cleavage of the phenolate group with the formation of isopropyl methyl phosphonic acid. These results seem to indicate that the presence of an ortho hydroxyl group on the phenol will tend to change the entire course of hydrolytic cleavage.

1.51 The effects of various nuclear substituents on the rate of hydrolysis of the phenolate esters were also determined. The data shows that the addition of an electron withdrawing group makes the phosphorous atom more positive and thus more prone to nucleophilic attack. The opposite effect was found when the substituent was an electron inducing group since the phosphorous atom is then less electron deficient and less positive.

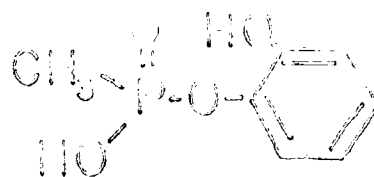
Appendix - Structure of Organophosphorus Compounds



G.C.

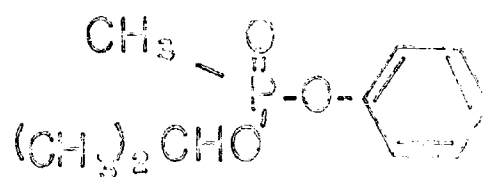


P.P.

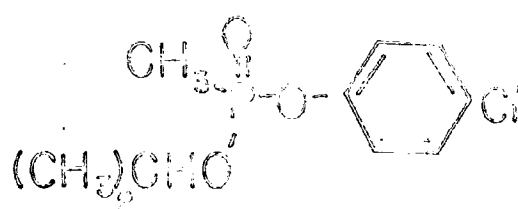


G.S.A.

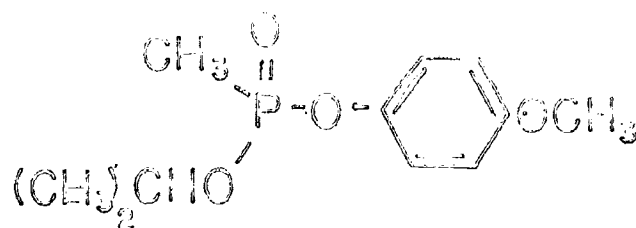
Appendix - continued



P.S.



C.P.S.



M.P.S.

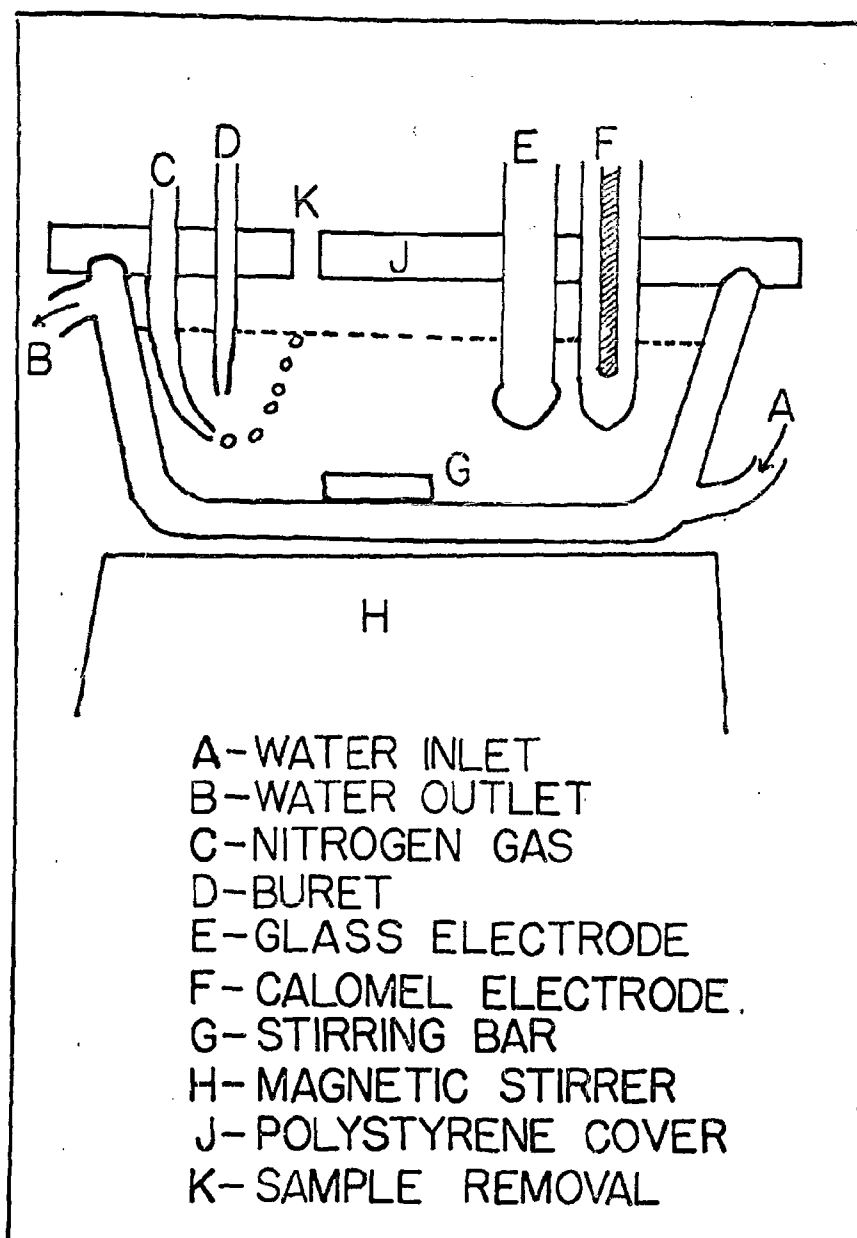


Figure 1
 REACTION ASSEMBLY

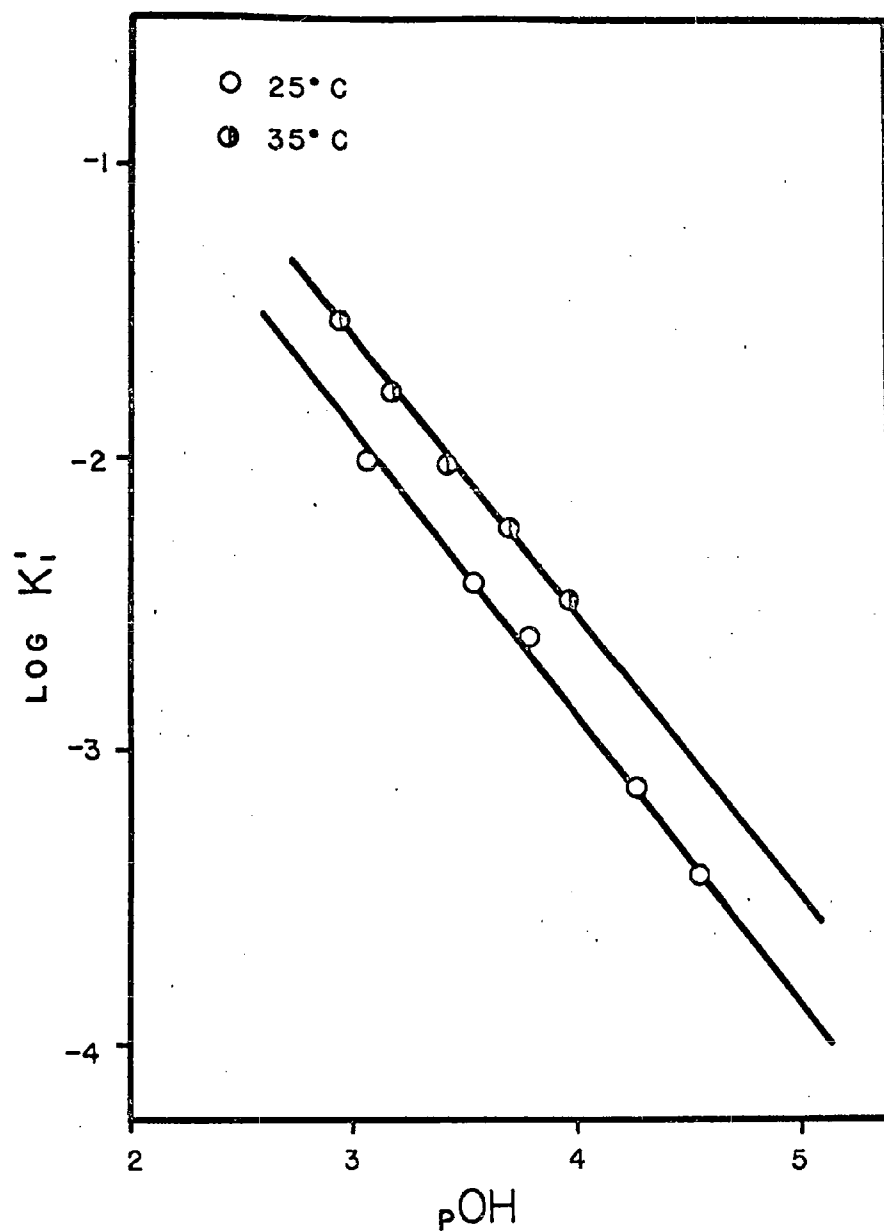


Figure 2

pOH Dependence of Hydrolysis of 1370

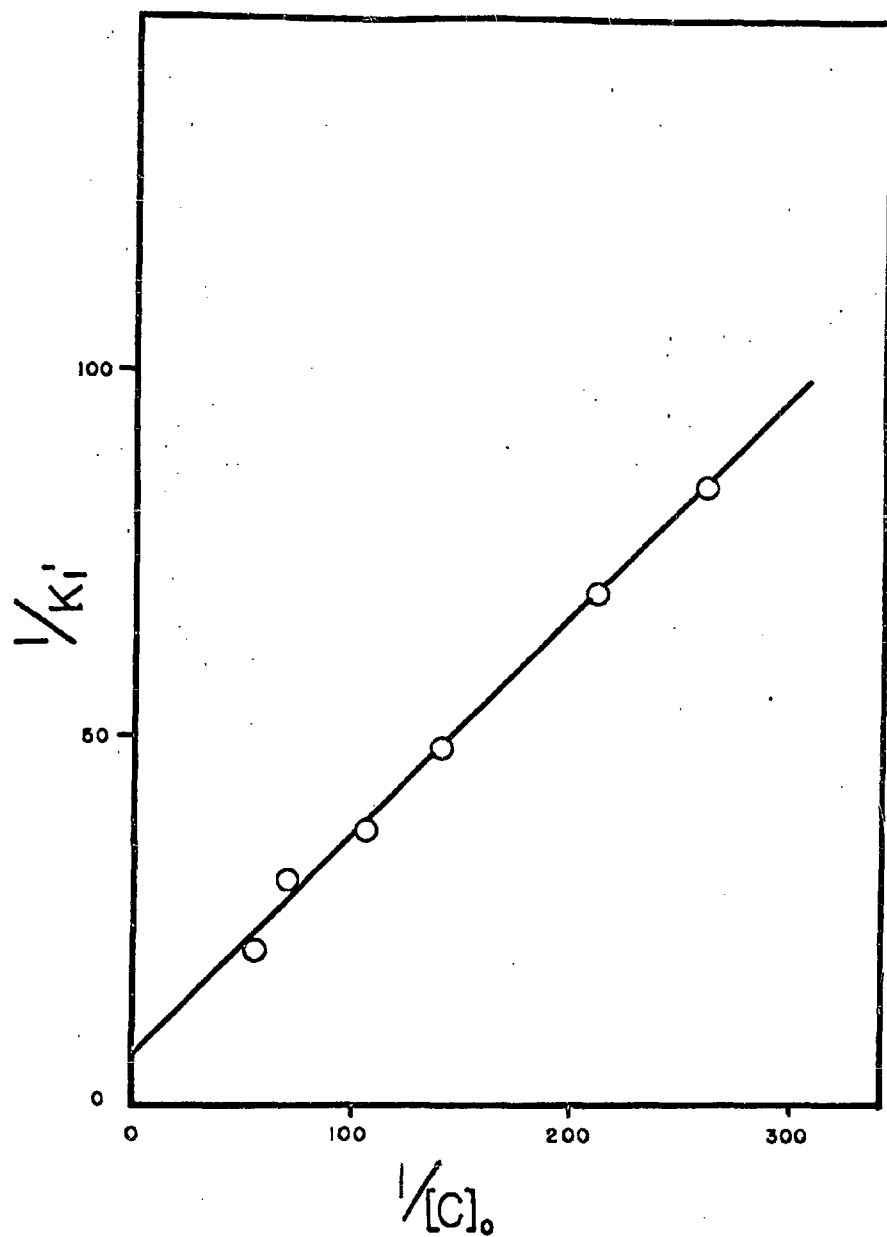


Figure 3

Reciprocal Plot for Reaction of 1370 with 1,2,3-Cyclohexane Trione Trioxime at 35° C.

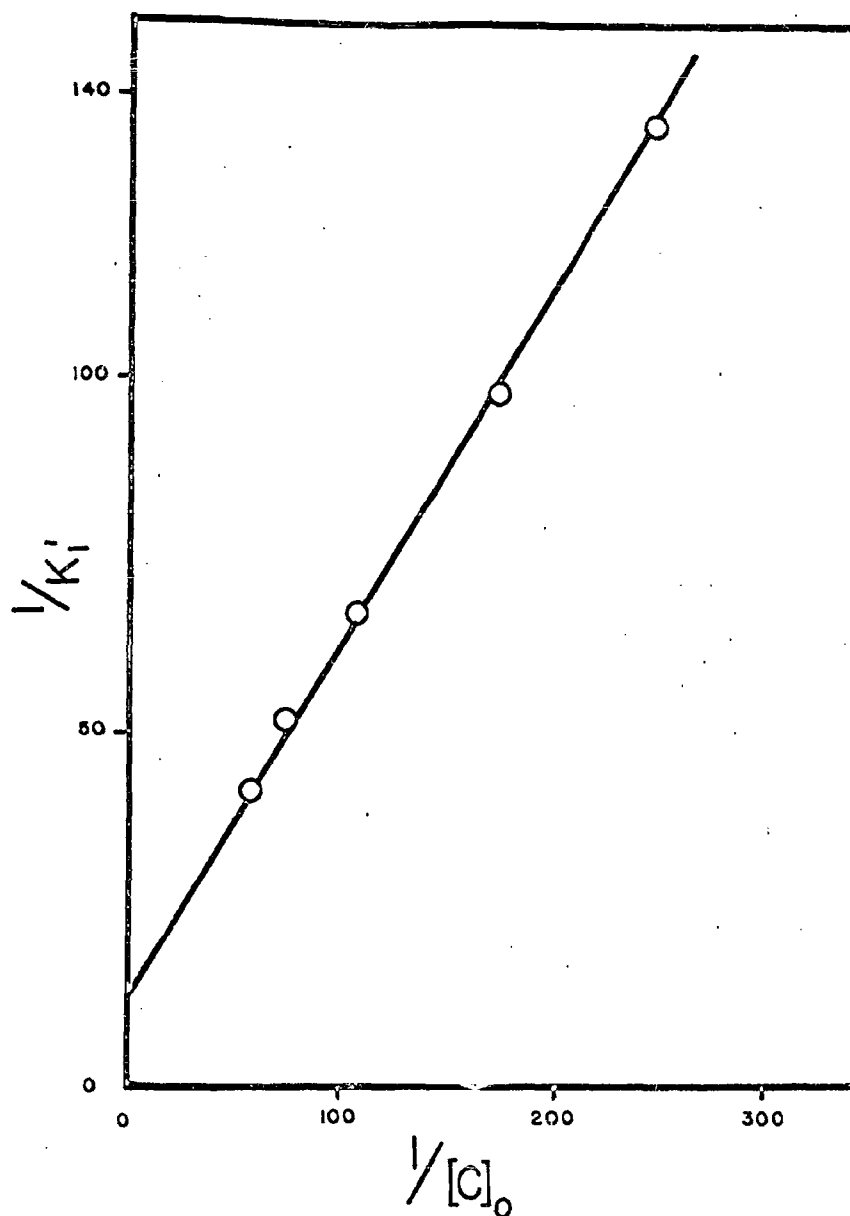


Figure 4

Reciprocal Plot for Reaction of 1370 with Methyl Gallate at 35°C.

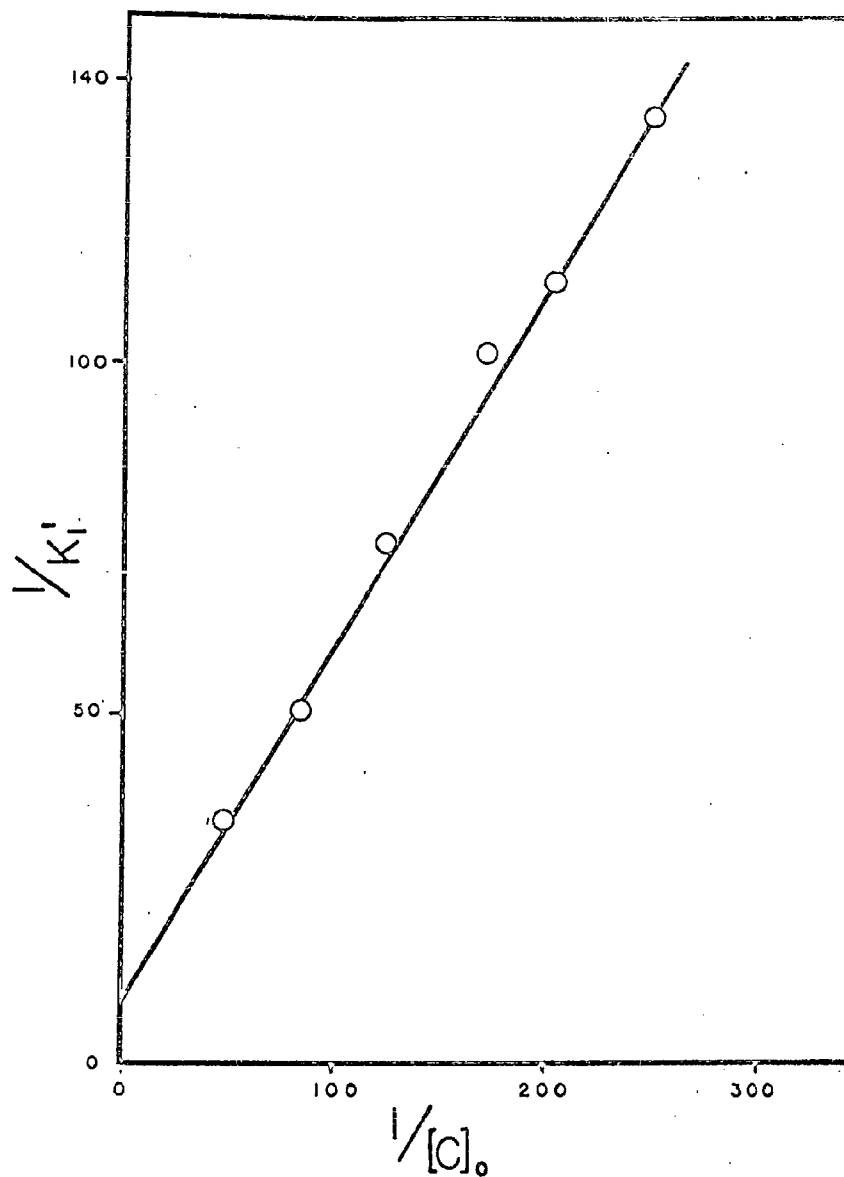


Figure 5

Reciprocal Plot for Reaction of 1370 with Propyl Gallate at 35°

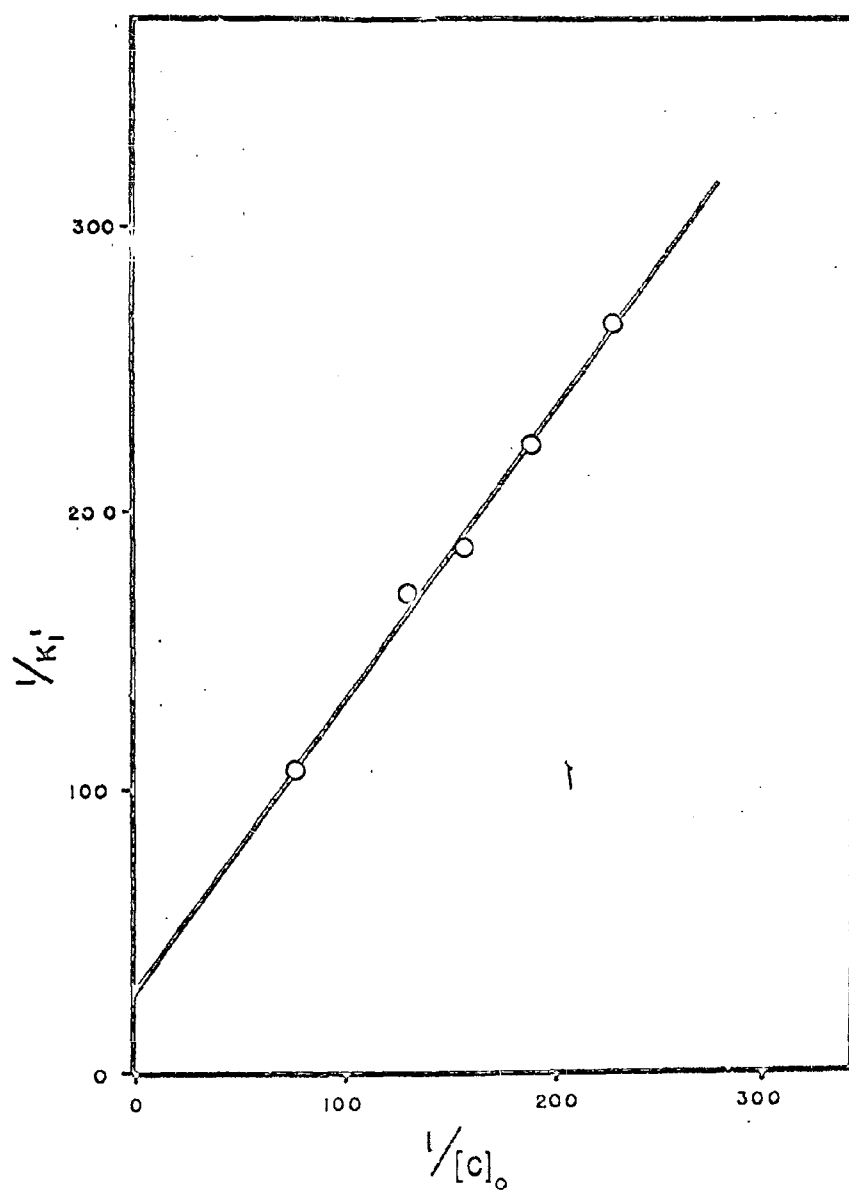


Figure 6

Reciprocal Plot for Reaction of 1370 with GME at 35°C.

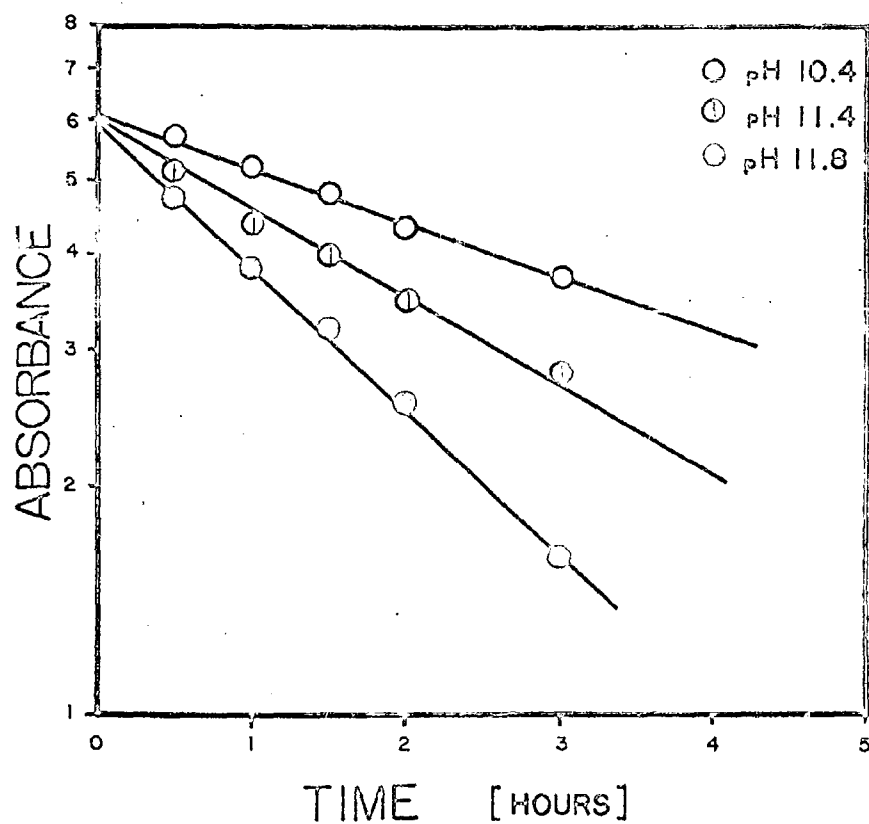


Figure 7

Semi Log Plot Showing Pseudo First Order Character of Hydrolysis of Isopropyl o-Hydroxyphenyl Methyl Phosphonate at 65°C.

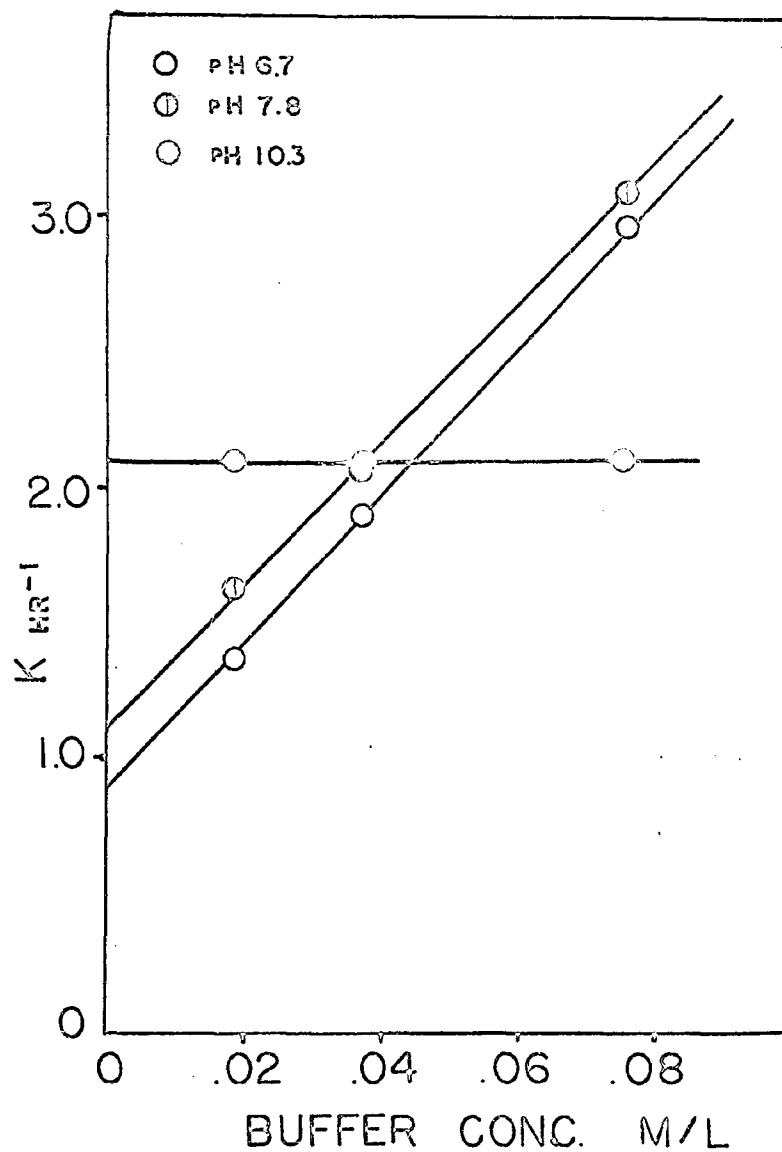


Figure 6

Effect of Phosphate Buffer Concentration on Rate of Hydrolysis of Isopropyl o-Hydroxyphenyl Methyl Phosphonate at 65°C.

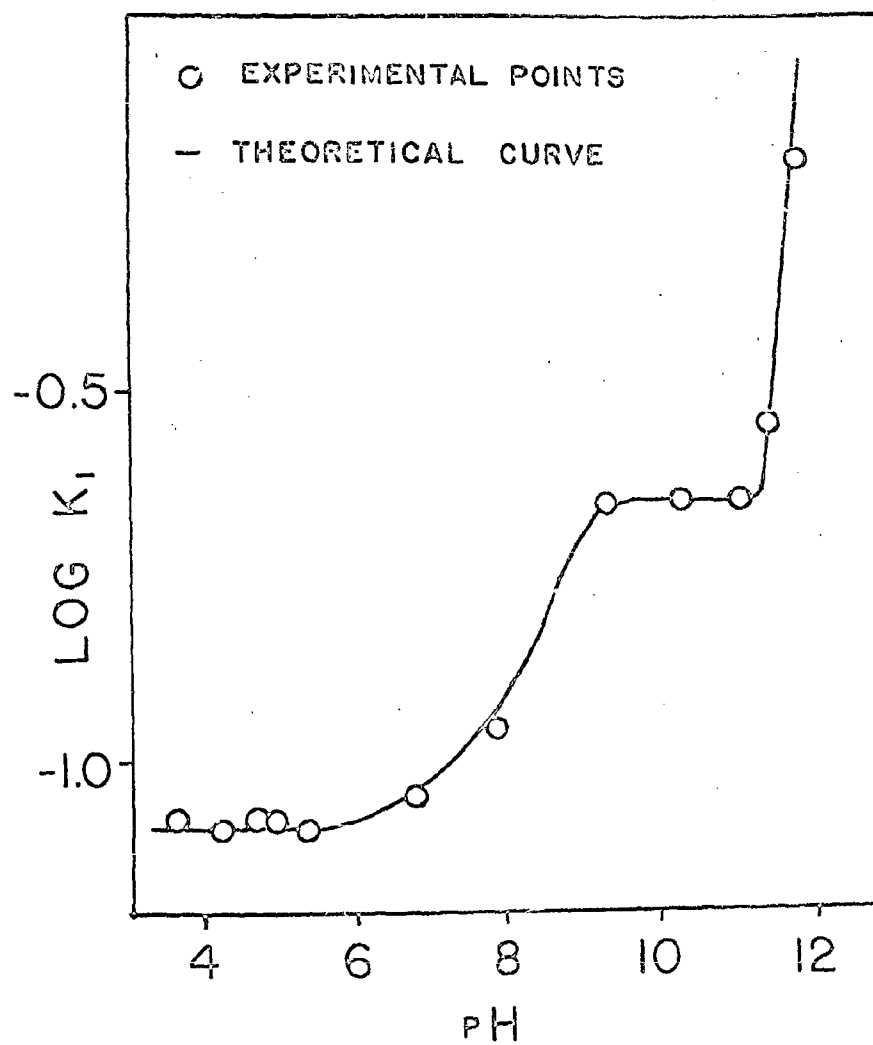


Figure 9

pH Profile of Isopropyl o-Hydroxyphenyl Methyl Phosphonate Hydrolysis at 65°C.

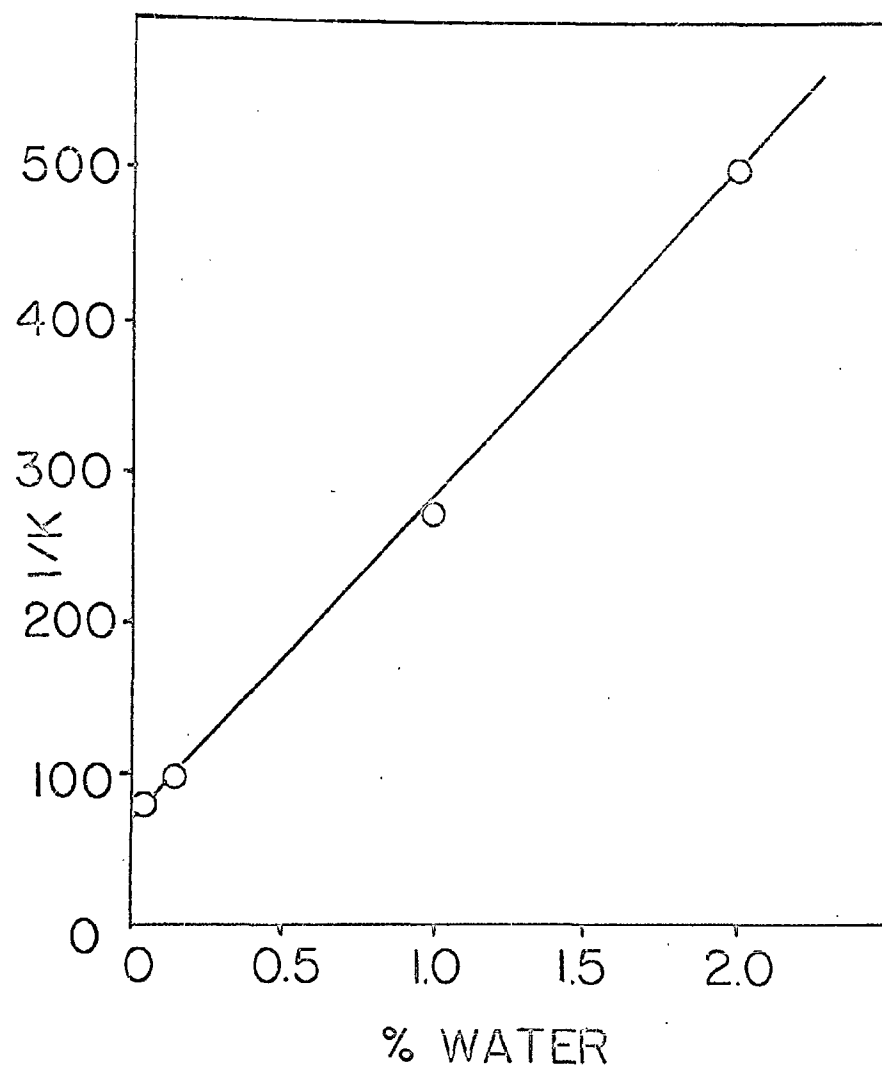


Figure 10

Effect of Water in Isopropanol on the Reciprocal of the Ratio of CS to CCA Formed from PP at 25°C.

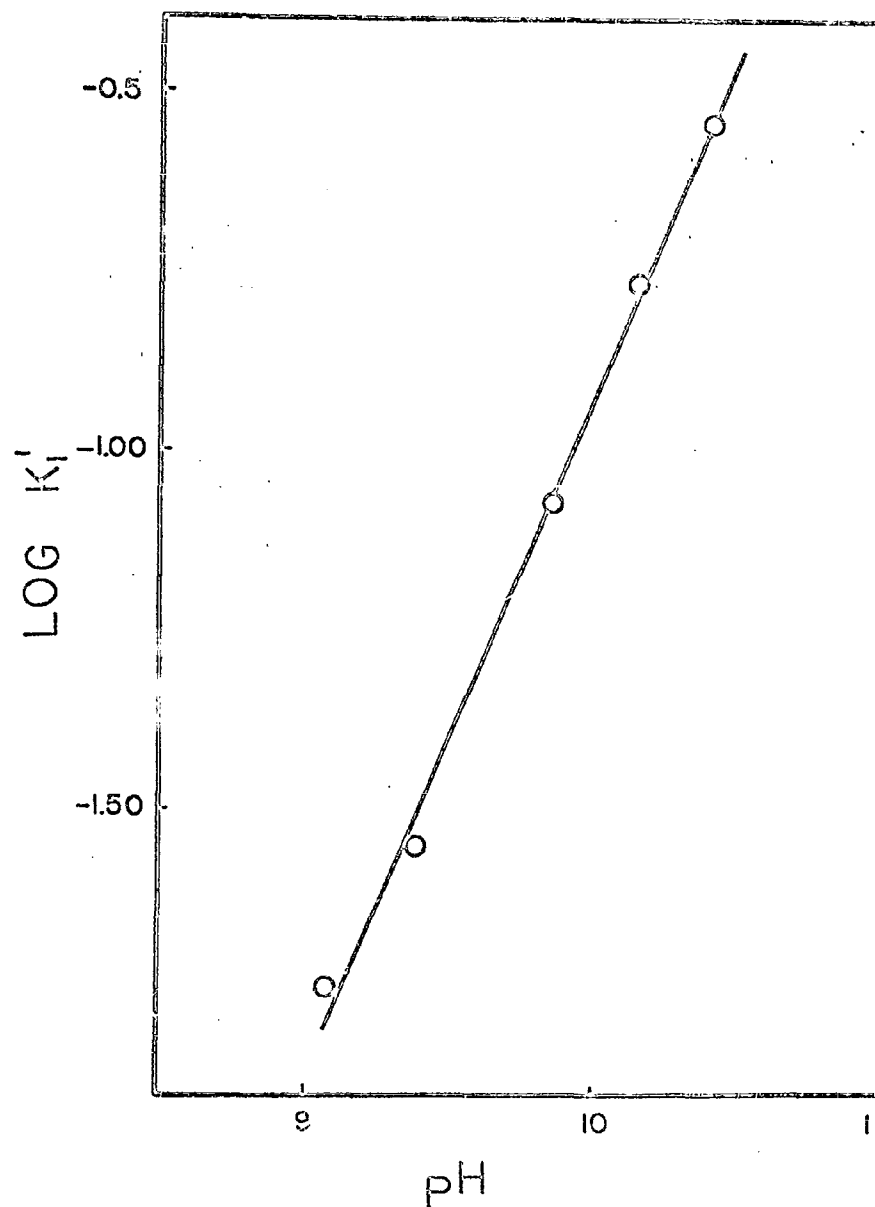


Figure 11

pH Profile of Isopropyl Phenyl Methyl Phosphonate
Hydrolysis at 65°C.

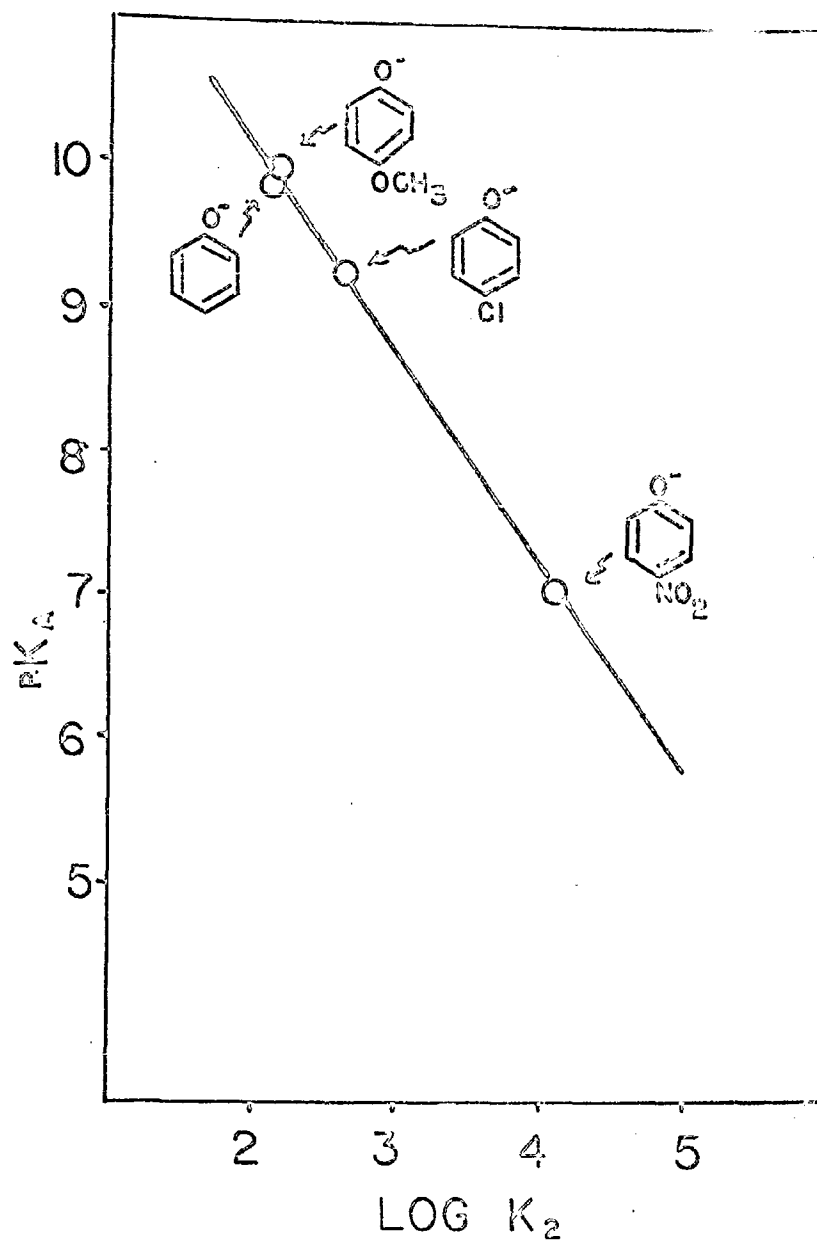
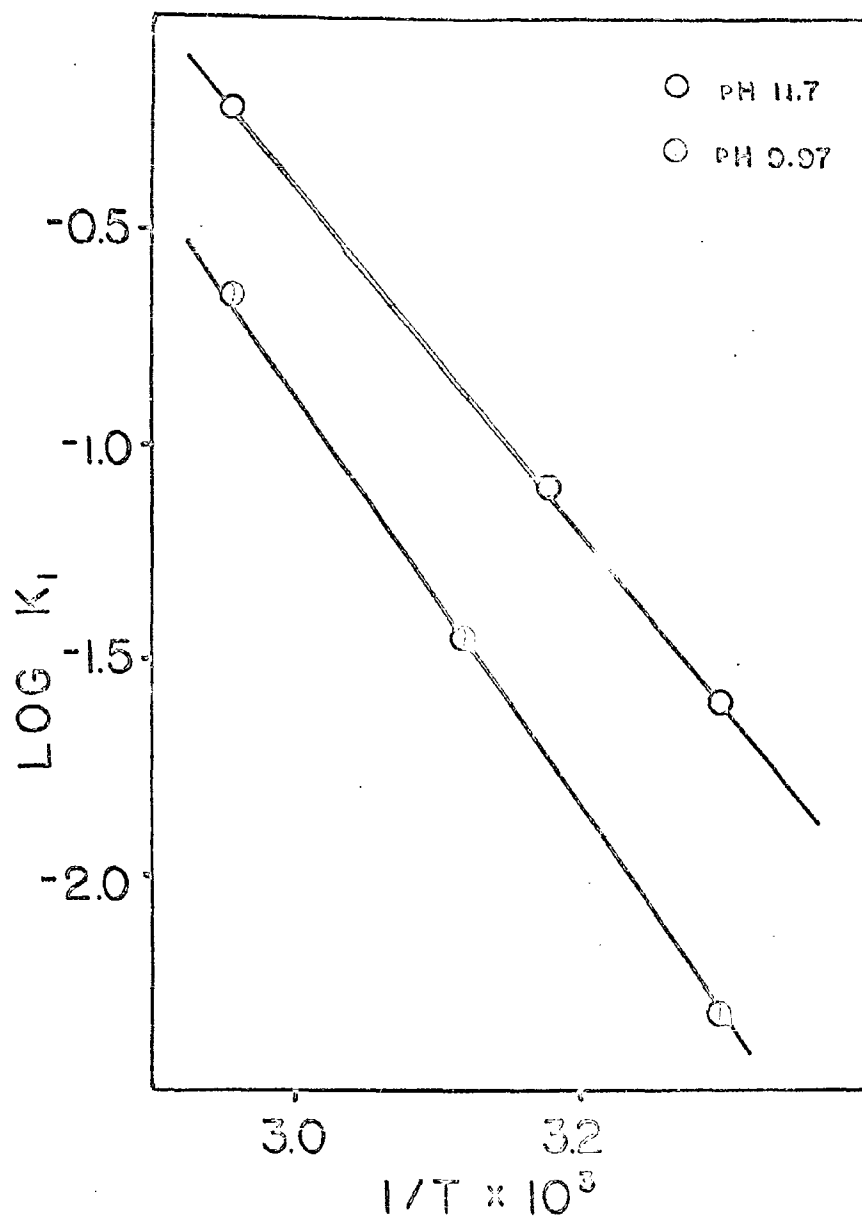
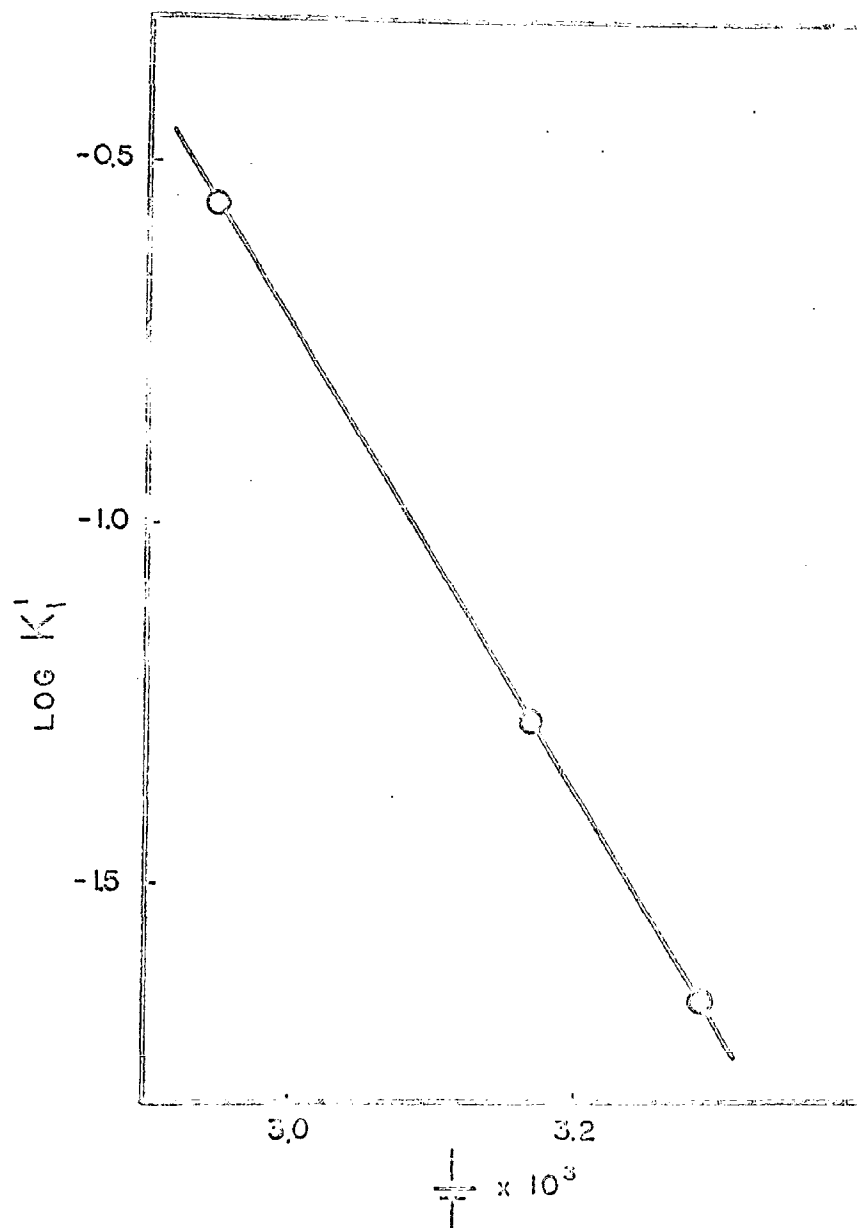


Figure 12

Effect of pK_a of Nuclear Substituents on Rates of Hydrolysis of Various Phosphonates at pH 10.2 and 65°C.



Arrhenius Plot of Rate of Hydrolysis of Isopropyl o-Hydroxyphenyl Methyl Phosphonate at Different pH's.



Arrhenius Plot of Rate of Hydrolysis of Propenyl Methyl Phosphonate at pH 10.4.

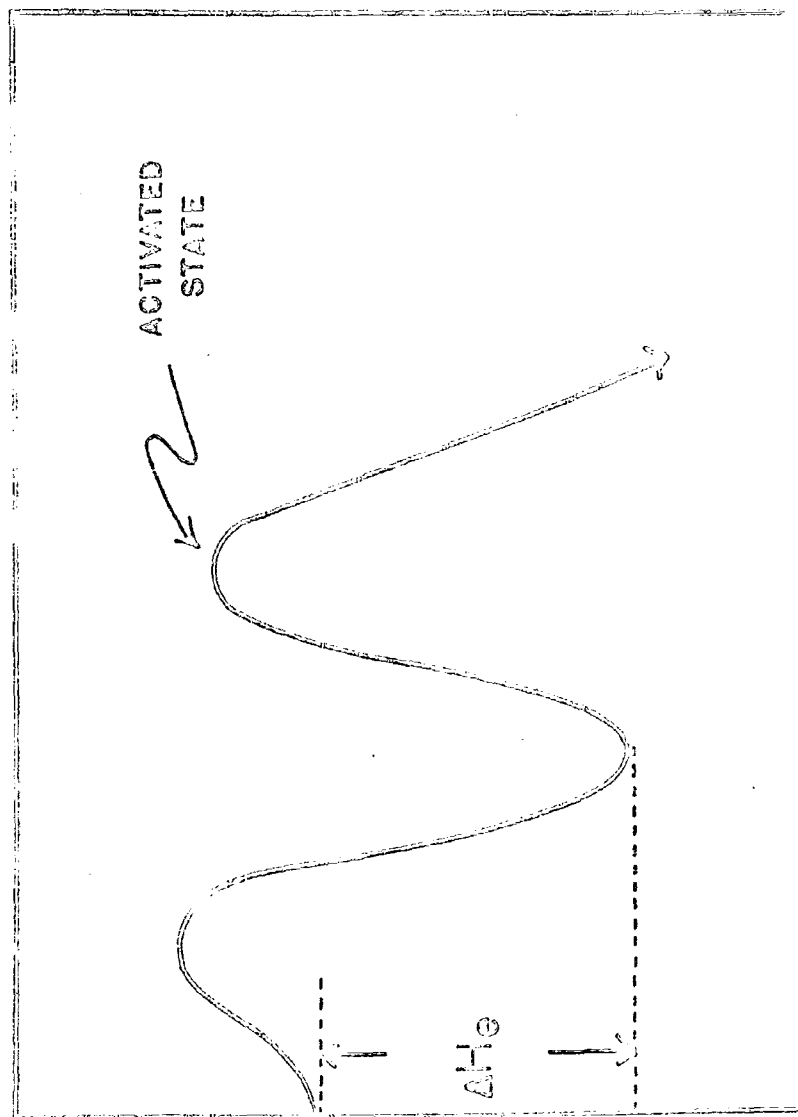


Figure 15
Energetics of the Reaction.

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