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INTRAMOLECULAR CATALYSIS IN THE HYDROLYSIS OF SOME PHOSPHONATE ESTERS

A thesis presented

by

Lorna Joan Williamson

to

The Department of Chemistry

in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

in the subject of

Chemistry

Harvard University

Cambridge, Massachusetts

July, 1973

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INTRAMOLECULAR CATALYSIS IN THE HYDROLYSIS OF SOME PHOSPHONATE ESTERS

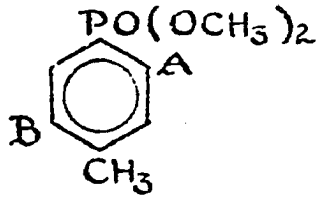
(Summary)

Research Director:
Professor F.H. Westheimer

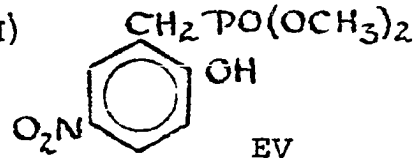
Lorna J. Williamson
July, 1973

The syntheses of four new phosphonate diesters (EII had previously been prepared) and of four new phosphonate monoesters are described.

Diesters:

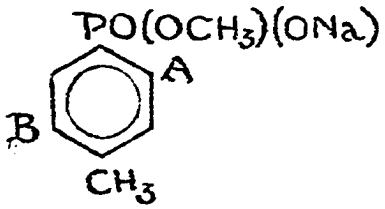


EI (A=-OH, B=-H)
EII (A=B=-H)
EIII (A=-OH, B=-NO_2)
EIV (A=-OCH_3, B=-NO_2)

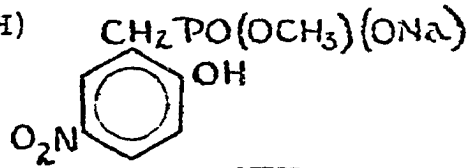


EV

Monoesters:



MEI (A=-OH, B=-H)
MEII (A=B=-H)
MEIII (A=-OH, B=-NO_2)



MEV

By uv and NMR kinetics, EV was found to hydrolyze up to 10^2 times faster than EII or EIII. MEV underwent hydrolysis an estimated 10^6 times faster than MEII at pD 7. The pH (pD)-rate profiles of the hydrolyses are given and the mechanistic possibilities discussed.

For both EV and MEV, intramolecular attack by the phenolic oxygen on phosphorus is assumed to be the source of internal catalysis. Strongly buffer-dependent rates in the hydrolysis of EV at neutral pH's were observed and attributed to general acid catalysis.

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Figure 1

Chrome alum crystal pictured with a 150 mm ruler

(see Appendix 3)

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ACKNOWLEDGEMENT

I wish to thank Professor Frank H. Westheimer for the opportunity of working under his direction. His helpfulness, particularly during some very frustrating last days, is greatly appreciated. His sense of humor and broad scope of interests enhanced the pleasure of days spent in these laboratories.

Professor William von E. Doering deserves special thanks for invaluable criticism of this thesis. Dr. Gary W. Allen gave valuable instruction and suggestions--heeded and otherwise. Mr. Hampar Janjigian made sure that cantankerous NMR machines were kept in line.

National Science Foundation supported me with two years of predoctoral fellowships (1969-1971) and supplied me with research assistantships (1971-1973) through a grant to Professor Westheimer. Harvard University gainfully employed me as a teaching fellow for three and one-half years (1969-1972).

Finally, my deepest gratitude must go to all of those many friends, both inside and outside of the Westheimer group, who helped a West Coast chauvinist adapt to life in Cambridge.

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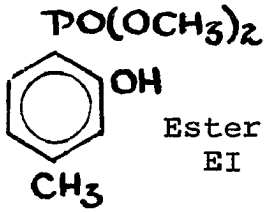
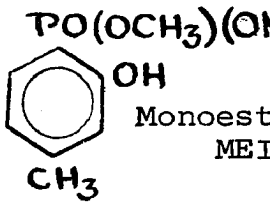
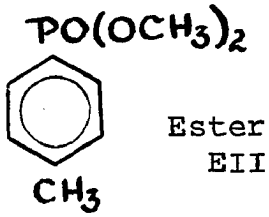
Abbreviations of Compounds Studied in this Work:



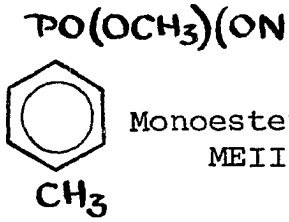
Ester I
EI



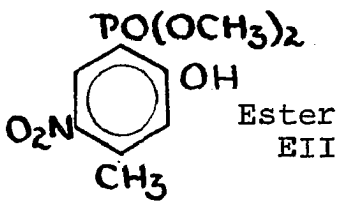
Monoester I
MEI



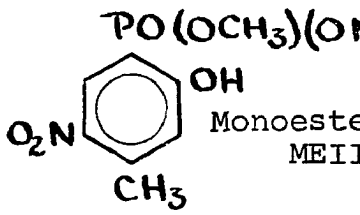
Ester II
EII



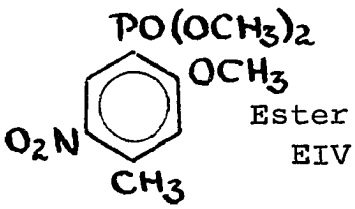
Monoester II
MEII



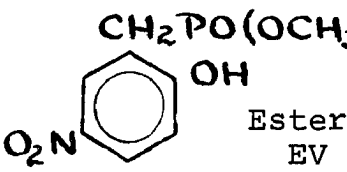
Ester III
EIII



Monoester III
MEIII



Ester IV
EIV



Ester V
EV

Monoester V
MEV

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CHAPTER I

GENERAL INTRODUCTION

The importance of phosphorus to life processes cannot, by any standards, be over-estimated. ATP and related molecules provide organisms with a convenient source of stored energy. Metabolic pathways and the messengers that trigger them rely upon phosphorus containing molecules. DNA and RNA, the basis for the genetic code, are built upon a backbone partially composed of this essential element.

Model systems have been devised by chemists to help elucidate the mechanisms by which enzymes act upon phosphorus-containing molecules. A popular theory for enzymic effectiveness is "approximation" described simply by Jencks as the enzyme's ability "to bring reactive molecules together at the active site..."[1] Chemical models for the approximation theory have two reactive moieties built into the same molecule.

---

[1]W.P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, 1969, p. 7.

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This thesis describes the synthesis of some dimethyl- and monomethylphosphonate esters which were designed to show intramolecular catalysis in hydrolysis reactions. The catalytic participation of the neighboring phenolic moiety in the hydrolysis of the phosphonic ester moiety and in the displacement on the methyl ester carbon by nucleophiles is discussed.

For the sake of organization, the phosphonate diesters and monoesters are treated separately.

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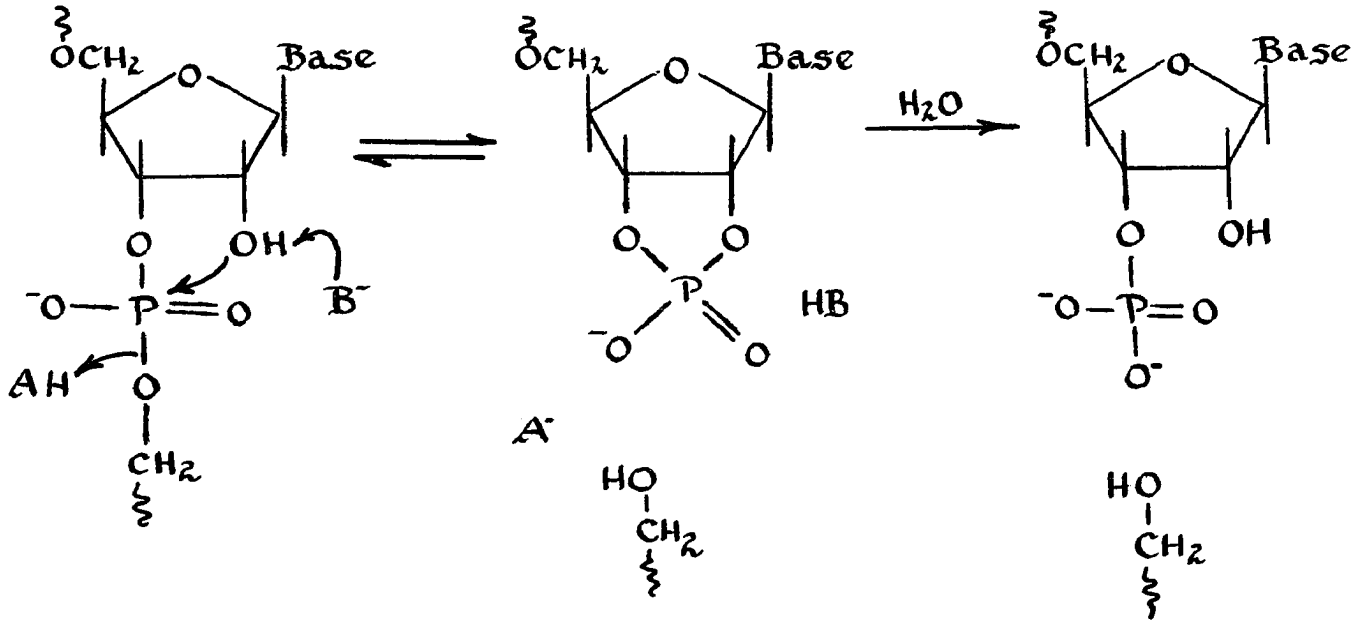
A. Background for Studies on the Phosphonate Diesters

Hydrolysis of phosphate di- and triesters is effectively catalyzed by intramolecular nucleophilic attack on phosphorus. The action of ribonuclease on ribonucleic acid involves intermediate formation of a cyclic phosphate which is then enzymically hydrolyzed by water:[2]



[2] a. J.R. Cox and O.B. Ramsay, Chem. Rev., 64, 317 (1964). b. R. Markham and J.D. Smith, Biochem. J., 52, 552 (1952). c. G.C.K. Roberts, E.A. Dennis, D.H. Meadows, J.S. Cohen, and O. Jardetzky, Proc. Natl. Acad. Sci., 62, 1151 (1969). d. D.A. Usher, D.I. Richardson, Jr., and F. Eckstein, Nature, 228, 663 (1970).

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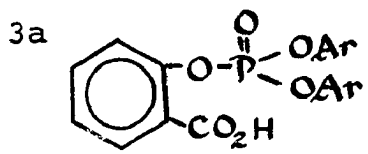
The model systems, 1, 2, and 3, investigated by Kirby and co-workers[3] gave additional evidence for nucleophilic



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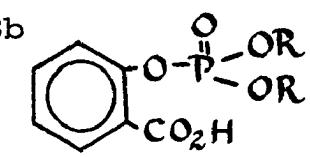

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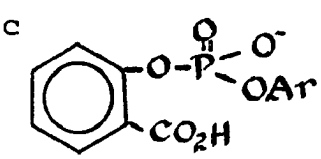
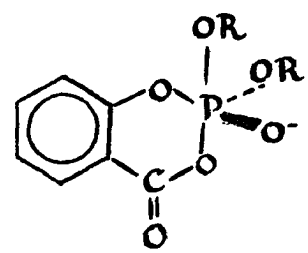


3

attack at phosphorus. These molecules underwent hydrolysis at rates of 10^7 to 10^8 times faster than their non-carboxylated counterparts. Convincing kinetic evidence as well as the identification of products consistent with the rules of pseudorotation[4] point to the existence of the penta-coordinated intermediate 4:



4

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[3] a. R.H. Bromilow, S.A. Khan, and A.J. Kirby, J. Chem. Soc.(B), 1972, 911. b. Ibid., 1971, 1091. c. S.A. Khan, A.J. Kirby, M. Wakselman, D.P. Horning, and J.M. Lawlor, ibid., 1970, 1182.

[4] F.H. Westheimer, Accts. Chem. Res., 1, 70 (1968).

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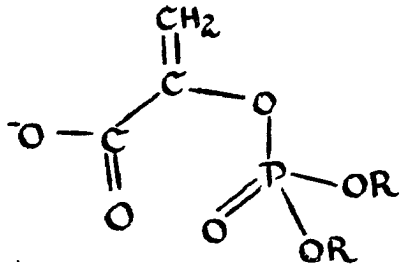
Derivatives of phosphoenol pyruvate 5 undergo hydrolysis about 150 times more readily than the carboxyl-esterified compounds.[5] The recent work of Simons on the more conformationally rigid norbornane systems 6, showed a rate catalysis



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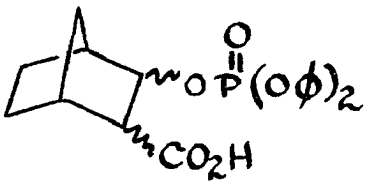

6

of up to 10^7 when compared to the non-carboxylated norbornanes. For both examples, nucleophilic catalysis via cyclic penta-coordinated intermediates was again postulated.

Intramolecular catalysis in phosphonate esters has also been investigated. Some models are of importance to the field of toxicology with respect to the process known as "aging" of cholinesterase.[7] Rapidly administered oximes cause

---

[5] a. V.M. Clark and A.J. Kirby, J. Amer. Chem. Soc., 85, 3705 (1963). b. K.J. Schray and S.J. Benkovic, J. Amer. Chem. Soc., 93, 2522 (1971).

[6] S.S. Simons, Ph.D. Thesis, Harvard University (1972).

[7] a. D.F. Heath, "Organophosphorus Poisons," Pergamon Press, New York (1961). b. A. Albert, "Selective Toxicity," 4th Ed., Methuen and Co., Ltd., London, 1968, pp. 400-402. c. D.B. Coult, D.J. Marsh, and G. Read, Biochem. J., 98, 869 (1966). d. J.C. Lamb, G.M. Steinberg, S. Solomon, and B.E. Hackley, Jr., Biochem., 4, 2475 (1965).

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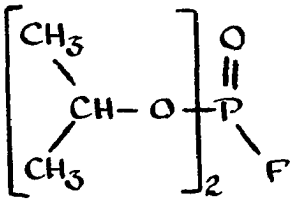
reversal of cholinesterase inhibition by such lethal nerve gases as DFP, 7, and Sarin, 8. The "aging" process occurs when oximes lose their ability to free the cholinesterase's active serine of its phosphorus-containing blocking group.



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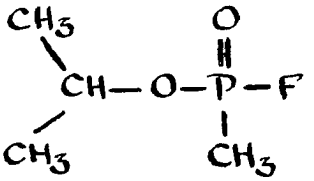

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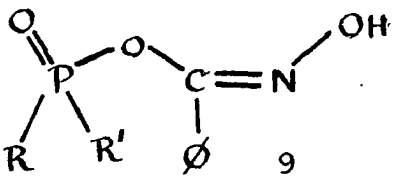
Cadogan and co-workers[8] have prepared phosphonate esters 9 equipped with their own "detoxifying" oxime moiety.



9

When compared to simple dialkyl phosphonates[9] in the pH range of 2-3.5, these α-hydroxyiminophosphonates hydrolyzed 10^7 times faster. The accompanying P-O bond cleavage

---

[8]a. J.I.G. Cadogan and J.A. Maynard, Chem. Comm., 1966, 854. b. J.I.G. Cadogan and D.T. Eastlick, ibid., 1970, 1546. c. J.I.G. Cadogan, J.A. (Maynard)Challis, and D.T. Eastlick, J. Chem. Soc. (B), 1971, 1988.

[9]a. R.F. Hudson and L. Keay, J. Chem. Soc., 1956, 2463. b. L. Keay, J. Org. Chem., 28, 1426 (1963). c. J.I.G. Cadogan, D.T. Eastlick, F. Hampson, and R.K. Mackie, J. Chem. Soc. (B), 1969, 144.

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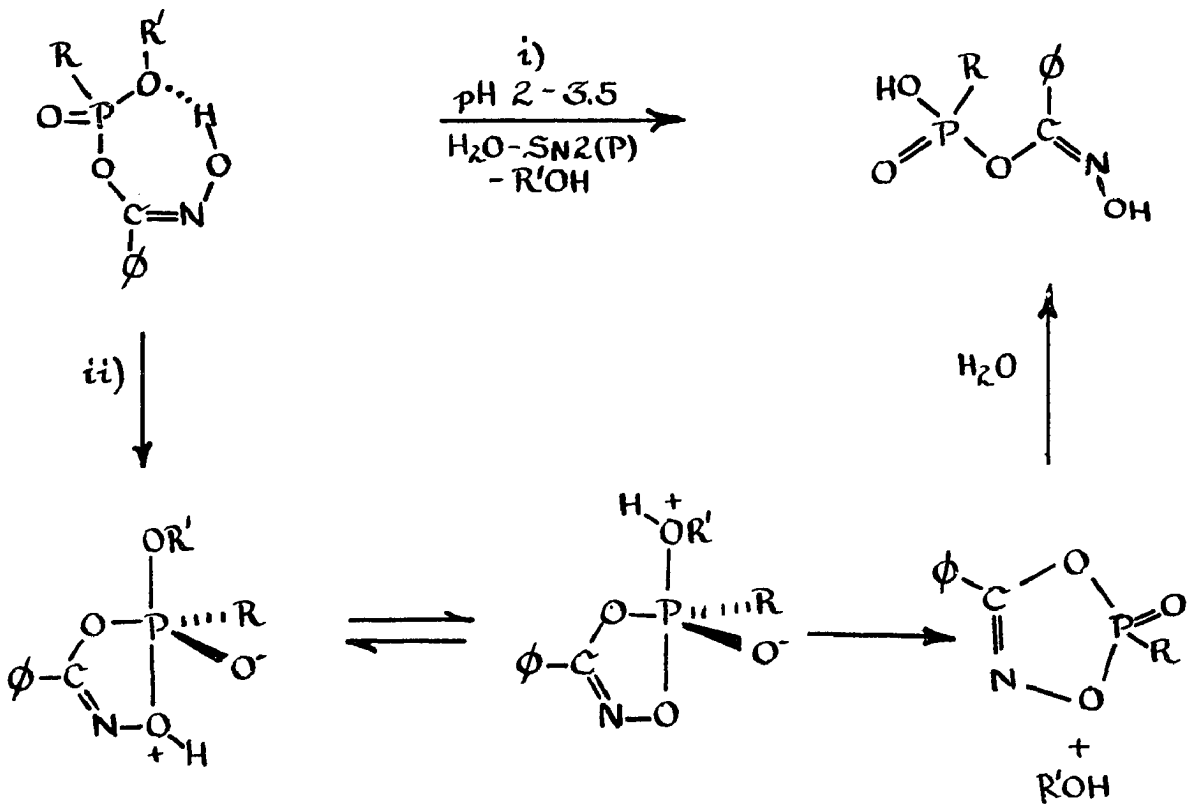
suggested two likely mechanisms:

i) an intramolecular acid-catalyzed $S_N2(P)$ reaction[10]

ii) an intramolecular nucleophilic attack by the oxime oxygen atom to give a penta-coordinated intermediate followed by rapid expulsion of the alcoholic group.



[10] A.J. Kirby and S.G. Warren, "The Organic Chemistry of Phosphorus," Elsevier Publishing Co., Amsterdam, 1967, p. 301 ff.

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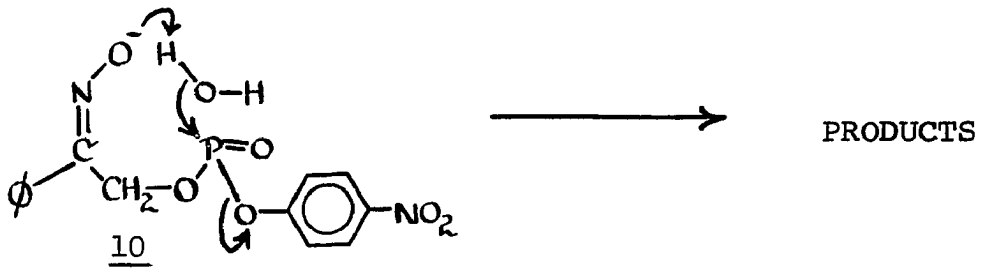
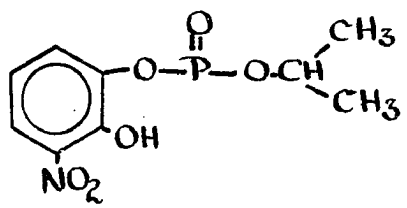
Another phosphonate bearing an oxime function, 10, also exhibited an accelerated hydrolysis of about 2×10^6 fold.[11] The mechanistic interpretation of these results invoked an "oximate anion-catalyzed water-mediated reaction:"



10

Preferential loss of the isopropyl group of the phosphonate ester 11 was facile at 30° and neutral pH's ($t_{1/2}$ ca. 30 min.); the 3-nitrocatechol group was hydrolyzed from 11 only under alkaline conditions.[12]



11

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[11] C.N. Lieske, J.W. Hovanec, G.M. Steinberg, and P. Blumbergs, Chem. Comm., 1968, 13.

[12] A.R. Mlodozeniec, Diss. Abst., 25, 1157 (1964).

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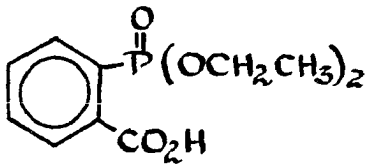
Structurally related to the salicyl phosphates studies by Kirby are the compounds 12 and 13. Diethyl 2-carboxyphenyl phosphonate, 12, first prepared by Gordon and co-workers,[13]



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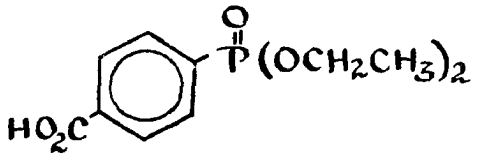
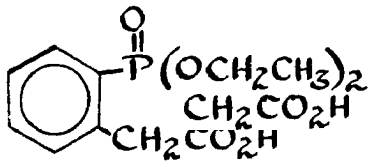


13

was found to hydrolyze an estimated 10^7 times faster than both its carboxyl ethyl ester and its para isomer, 13; 12 also hydrolyzed more readily than its sodium salt. This rate-enhancement was attributed to an internal proton transfer (via a seven-membered ring) followed by an $S_N2(P)$ attack by water. Later studies by Blackburn and Brown[14] revealed that 12 hydrolyzed about 10^5 times faster than 14. They preferred a mechanistic argument involving nucleophilic



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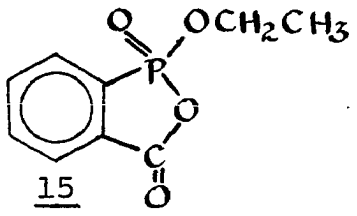

15

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[13]M. Gordon, V.A. Notaro, and C.E. Griffin, J. Amer. Chem. Soc., 86, 1898 (1964).

[14]G.M. Blackburn and M.J. Brown, ibid., 91, 525, (1969).

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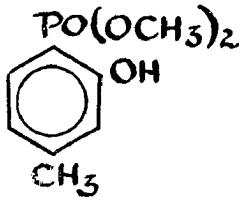
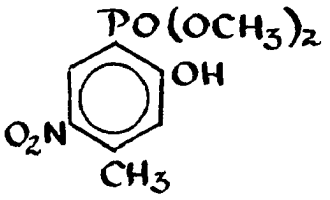
catalysis by the carboxyl group. Synthesis and the subsequent hydrolysis of the anhydride 15 showed it to have the proper kinetic requirements as an intermediate in the hydrolysis of 12.

The preceeding, somewhat lengthy historical discussion was intended to show the variety of structural approaches to the question of intramolecular catalysis in phosphate and phosphonate ester hydrolysis. Accompanying the various approaches is an almost equally varied set of possible interpretations suggested to explain the observed rate accelerations. This thesis may serve only to add to the list of possible structural and mechanistic approaches to intramolecular catalysis.

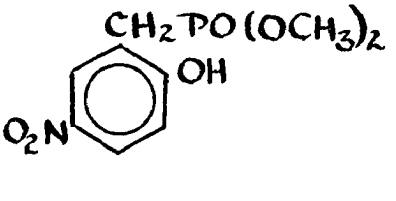
Five phosphonate diesters were prepared. Three of these, imaginatively nick-named Ester I (EI), Ester III (EIII), and Ester V (EV), were designed to show effective intramolecular



EI



EIII



EV

acid-catalysis during hydrolysis; two, Ester II (EII) and

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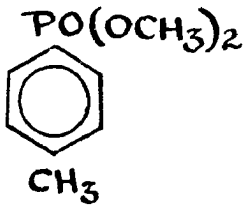
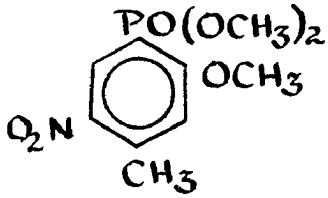
Ester IV (EIV) were prepared for comparative purposes only.

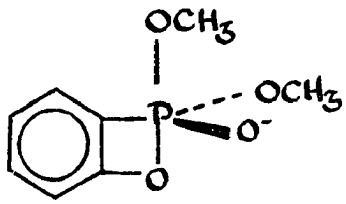


EII



EIV

The first model compound prepared, dimethyl 2-hydroxy-4-methylphenyl phosphonate,[15] EI, precludes any possibility of nucleophilic catalysis by the hydroxyl oxygen--the very strained four-membered ring makes intermediate 16 seem very



16

unlikely. In preliminary kinetic studies (Section A, Chapter III), EI failed to show any significant rate acceleration under hydrolytic conditions when compared to the "control molecule," dimethyl 4-methylphenyl phosphonate, EII.

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[15] The 4-methyl group has no function other than to protect the investigator. The synthetic intermediate, the bromophosphonic acid, has a higher anticholinesterase I_{50} ($> 10^{-2}$) than the unmethylated bromophosphonic acid ($I_{50} = 4 \times 10^{-3}$ mole/l.) See L.D. Freedman, H. Tauber, G.O. Doak, and H.J. Magnuson, J. Amer. Chem. Soc., 75, 1379 (1953).

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The basic structural plan of EI was believed to be worth salvaging. Dimethyl 2-hydroxy-4-methyl-5-nitrophenyl phosphonate, EIII, was, therefore, synthesized. It was hoped that by lowering the pK_a of the hydroxyl moiety, more facile proton transfer from the phenolic function to the phosphonate function would result in an increased rate of hydrolysis. To insure that any large catalytic effects (if observed) could not be attributed to inductive effects by the nitro or hydroxyl moieties, dimethyl 2-methoxy-4-methyl-5-nitrophenyl phosphonate, EIV, was also synthesized.[16] EIII, however, also showed stubborn resistance to hydrolysis although it did exhibit significant rate enhancement over EII during $S_N2(C)$ reactions (see Section B and C, Chapter III).

In a final, but not futile, attempt to achieve the dream of significant intramolecular catalysis, dimethyl 2-hydroxy-5-nitrobenzyl phosphonate, EV, was prepared. Preliminary studies revealed the long-awaited lability towards water. For the hydrolysis of Ester V, the preferred mechanism proposes nucleophilic attack by the phenolic oxygen on phosphorus.

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[16]I would like to thank Dr. Gary W. Allen for this suggestion.

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In neutral solutions, buffer catalysis in the hydrolysis of Ester V was observed.

Chapter II of this thesis presents the syntheses of these phosphonate diesters; only one (EII) had been previously prepared. Chapter III relates the experiments performed on these compounds; the "discussion" contained therein attempts to offer mechanistic interpretations.

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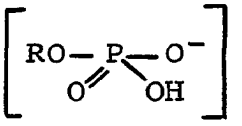
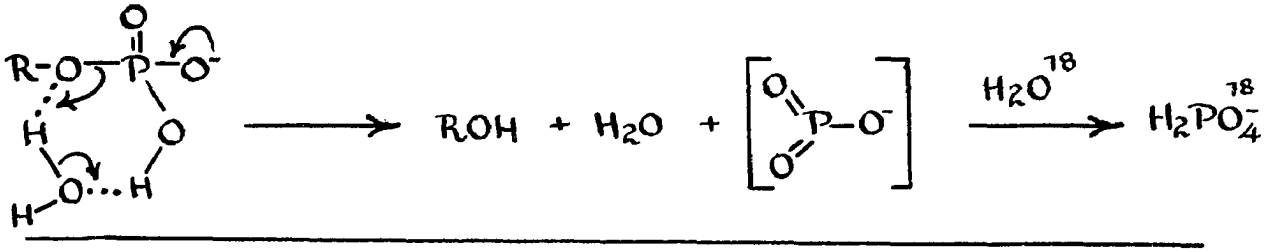
B. Background for Studies on the Phosphonate Monoesters

Intramolecular catalysis in the hydrolysis of phosphonate monoesters has apparently received little attention.[14] Examination of such catalysis in phosphate monoesters, however, suggests some interesting possibilities.

1. The Monomeric Metaphosphate Intermediate

Monoesters of phosphoric acid typically show bell-shaped pH-rate profiles with rate maxima at pH 4[17]. The predominant species at this pH is the monoanion  . Tracer studies with ^{18}O showed its incorporation in the resultant phosphoric acid rather than in the alcohol. To account for these facts, a mechanism invoking the existence of monomeric metaphosphate has been postulated:[17a]



[17] a. W.W. Butcher and F.H. Westheimer, J. Amer. Chem. Soc., 77, 2420 (1955). b. M.C. Bailly, Bull. Soc. Chim., 9, 421 (1942). c. A. Desjobert, Compt. Rend., 224, 575(1947); Bull. Soc. Chim. 14, 809 (1947). d. A.J. Kirby and S.G. Warren, "The Organic Chemistry of Phosphorus," p. 284. e. A.J. Kirby and A.G. Varvoglis, J. Amer. Chem. Soc., 89, 415 (1967) f. T.C. Bruice and S.J. Benkovic, "Biorganic Mechanisms," Vol. II, W.A. Benjamin, Inc., New York, 1966, p. 3 ff.

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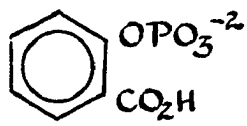
The extensively studied salicyl phosphate 17 like its derivative di- and triesters (1, 2, and 3) exhibits intramolecular catalysis by the carboxyl group.[18] Likewise, the



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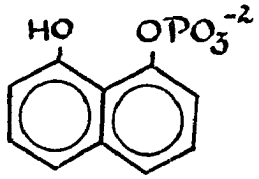

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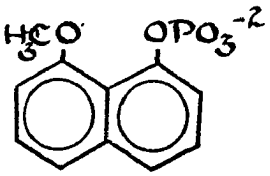
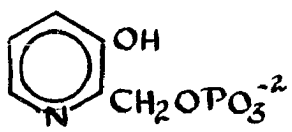


19

the hydroxy naphthoyl derivative 18 is hydrolyzed some ten times faster than 19.[18b] The substituted pyridine 20 of Murakami and co-workers[19] hydrolyzed from two to 125 times faster than 21, depending upon pH. The ionic species shown below exhibited the greatest rate enhancement.



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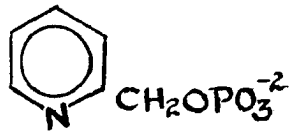

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[18]a. J.D. Chanley and E.M. Gindler, J. Amer. Chem. Soc., 75, 4035 (1953). b. M.L. Bender and J.M. Lawlor, ibid., 85, 3010 (1963). c. R.H. Bromilow and A.J. Kirby, J. Chem. Soc. (B), 1972, 149.

[19]Y. Murakami, J. Sunamoto, and H. Ishizu, Bull. Chem. Soc., Japan, 45, 590 (1972).

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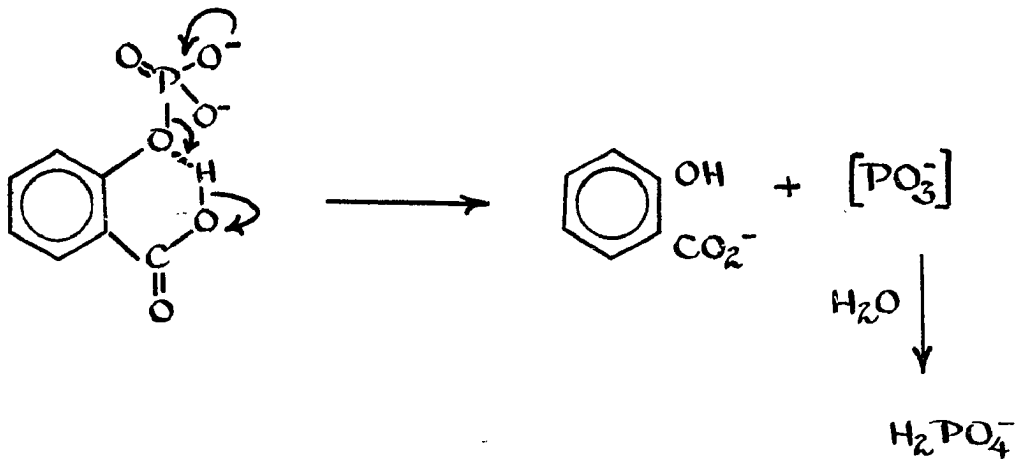
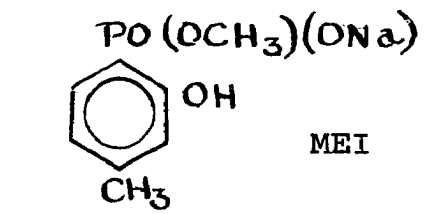
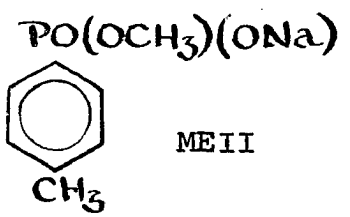
For all of these cases, a mechanism utilizing the monomeric metaphosphate intermediate has been invoked:



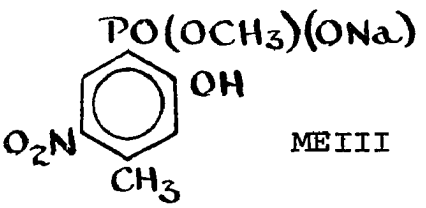
In this work, four phosphonate monoesters, (MEI, MEII, MEIII, and MEV) were prepared.[20] Their syntheses are described in Chapter II.



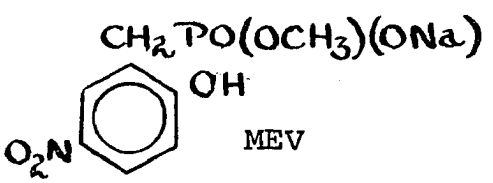
MEI



MEII



MEIII



MEV

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[20]The sodium salts were preferred because of their solubility in water.

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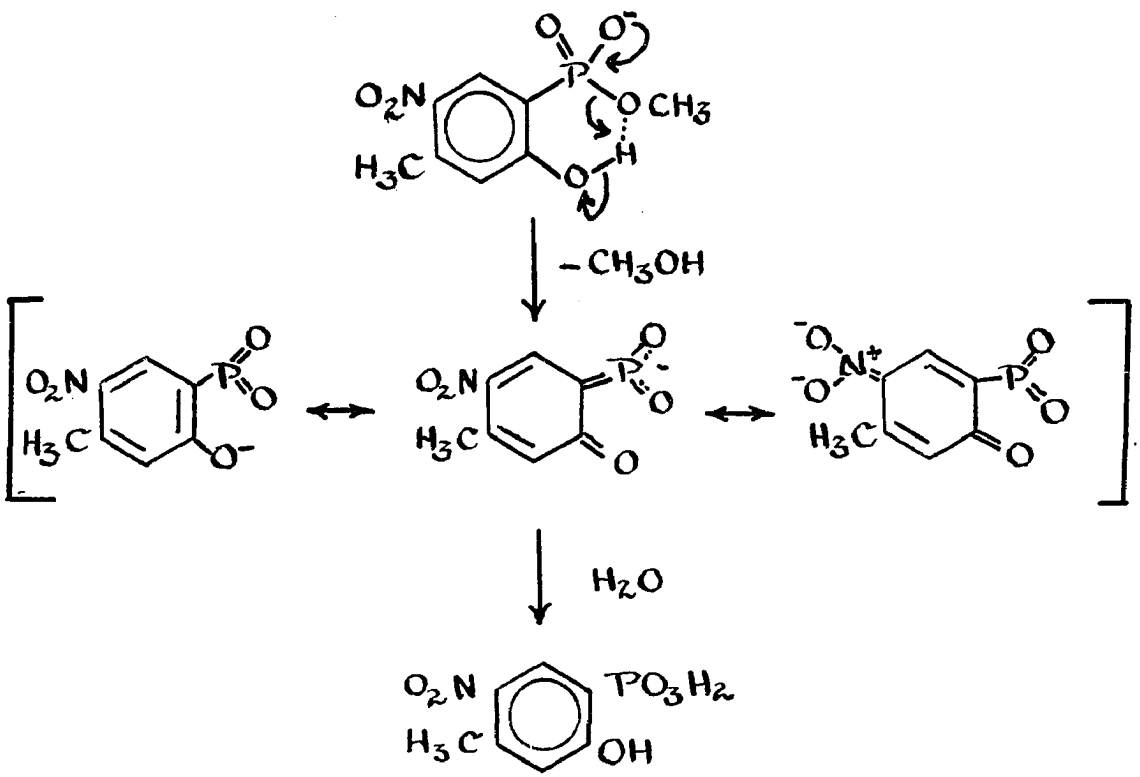
In analogy to the monomeric metaphosphate mechanism discussed above, a similar intermediate may be postulated for the hydrolysis of MEI and MEIII:



If this mechanism were operative, bell-shaped pH-rate profiles with rate maxima corresponding to the pH's at which the monoanions of MEI and MEIII exist would be expected.

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Sections A and B of Chapter IV describe the experiments and results of hydrolysis of MEI, MEII, MEIII, and MEV in buffered solutions.

2. Metal Ion Catalysis

Frequently fundamental to the action of enzymes,[21] metal ions probably maintain a productive geometry between the substrate and the enzyme.[22] Metal ions are also effective in catalyzing the hydrolysis of some phosphate esters. Here, they probably act as effective electron sinks, making phosphorus more susceptible to nucleophilic attack by water or by allowing for a better leaving group.[22]

Hofstetter and co-workers[23] discovered that heavy metals such as Cu^{+2} and Fe^{+3} catalyzed the hydrolysis of

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[21]H.R. Mahler and E.H. Cordes, "Biological Chemistry," 2nd Ed., Harper and Row, New York, 1971, pp. 16-19.

[22]W.P. Jencks, "Catalysis in Chemistry and Enzymology," pp. 111-115.

[23]R. Hofstetter, Y. Murakami, G. Mont, and A.E. Martell, J. Amer. Chem. Soc., 84, 3041 (1962).

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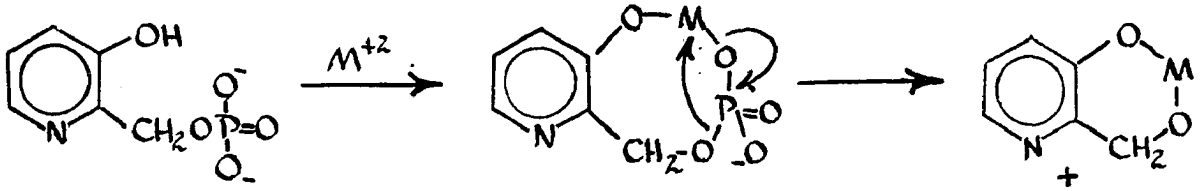
salicyl phosphate 17. Murakami[19] studied the effect of many divalent metal ions in the hydrolysis of 3-hydroxy-2-pyridyl methyl phosphate 20. The mechanisms suggested for the metal's participation are somewhat obscure, generally picturing some sort of chelated metal ion as shown below:



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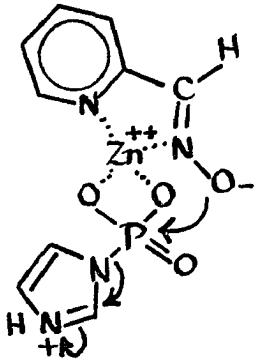
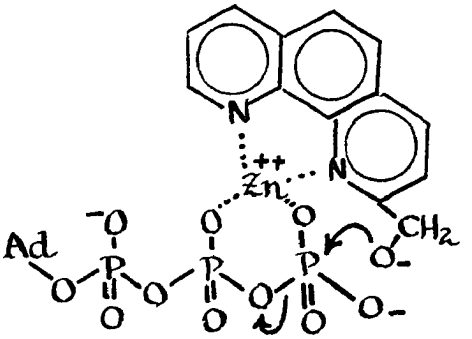
Cooperman[24] and Sigman[25] reported an absolute requirement for a metal ion (Zn^{+2}) in the phosphorylation respectively of Zn^{+2}-pyridine-2-carbaldoxime by phosphoryl imidazole and of 1,10-phenanthroline-2-carbinol by ATP. Ternary complexes 22 and 23 were suggested.

22





23

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[24]G.J. Lloyd and B.S. Cooperman, J. Amer. Chem. Soc., 93 4883 (1971).

[25]D.S. Sigman, G.M. Wahl, and D.J. Creighton, Biochem., 11, 2236 (1972).

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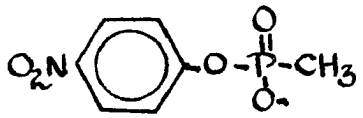
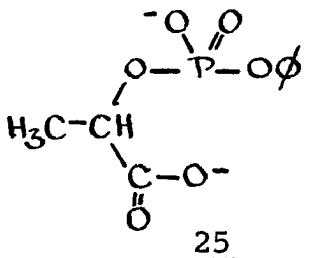
Blewett and Watts[26] reported a 10^5-10^6 catalytic factor produced by ytterbium III in the hydrolysis of 24. For the



24

same monoaryl phosphonate, Edwards and co-workers[25] saw a 12-fold rate enhancement by Ca^{+2}.

Very recently, Benkovic[28] noted up to a 300-fold catalysis produced by Zn^{+2} and up to a 40-fold catalysis produced by Mg^{+2} in the hydrolysis of phosphate diesters 25 and 3. Benkovic suggested involvement of the metal ion



25

at the penta-coordinate level of phosphorus through an intermediate of type 26.

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[26]F. McC. Blewett and P. Watts, J. Chem. Soc. (B), 1971, 881.

[27]E.J. Behrman, M.J. Biallas, H.J. Brass, J.O. Edwards, and M. Isaks, J. Org. Chem., 35, 3063 (1970).

[28]J.J. Steffens, E.J. Sampson, I.J. Siewers, and S.J. Benkovic, J. Amer. Chem. Soc., 95, 936 (1973).

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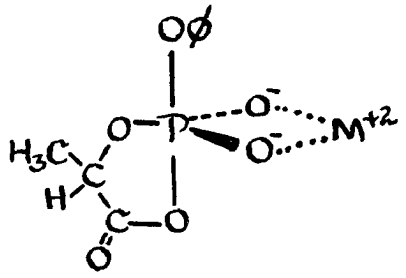

26

Unfortunately, an insufficiency of time prevented an even half-thorough investigation of the interesting possibility of metal-ion catalysis in the hydrolysis of the phosphonate monoesters prepared in this study. The little that was done in this direction is reported in Section C of Chapter IV.

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CHAPTER II

SYNTHESES

The NMR spectra were taken on Varian spectrometers A-60 and T-60. IR spectra were taken on a Perkin-Elmer Infracord. A Thomas-Hoover capillary melting point apparatus and a Nalge hot-plate melting point apparatus were used. All reported melting points are corrected.

All chemicals were reagent grade with the exception of p-toluidine and sodium fluoborate which were technical grade, and trimethyl phosphite which was practical grade.

Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

Toxicity tests were performed by Leberco Laboratories, Roselle Park, N.J.

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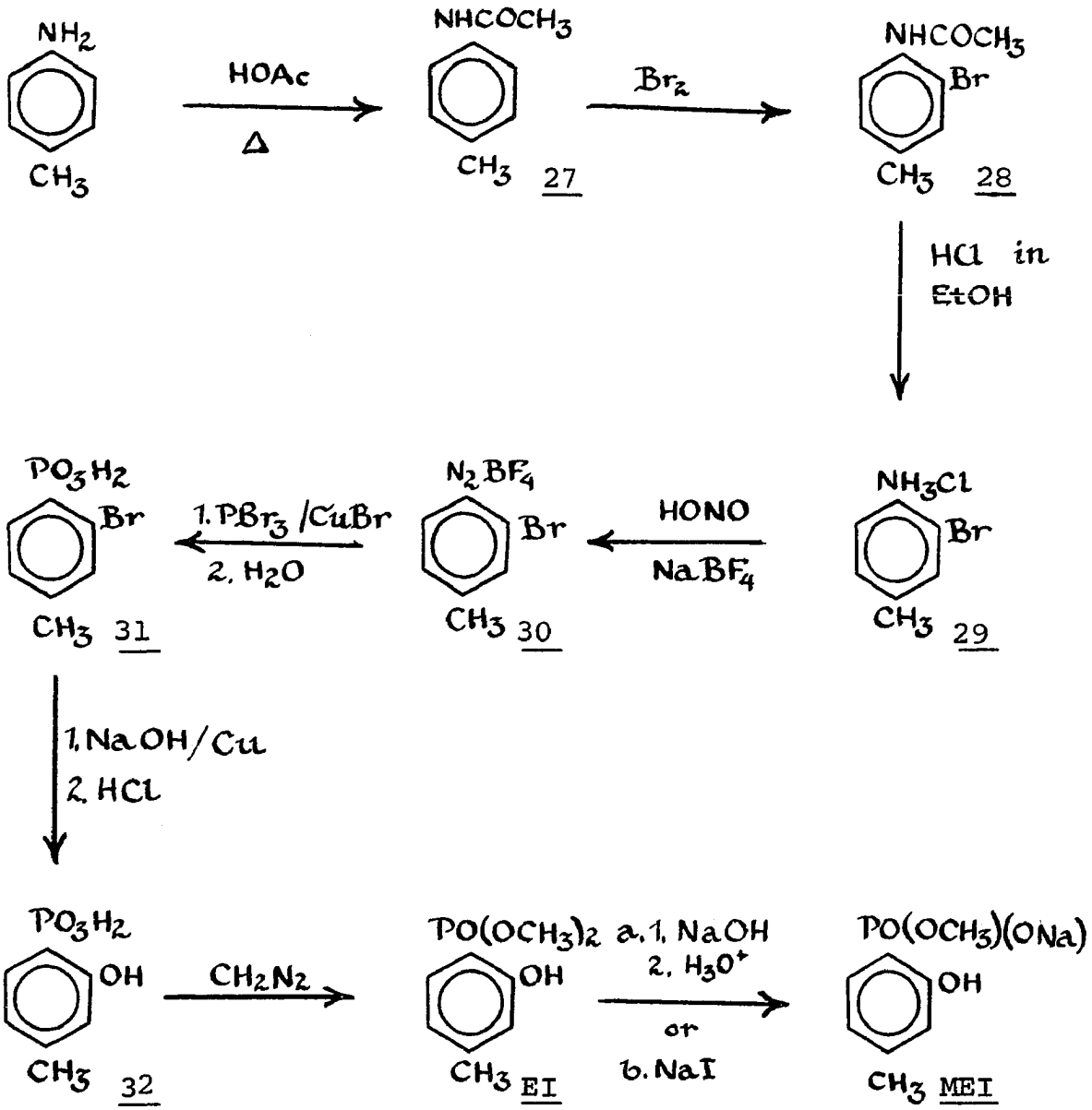
A. Synthesis of Dimethyl 2-Hydroxy-4-Methylphenylphosphonate (Ester I) and of Methyl 2-Hydroxy-4-Methylphenylphosphonate, Monosodium Salt (Monoester I)

The following scheme was used in the synthesis of Ester I and Monoester I:



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1. 3-Bromo-4-Aminotoluene, Hydrochloride Salt (29)

The procedure outlined in "Organic Syntheses"[1] was followed with slight modifications. After refluxing the acetic acid and p-toluidine, the acylated product 27 was allowed to precipitate from solution. It was then filtered; the solvent, containing highly colored impurities, was discarded. Fresh acetic acid was used to dissolve 27 for the subsequent bromination. This slight modification produced a substantially purer brominated product 28 and alleviated the need for the recommended recrystallization of product 28 from ethanol. The hydrochloride salt 29 was easily recrystallized from water; m.p. with decomp. 223-226°, reported 221°.[2]

2. 2-Bromo-4-Methylbenzenediazonium Fluoborate (30)

Method II described by Roe[3] was used in this preparation. Due to the insolubility of hydrochloride salt 29 in the

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[1] J.R. Johnson and L.T. Sandborn in "Organic Syntheses," Collective Volume I, John Wiley and Sons, Inc., New York, 1941, p. 111.

[2] I. Heilbron (edit.), "Dictionary of Organic Compounds," 4th Ed., Oxford University Press, New York, 1965, p. 492.

[3] A. Roe in "Organic Reactions," Volume V, John Wiley and Sons, Inc., New York, 1949, p. 205.

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aqueous reaction medium, the salt was finely ground with a mortar and pestle to allow for greater surface reaction. Ten grams of the salt, 10 ml of conc. HCl, and 5 gm of $NaBF_4$ were suspended in 20 ml of water and cooled in an ice bath to 0°C. A pre-cooled solution of 3.1 gm $NaNO_2$ in 8 ml water was added slowly to the stirred suspension. The temperature of the reaction mixture was kept below 10°C. The progress of the reaction was checked with potassium iodide-starch paper. The resultant slurry was filtered through a sintered glass funnel and washed with cold ether. The white granular product was then thinly spread on filter paper and allowed to dry overnight. Attempted recrystallization of the diazonium salt 30 in a variety of solvents was unsuccessful. Yield was 60%; m.p. with decomp. 121-123°.

3. 2-Bromo-4-Methylphenylphosphonic Acid (31)

The procedure of Doak and Freedman,[4] with modifications, was followed. After addition of the reagent (PBr_3) and the catalyst (CuBr) to the stirred suspension of the diazonium

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[4]G.O. Doak and L.D. Freedman, J. Amer. Chem. Soc., 73, 5658 (1951).

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salt 30 in ethyl acetate,[5] the mixture was slowly heated to about 40°C at which point evolution of gas became apparent. Heating was continued until gaseous evolution ceased. After the removal of the ethyl acetate by steam distillation, the remaining liquid was filtered to remove a gummy oil. The filtrate was then evaporated on a Büchi rotary evaporator. The remainder of the preparation followed that described in reference 4. Acidification of the final filtrate usually resulted in an oiling out of the phosphonic acid 31, probably due to the presence of small amounts of the diarylphosphinic acid, a side product of the synthesis. Heating the acidified solution to boiling followed by filtration removed the contaminant and allowed crystallization of the product. The yield (15-20%) was less than that reported (27%); m.p. 194-196°, reported 199-203°.[5]

4. 2-Hydroxy-4-Methylphenylphosphonic Acid (32)

Following similar procedures,[6] one gram of the phenyl-

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[5] L.D. Freedman, H. Tauber, G.O. Doak, and H.J. Magnuson, ibid., 75, 1379 (1953). Note: the phosphonic acid is incorrectly named in this reference.

[6] a. A.M. Lukin and I.D. Kalinina, Zhur. Obsc. Khim., 30, 1597 (1960); see Chem. Abstr. 55, 1487i (1961). b. L.D. Freedman and G.O. Doak, J. Org. Chem., 23, 769 (1958). c. V.L. Bell, Jr. and G.M. Kosolapoff, J. Amer. Chem. Soc., 75, 4902 (1953).

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phosphonic acid 31 was dissolved in 20 ml 4 N NaOH containing a catalytic amount of fine copper powder (0.1 gm; Baker and Adamson, Code 1618, lot number D322). The reaction mixture was refluxed for four hours, filtered, and acidified to pH 8. The resultant gelatinous precipitate (possibly due to the leaching action of the NaOH on the glassware) was filtered from the solution. The solution was further acidified to pH 1; any further precipitate (unreacted starting material), was removed by filtration. The solution was then continuously extracted with ether. The ether extract was evaporated in vacuo to yield a clear yellow oil which solidified upon cooling and scratching or upon trituration with chloroform. The white granular solid was then recrystallized from hot 4 N HCl.

The resultant white needles gave a positive phenolic reaction when tested with ferric chloride solution. Yield was 25%, m.p. 138-139°.

Elemental Analysis $C_7H_9O_4P$ (M. Wt. 188)

	%C	%H	%P
Calcd.	44.69	4.82	16.46
Exptl.	44.68	4.89	16.67

Mass. Spect. parent ion 188.

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IR, KBr pellet, spectrum 1a.

NMR in NaOD/D_2O with TSP (sodium 3-trimethyl silyl propionate-2,2,3,3-d_4) as internal standard, spectrum 1b.

5. Dimethyl 2-Hydroxy-4-Methylphenylphosphonate (EI)

The diazomethane used in methylating the hydroxyphosphonic acid 32 was prepared by the method of Moore and Reed[7] using Aldrich N,N'-dimethyl-N,N'-dinitrosoterephthalimide, 70% in mineral oil.

Five grams of the diazomethane precursor suspended in 100 ml ether and 15 ml carbitol was reacted with 17 ml 30% NaOH. The resultant diazomethane was carefully co-distilled with ether and collected in a chilled flask. Two grams of the hydroxyphosphonic acid 32 was dissolved in ether and cooled in an ice bath. To this was slowly added two equivalents of diazomethane.

In the preliminary preparations of the diester, the molarity of the distilled diazomethane solution was determined by reaction with an excess but known amount of

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[7] J.A. Moore and D.E. Reed in "Organic Syntheses," Vol. 41, John Wiley and Sons, Inc., New York, 1961, p. 16.

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benzoic acid in water and back titrated with NaOH solution.[8] The phenolphthalein endpoint was sharper if the ether from the diazomethane solution was boiled off prior to back titration. A calculated volume of diazomethane was then added to the hydroxyphosphonic acid. In later preparations, the methylation of the phosphonic acid was followed by thin layer chromatography using ethyl acetate as eluent and a spray of $FeCl_3$ in ethanol and iodine vapor as developers. Diazomethane solution was added slowly to the ether solution of 32. Periodic checks of this solution by TLC revealed only two spots. The starting material 32 remained tightly bound at the origin whereas Ester I had an R_f value of 0.85. Under the conditions of slow diazomethane addition and low temperature, the unwanted methyl ether was apparently not formed.

After the methylation was completed, the ether solution was evaporated *in vacuo* to yield a yellow oil. Recrystallization from water gave lustrous white flakes; yield was quantitative depending upon the amount of

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[8] L.F. Fieser and M. Fieser, "Reagents for Organic Synthesis," Vol. I, Wiley, New York, 1967, p. 191.

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diazomethane used; m.p. 93-94°.

Elemental Analysis $C_9H_{13}O_4P$

	%C	%H	%P
Calcd.	50.00	6.06	14.33
Exptl.	49.97	6.02	14.57

IR, KBr pellet, spectrum 2a.

NMR in CCl_4, TMS as internal standard, spectrum 2b.

Ester I showed some physiological activity, causing death, when dissolved in corn oil and injected subcutaneously into mice. It has an LD_{50} between 50 and 20 mg/kg of mouse.

6. Methyl 2-Hydroxy-4-Methylphenylphosphonate, Monosodium Salt (MEI)

a. By Hydrolysis

A greatly modified version of a hydrolysis preparation of monoesters from diesters described by Cherbuliez et al.[9] was used in the synthesis of MEI. All attempts to prepare MEI by methylation of the parent phosphonic acid 32 by

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[9]E. Cherbuliez, B.Baehler, H. Probst and J. Rabinowitz, Helv. Chim. Acta, 45, 2656 (1962). See Chem. Abst. 58, 9129c (1963).

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methyl iodide or dimethyl sulfate failed.

To obtain the correct ionic species, pH adjustments were required. Assuming for the monoester-monoacid, the acid dissociation constants $K_1 = 10^{-2}$ and $K_2 = 10^{-10}$[10] calculations for a polybasic acid equilibrium were performed using the treatment of Butler.[11] By plotting mole fraction of ionic species vs. pH, the desired monosodium salt, MEI, was found to be the dominant species in the pH range 4-8.

A 0.50 gm sample of Ester I was dissolved in two equivalents of NaOH solution (4.60 ml, 1.0 N soln.) and refluxed until hydrolysis was complete as shown by TLC (ethyl acetate as eluent, a spray of $FeCl_3$ in ethanol was used as developer). Adjustment of the pH to 7-8 was accomplished by introduction of CO_2 in the form of dry ice. A gelatinous white precipitate was removed by filtration and discarded. The filtrate was evaporated *in vacuo* to

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[10] H.H. Jaffé, L.D. Freedman and G.O. Doak , J. Amer. Chem. Soc., 75, 2209 (1953).

[11] J.N. Butler, "Ionic Equilibrium: a Mathematical Approach," Addison-Wesley, Reading, Mass., 1964, p. 210.

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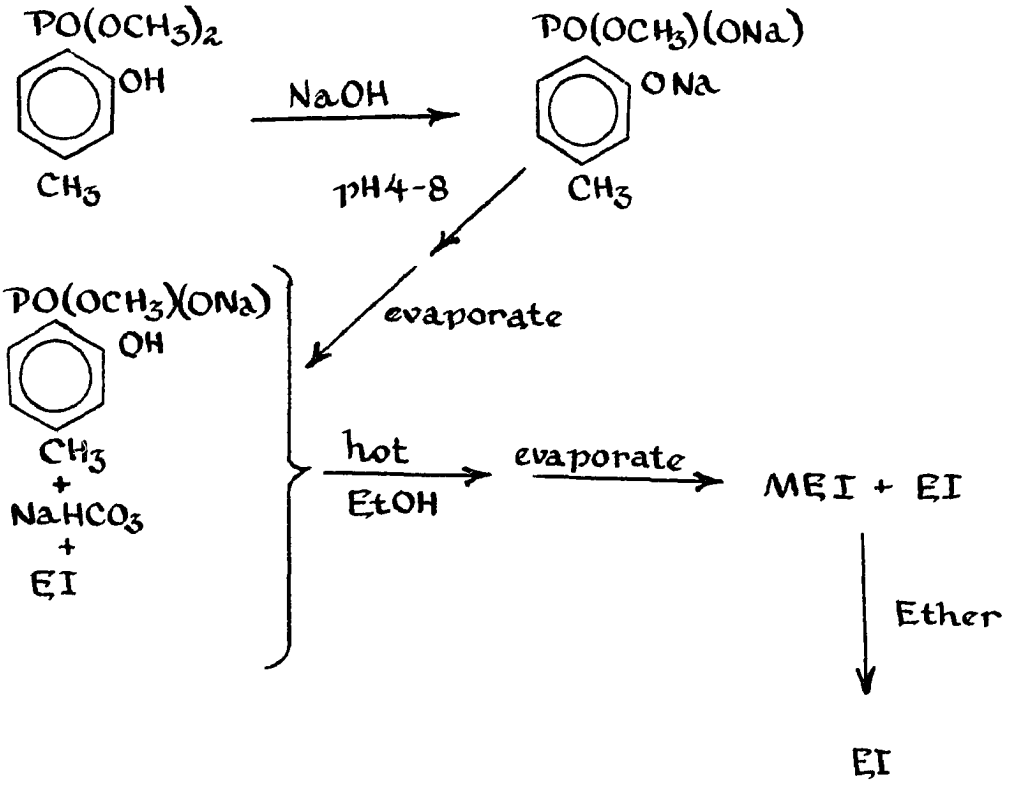
give a quantity of white solid containing the desired product as well as inorganic sodium salts (eg. $NaHCO_3$). The solid was swirled with hot ethanol and filtered. The ethanol filtrate was evaporated to yield an air-filled, slightly gummy solid which was rinsed with anhydrous ether to remove any starting material. The product MEI was next dissolved in methanol, treated with charcoal, filtered, and evaporated. MEI is quite hygroscopic.

The following scheme diagrams the above purification procedure:



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b. By Displacement

Later experiments performed on the phosphonate diesters showed their great susceptibility to nucleophilic displacement on the methyl ester carbon. (See Sections B and C,,Chapter III.) This reactivity toward nucleophiles was used in the preparation of the sodium salts of the phosphonate monomethyl esters. The advantages of this method include:

1. the use of a non-aqueous solvent (acetone) thus preventing hydrate formation, a problem in the synthesis of Monoester II, and
2. elimination of required pH adjustment as noted in the hydrolytic synthesis of Monoester I.

A 0.1 gm sample of EI was dissolved in acetone to which one equivalent of vacuum-dried NaI was added (0.0695 gm). The solution was refluxed for about two hours. The resultant solid was rinsed with ether to remove unreacted EI. The IR spectrum of the material prepared this way was identical to that of the hydrolysis product. The elemental analysis for carbon, however, was more satisfactory; m.p. was 195-201°.

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IR, KBr pellet, spectrum 3a.

NMR in D_2O, TSP as internal standard, spectrum 3b.

Elemental Analysis $C_8H_{10}O_4PNa$

	%C	%H	%P	%Na
Calcd.	42.87	4.49	13.82	10.26
Exptl.(hydrolysis)	41.70	4.67	13.93	10.61
(displacement)	42.94	5.09	13.34	10.13

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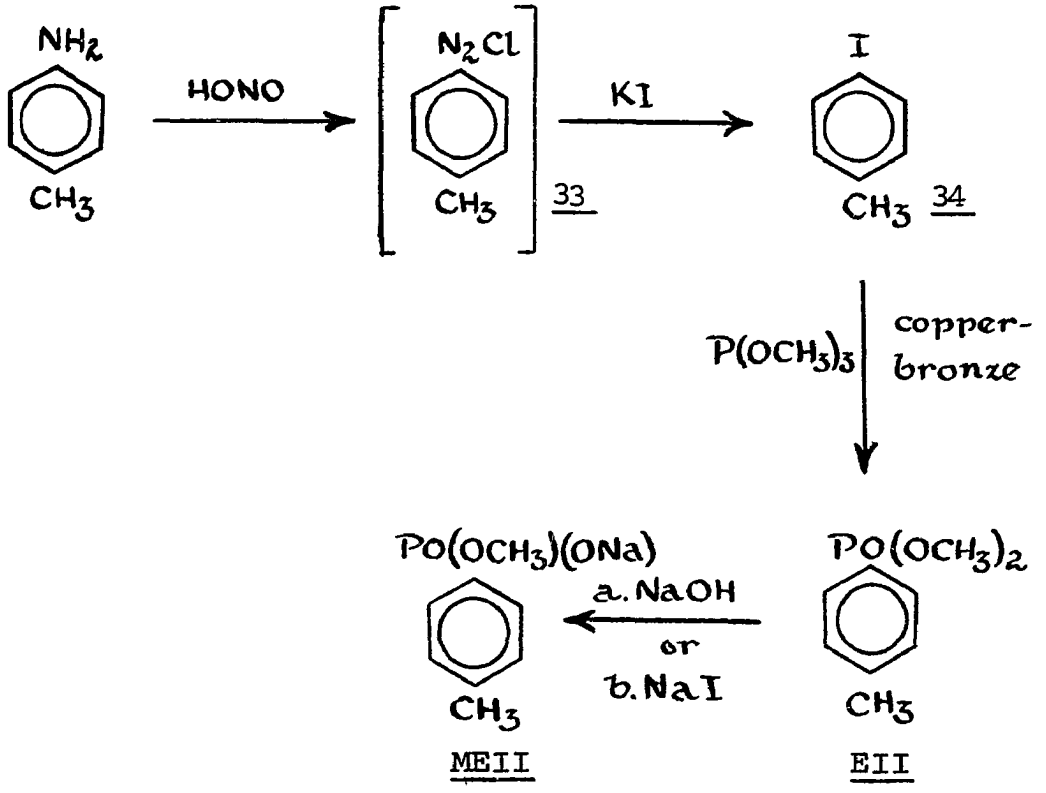
B. Synthesis of Dimethyl 4-Methylphenylphosphonate (Ester II) and of Methyl 4-Methylphenylphosphonate, Monosodium Salt (Monoester II)

The following scheme was used in the synthesis of Ester II and Monoester II:



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1. Iodotoluene (34)

The procedure outlined in Vogel[12] was followed exactly. Technical grade p-toluidine was first recrystallized from ethanol-water. Extreme caution should be observed when placing the solution containing KI and the diazonium salt 33 on the steam bath-- very rapid evolution of nitrogen results. M.p. of p-iodotoluene 34-36°, reported 35°.[12]

2. Dimethyl 4-Methylphenylphosphonate (EII)

A modified Michaelis-Arbuzov reaction outlined by Tavs and Korte[13] was followed. Ammonia, however, was not added following completion of the reaction, for the copper salts and the excess copper-bronze were easily removed by filtration. The crude phosphonate (EII) was fractionally distilled, coming over at the reported temperature and pressure (100°/ 0.1 mm). The NMR of EII in CCl_4 corresponded to that previously described by Obrycki and Griffin.[14] The NMR of twice

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[12] A.I. Vogel, "Practical Organic Chemistry," 3rd Ed., John Wiley and Sons, Inc., New York, 1956, p. 599-600.

[13] P. Tavs and F. Korte, Tetrahedron, 23, 4677 (1967).

[14] R. Obrycki and C.E. Griffin, J. Org. Chem., 33, 632, (1968).

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distilled EII showed no contaminants, nor did thin layer chromatography (ethyl acetate eluent, iodine vapor developer) reveal any spots other than that of EII (R_f = 0.75).

IR, liquid film, spectrum 4a.

NMR in CCl_4, TMS as internal standard, spectrum 4b.

3. Methyl 4-Methylphenylphosphonate, Monosodium Salt (MEII)

a. By Hydrolysis

One gram of Ester II was dissolved in 10 ml methanol to which was added one equivalent of aqueous NaOH. The solution was refluxed for a few hours until hydrolysis of the diester was complete as shown by TLC.[15] Extraction by ether removed any unreacted starting material. The pH was checked and found to be at 7; the solution was filtered and evaporated to dryness. The solid obtained was rinsed with anhydrous ether and allowed to dry. The white chalky powder was obtained in near quantitative yield, m.p. $> 300^{\circ}$. Recrystallization attempts of MEII from many solvents were futile. The IR

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[15]Later NMR kinetic studies showed that alkaline saponification of EII occurred very readily so that less than 30 min. at reflux is probably all that is required.

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of this material showed water contamination despite overnight drying in vacuo at 100°.

b. By Displacement

A 0.1 gm sample of EII was dissolved with one equivalent of NaI (0.075 gm) in specially dried acetone.[16] The flask and condenser were flushed with dry N_2; the system was sealed off by means of a balloon attached to the top of the condenser. The mixture was refluxed for four hours; white solid was formed in the reaction flask. The solid was filtered and dried in vacuo at 100°C. The IR of this material lacked the absorption at 3500 cm^{-1} observed in the substance prepared by the hydrolytic method; m.p. > 300°.

IR, KBr pellet, spectrum 5a.

NMR in D_2O, TSP as internal standard, spectrum 5b.

Elemental Analysis $C_8H_{10}O_3PNa$

	%C	%H	%P	%Na
Calcd.	46.17	4.84	14.88	11.05
Exptl. (displacement)	45.96	4.95	15.09	10.84
Calcd.($\cdot H_2O$)	42.49	5.34	13.70	10.17
Exptl. (hydrolysis)	42.10	4.48	14.18	10.41

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[16] A. Weissberger, "Technique of Organic Chemistry", Vol. VII, "Organic Solvents," Interscience Publishers, Inc., New York, 1955, p. 380 (Timmermans and Gillo method).

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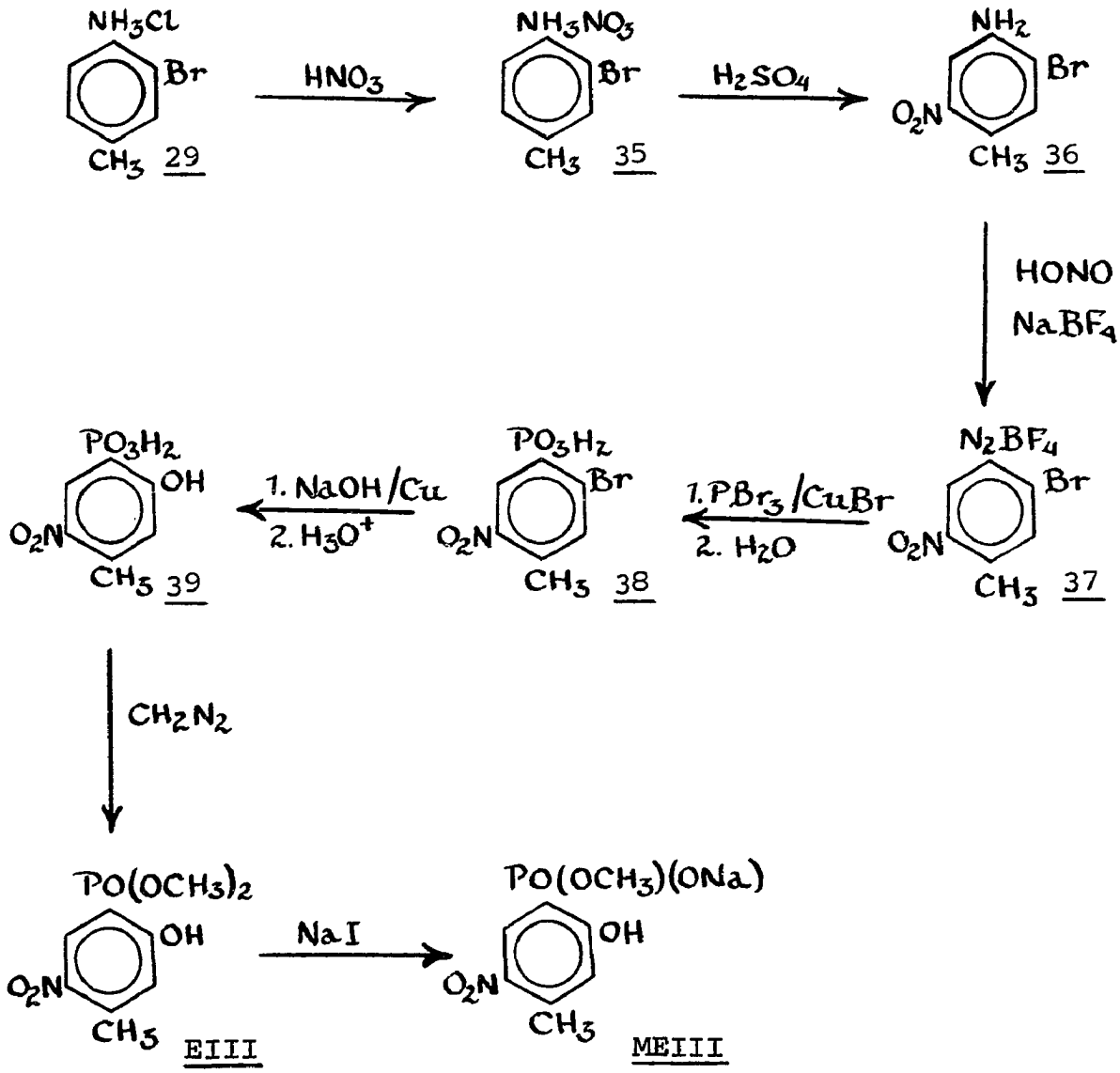
C. Synthesis of Dimethyl 2-Hydroxy-4-Methyl-5-Nitrophenylphosphonate (Ester III) and of Methyl 2-Hydroxy-4-Methyl-5-Nitrophenylphosphonate, Monosodium Salt (Monoester III)

The following scheme, essentially that for the preparation of EI, was used in the synthesis of Ester III and Monoester III:



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1. 2-Nitro-5-Bromo-p-Toluidine (36)

The nitrate salt 35, precursor to the nitro compound 36, was prepared by the method of Cohen and Dakin.[17] The hydrochloride salt 29 was dissolved in hot water and poured into an excess of nitric acid-water solution (1:2). The nitrate salt 35 precipitated immediately as white, flat flakes (m.p. with decomp. 192-197°). The product was filtered and allowed to dry.

In preparing nitro compound 36, the procedure of Morgan and Clayton[18] was followed with some modification. Pouring the crude nitro compound/sulfuric acid solution directly onto crushed ice seemed to work better than pouring it into ice water. With neutralization, the product separated as yellow-brown granules. Recrystallization from ethanol or acetic acid usually resulted in substantial loss of material. Golden-yellow needles, m.p. 119-121° (reported 121°[18]) were obtained in about 40% yield.

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[17]J.B. Cohen and H.D. Dakin, J. Chem. Soc., 81, 1324, (1902).

[18]G.T. Morgan and A. Clayton, J. Chem. Soc., 87, 944, (1905).

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2. 2-Bromo-4-Methyl-5-Nitrobenzenediazonium Fluoborate (37)

The same procedure as that used to prepare diazonium salt 30 was followed. Ten grams of the nitro compound 36 (finely ground) was suspended in 20 ml water with 9 ml conc. HCl and 6 gm $NaBF_4$ and reacted with 3 gm $NaNO_2$. A golden-yellow powder in near quantitative yield, m.p. with decomp. 191-192°, was obtained.

IR, KBr pellet, spectrum 6a.

3. 2-Bromo-4-Methyl-5-Nitrophenylphosphonic Acid (38)

The Doak and Freedman procedure used in the preparation of phosphonic acid 31 was followed. Addition of PBr_3 to the stirred suspension of diazonium salt 37 and cuprous bromide in ethyl acetate caused immediate evolution of gas. Subsequent heating of the reaction mixture produced no further gaseous evolution. Precipitation of the crude phosphonic acid by acidification with HCl (conc.) was facile compared to the usual frustrating oiling out of product 31 observed earlier.

The light yellow powder obtained was filtered and recrystallized from dil. HCl to give pale yellow, lustrous

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flakes (m.p. 213-216°, yield 26%).

Elemental Analysis $C_7H_7O_5NPBr$

	%C	%H	%N	%P	%Br
Calcd.	28.40	2.38	4.73	10.46	27.00
Exptl.	28.26	2.25	4.64	10.26	26.74

IR, KBr pellet, spectrum 7a.

NMR in $NaOD/D_2O$, TSP as internal standard, spectrum 7b.

4. 2-Hydroxy-4-Methyl-5-Nitrophenylphosphonic Acid (39)

The same procedure used to prepare the hydroxyphosphonic acid 32 was followed. The solution containing bromophosphonic acid 38 and 4 N NaOH was allowed to reflux for only two hours with fine copper powder as catalyst. The resultant deeply colored red-brown solution was continuously extracted overnight. Evaporation in vacuo of the ether gave brown crystals which, when recrystallized from dil. HCl, became fine, golden-yellow needles, giving a positive $FeCl_3$ test. Yield was 60%, m.p. 197-198°.

Elemental Analysis $C_7H_8O_6NP$

	%C	%H	%N	%P
Calcd.	36.06	3.46	6.01	13.29
Exptl.	35.98	3.35	5.94	13.22

IR, KBr pellet, spectrum 8a.

NMR in $acetone_{d_6}$, TMS as internal standard, spectrum 8b.

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5. Dimethyl 2-Hydroxy-4-Methyl-5-Nitrophenylphosphonate (EIII)

As in the preparation of Ester I, Ester III was formed from the parent phosphonic acid by slow, dropwise addition of two equivalents of diazomethane. EIII precipitated from its ether solution as the methylation was carried out. Recrystallization of EIII in water gave yellow fibrous needles. The NMR showed EIII to be contaminated with a few percent of the totally methylated compound, EIV, which could not be removed by the recrystallization from water. Yield was 70%; m.p. of twice recrystallized (and slightly EIV contaminated) material, 135-138°.

Elemental Analysis $C_9H_{12}O_6NP$

	%C	%H	%N	%P
Calcd.	41.39	4.63	5.36	11.86
Exptl.	41.50	4.64	5.12	11.80

IR, KBr pellet, spectrum 9a.

NMR in acetone$_{d_6}$, TMS as internal standard, spectrum 9b. In acetone$_{d_6}$, the phenolic proton is not observable; in CCl_4, it appears at 10.7 δ.

Toxicity tests administered with doses of 50 mg/kg of mouse showed no physiological activity for EIII.

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6. Methyl 2-Hydroxy-4-Methyl-5-Nitrophenylphenyl-phosphonate, Monosodium Salt, (MEIII)

A 0.1 gm sample of EIII and one equivalent of NaI (0,057 gm) were dissolved in five ml of acetone. The solution was refluxed for less than thirty minutes. The acetone was removed in vacuo, yielding a hygroscopic, light yellow, glassy powder. The melting point was broad, starting at 120°; this was probably due to the non-crystalline nature of the material.

Elemental Analysis $C_8H_9O_6NPNa$

	%C	%H	%N	%P	%Na
Calcd.	35,70	3.37	5.21	11.51	8.54
Exptl.	35.68	3.29	5.09	11.34	8.36

IR, KBr pellet, spectrum 10a.

NMR in acetone$_{d_6}$, TMS as internal standard, spectrum 10b.

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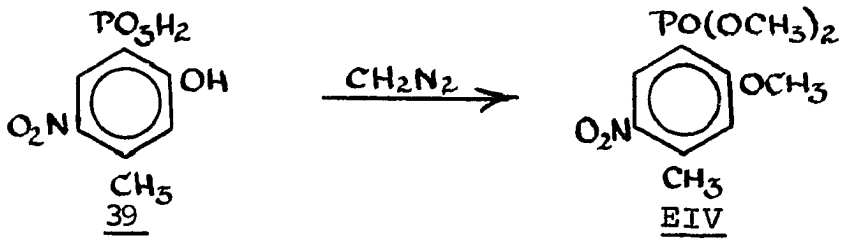
D. Synthesis of Dimethyl 2-Methoxy-4-Methyl-5-Nitrophenyl-phosphonate (Ester IV)

Ester IV was easily prepared from phosphonic acid 39 by this reaction:



39 EIV

As noted above, EIII precipitated upon formation, but further addition of diazomethane redissolved the precipitate to give Ester IV. The ether was evaporated in vacuo yielding creamy colored granules. Recrystallization from water gave very pale yellow, lustrous flakes. Yield was 60%; m.p. 102-104°.

Elemental Analysis $C_{10}H_{14}O_6NP$

	%C	%H	%N	%P
Calcd.	43.64	5.13	5.09	11.25
Exptl.	43.49	5.10	4.90	11.33

IR, KBr pellet, spectrum 11a.

NMR in acetone$_{d_6}$, TMS as internal standard, spectrum 11b.

Toxicity tests were negative at doses of 50 mg/kg of mouse.

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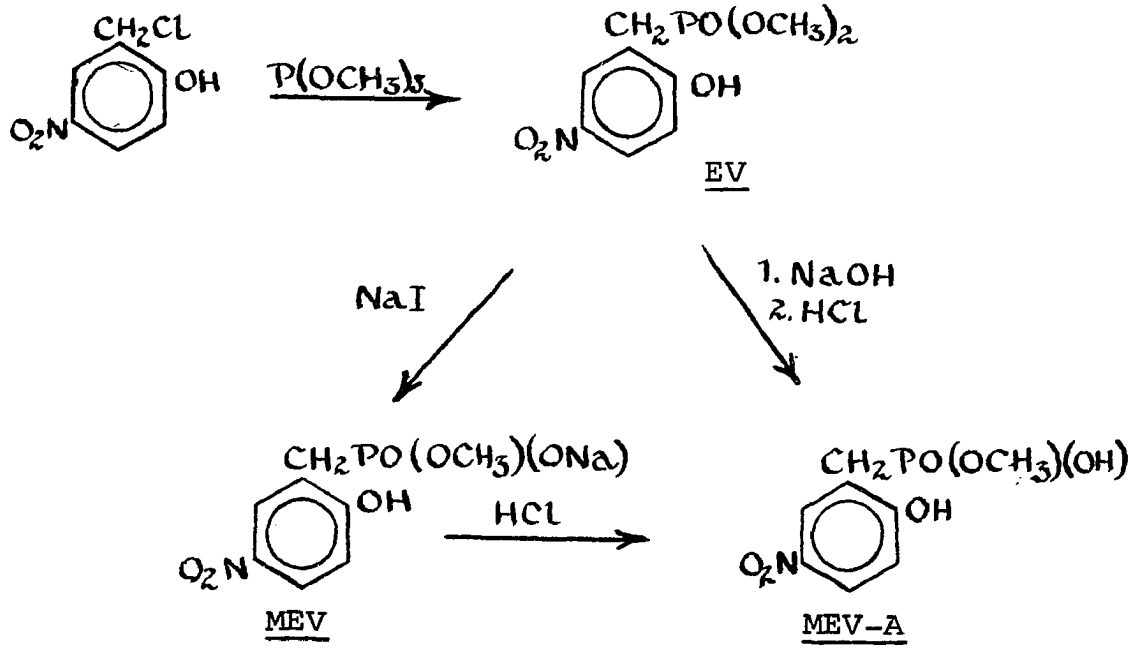
E. Synthesis of Dimethyl 2-Hydroxy-5-Nitrobenzylphosphonate (Ester V) and of Methyl 2-Hydroxy-5-Nitrobenzylphosphonate, Monosodium Salt and Acid (Monoester V)

Ester V and Monoester V were prepared by these reactions:



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1. Dimethyl 2-Hydroxy-5-Nitrobenzylphosphonate (EV)

The method of Arbuzov and Lugovkin[19] for the corresponding ethyl ester was followed. To 3.4 gm 2-hydroxy-5-nitrobenzyl chloride in 20 ml toluene was added 2.8 gm trimethylphosphite. The mixture was refluxed for 15-20 minutes with evolution of gas. Upon cooling, a copious precipitate formed. The material was recrystallized from toluene, yielding pale yellow, fibrous needles in 70% yield, m.p. 145-148°.

Elemental Analysis $C_9H_{12}O_6NP$

	%C	%H	%N	%P
Calcd.	41.39	4.63	5.36	11.86
Exptl.	41.25	4.86	5.24	11.75
	41.21	4.80	5.38	11.70

IR, KBr pellet, spectrum 12a.

NMR in $DMSO_{d_6}$, TMS as internal standard, spectrum 12b.

Toxicity tests were negative at doses of 50 mg/kg of mouse.

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[19] B.P. Lugovkin and B.A. Arbuzov, Dokl. Akad. Nauk SSSR, 59, 1301 (1948); Izvest. Akad. Nauk SSSR Otdel Khem. Nauk, 1950, 56; see Chem. Abst., 42, 7265g (1948); 44, 7256e (1950).

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2. Methyl 2-Hydroxy-5-Nitrobenzylphosphonate, Monosodium Salt and Acid (MEV and MEV-A)

a. By Displacement (MEV)

Because the displacement reactions of iodide ion on the previous phosphonate diesters had worked so well in the preparation of their monoesters, this method was tried first in the preparation of MEV.

One gram of Ester V and one equivalent of NaI (0.575 gm)[20] were dissolved in specially dried acetone.[16] The system was flushed with nitrogen, sealed with a balloon, and refluxed for 3.5 hours. Evaporation of the acetone *in vacuo* yielded a bright yellow, glassy solid. Redissolving this solid in more acetone caused precipitation of a golden-yellow powder.

Despite efforts to exclude water from the system, the NMR spectrum of the above powder showed the material to be approximately 65%monoester-35% phosphonic diacid; attempts to purify this sodium salt failed.

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[20] Both EV and NaI were dried *in vacuo* at 100°C for 12 hours. The glassware was also oven-dried prior to use.

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A few milligrams of the pure (by NMR) monosodium salt were obtained by this method of synthesis (m.p. $> 300^{o}$). The procedure for purification, however, was not reproducible.

Elemental Analysis $C_8H_9O_6NPNa$

	%C	%H	%N	%P	%Na
Calcd.	35.70	3.37	5.21	11.51	8.54
Exptl.	35.34	3.51	5.02	11.38	8.26

b. By Hydrolysis (MEV-A)

In a test tube, 0.3 gm of EV was dissolved in about 4 ml 1 N NaOH, the final pH of the solution being greater than ten. The test tube was then placed in an oil bath at 95^{o} for 15 to 20 minutes. The test tube was removed from the bath and the deep orange solution was neutralized to pH 1 with conc. HCl. Vigorous shaking of the test tube caused white needles to form. The NMR of this material showed no contaminants. Yield was 83%; m.p. 149-153°C.[21]

Elemental Analysis $C_8H_{10}O_6NP$

	%C	%H	%N	%P
Calcd.	38.87	4.07	5.67	12.53
Exptl.	38.74	4.08	5.56	12.48

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[21] A pure (by NMR) sample of MEV-A was also prepared by acidification of MEV by Prof. F.H. Westheimer.

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IR, KBr pellet, spectrum 13 a.

NMR in acetone$_{d_6}$, TMS as internal standard, spectrum 13b.

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F. IR and NMR Spectra

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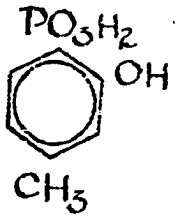
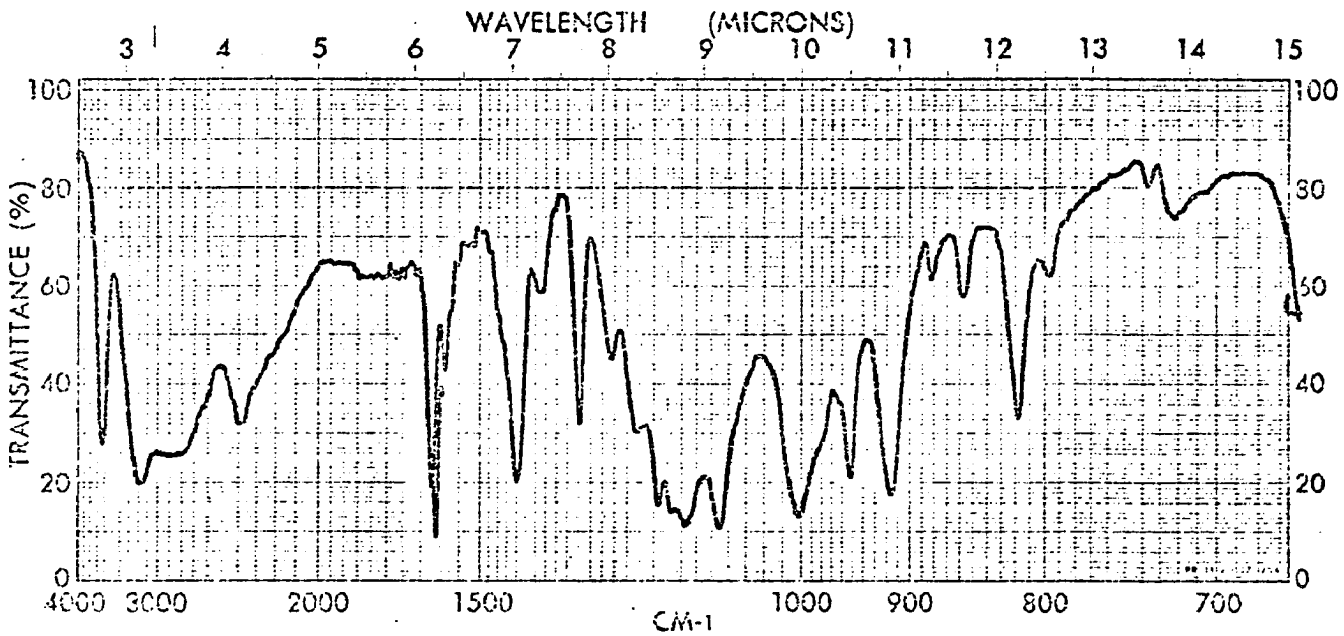
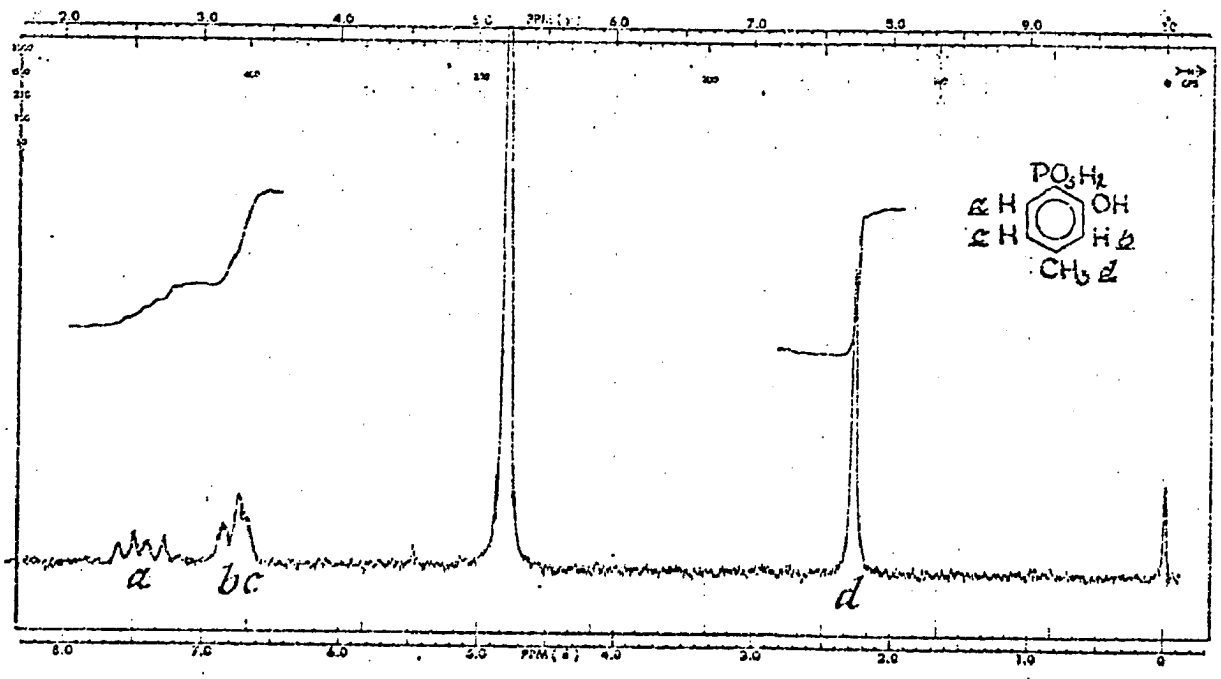
IR and NMR Spectra of 



Spectrum 1a: KBr pellet



Spectrum 1b : NaOD/D_2O with TSP

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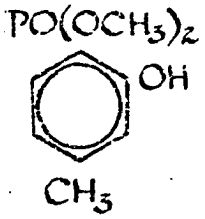
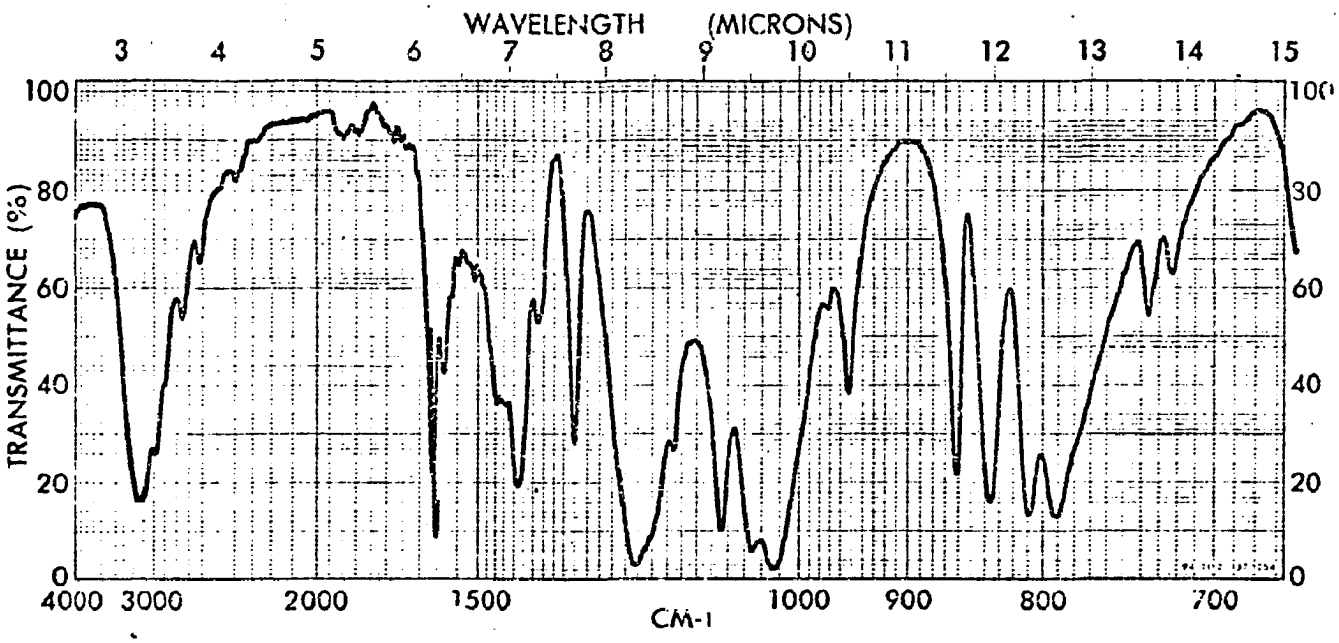
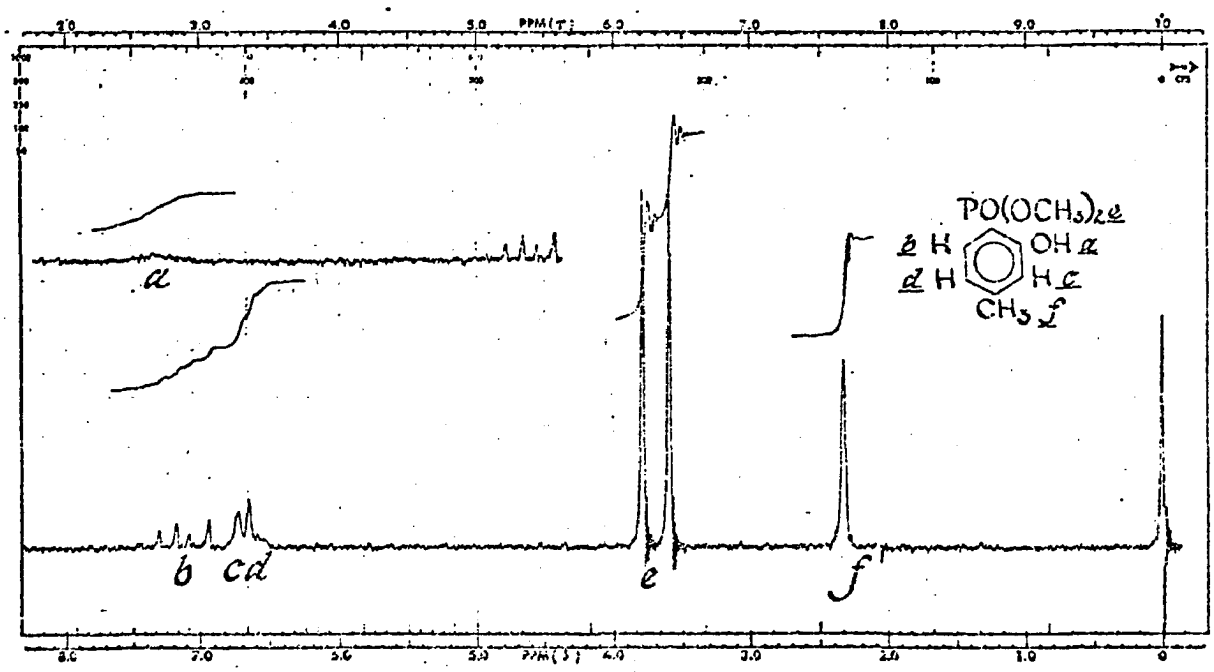
IR and NMR spectra of





Spectrum 2a: KBr pellet



Spectrum 2b: CCl_4 with TMS, offset 175 Hz.

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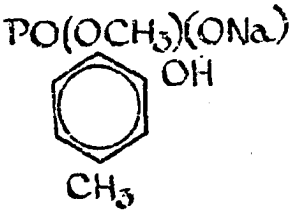
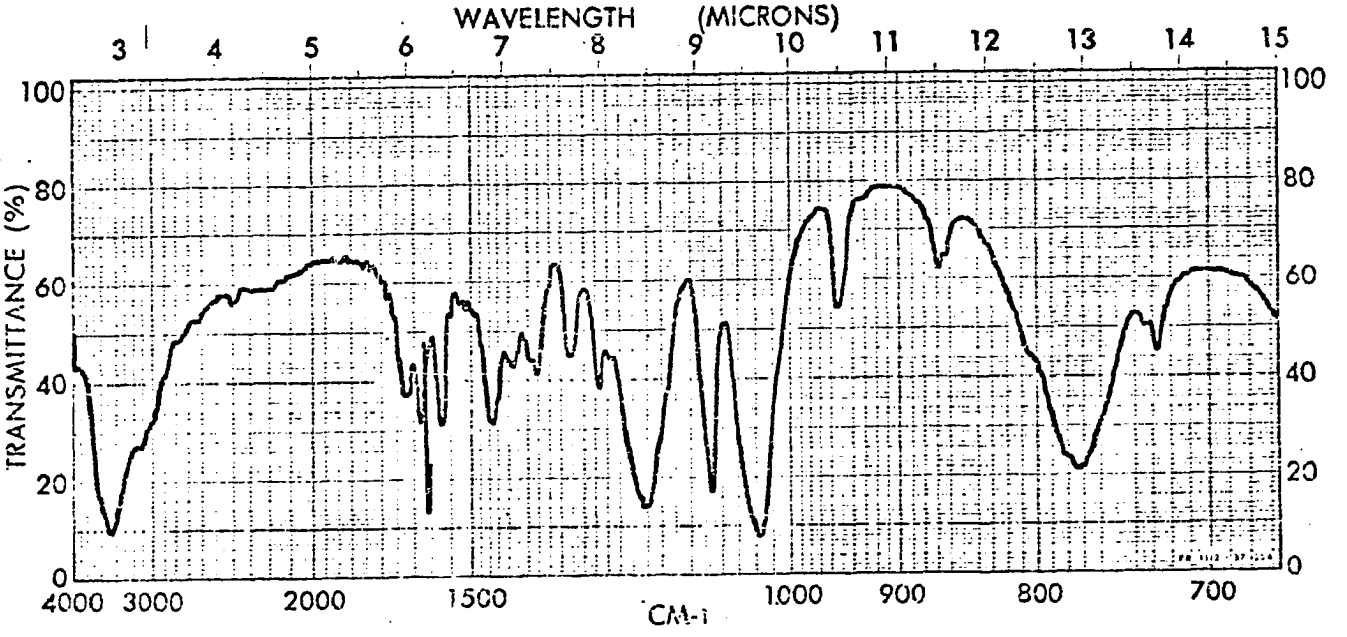
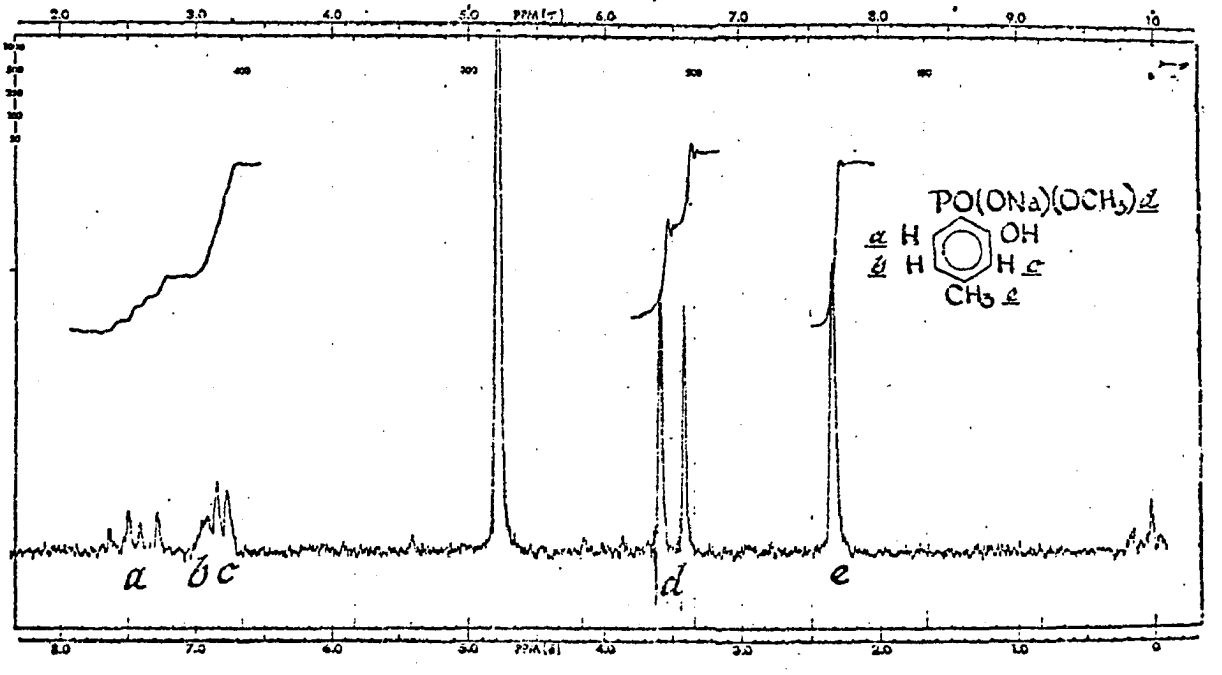
IR and NMR Spectra of





Spectrum 3a: KBr pellet



Spectrum 3b: D_2O with TSP

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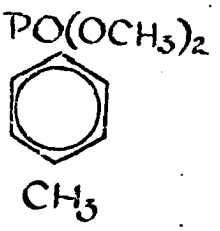
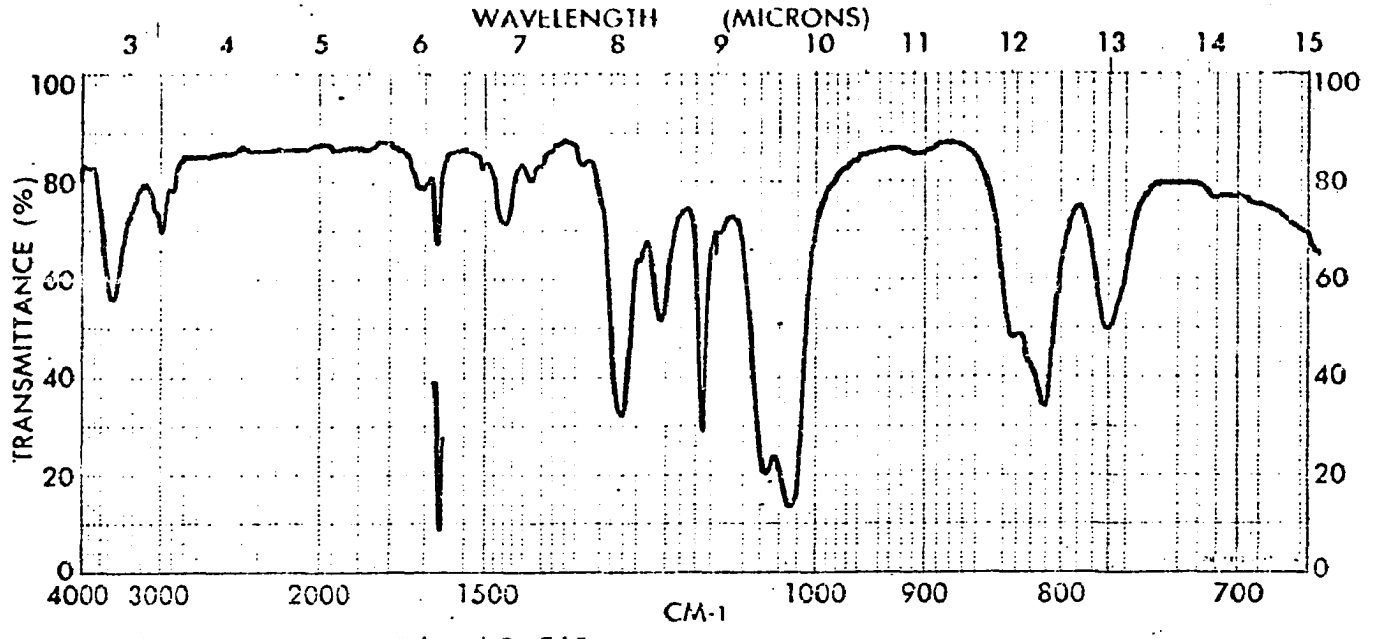
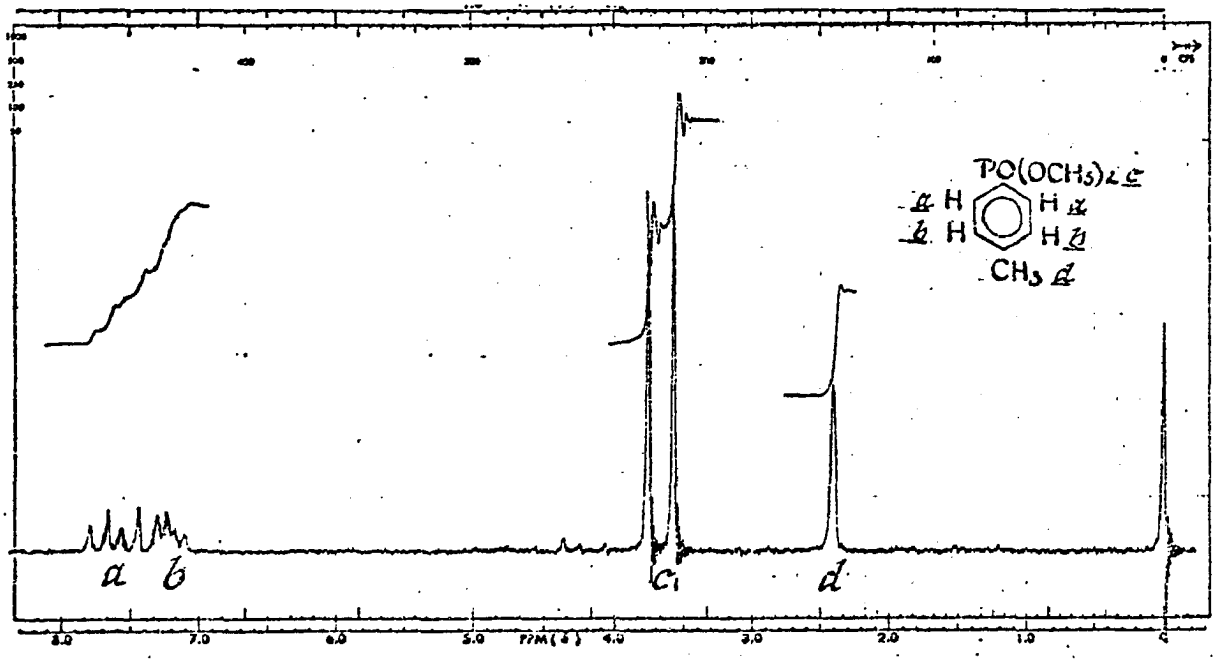
IR and NMR Spectra of 



Spectrum 4a: Liquid film



Spectrum 4b: CCl_4 with TMS

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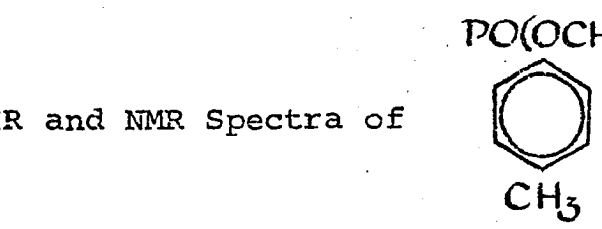
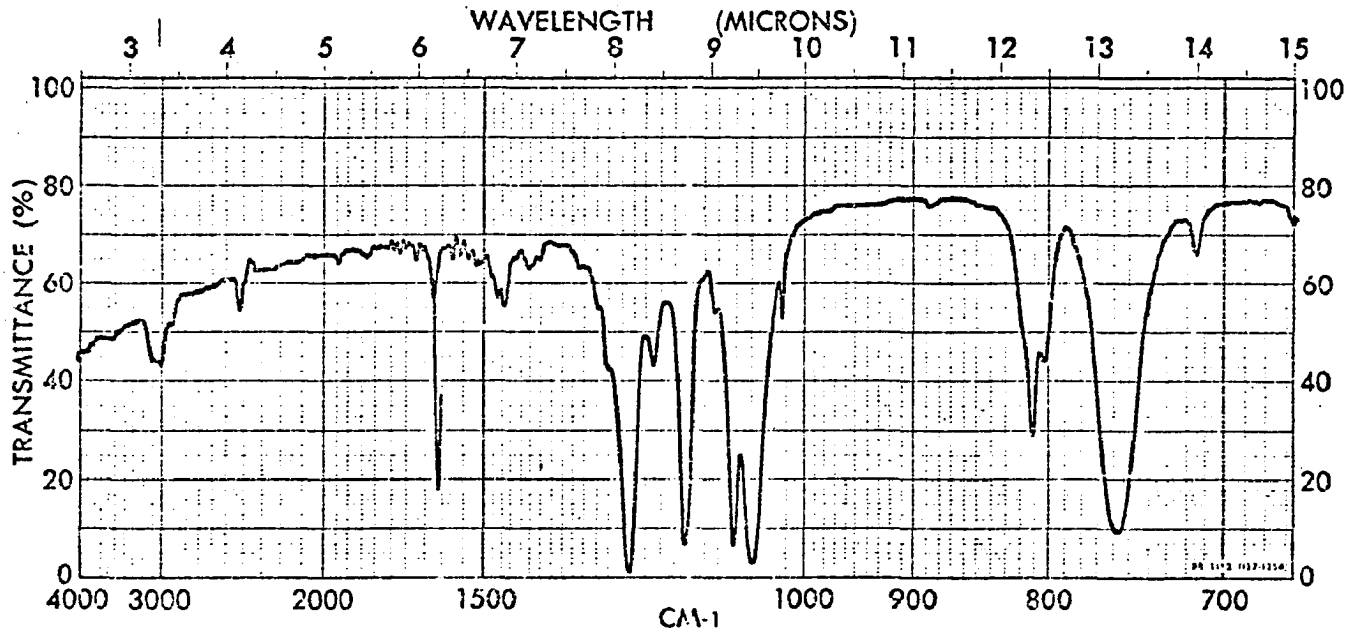
IR and NMR Spectra of $PO(OCH_3)(ONa)$





Spectrum 5a: KBr pellet

Spectrum 5b : D_2O with TSP

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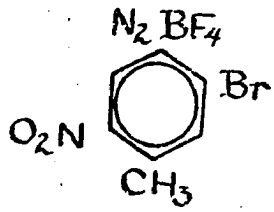
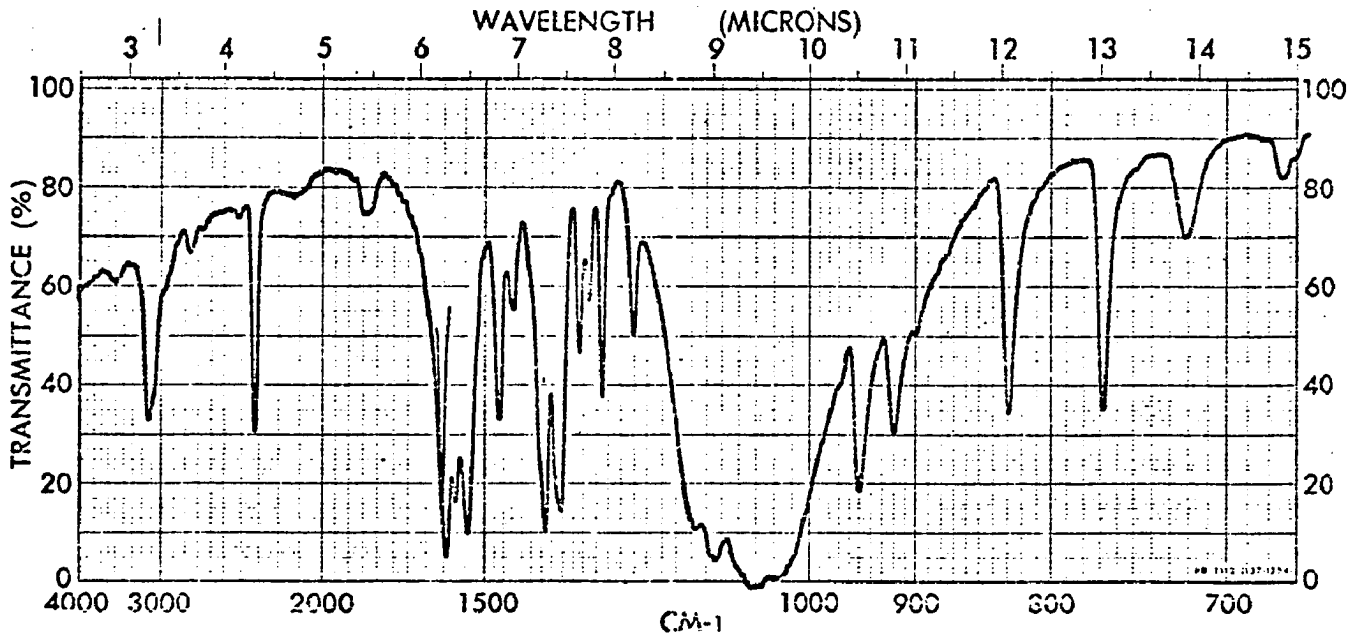
IR Spectrum of 



Spectrum 6a: KBr pellet

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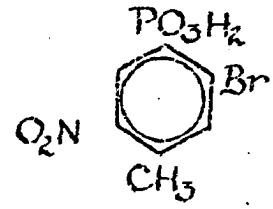
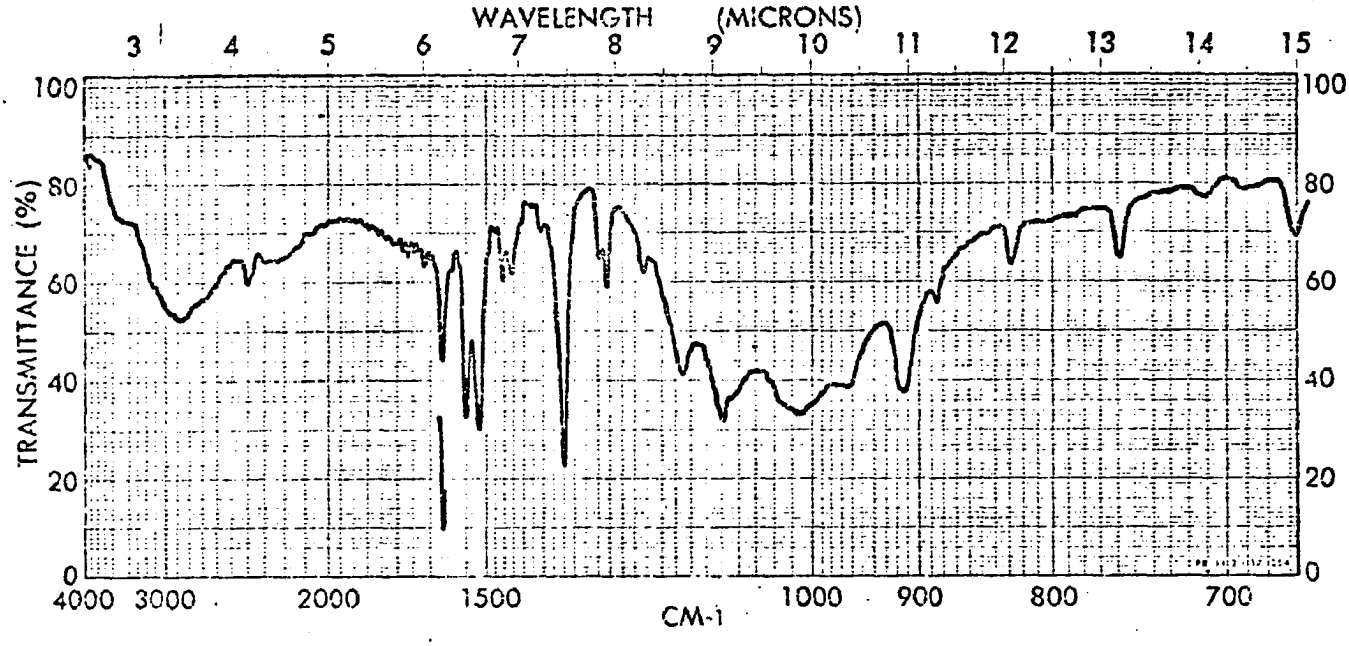
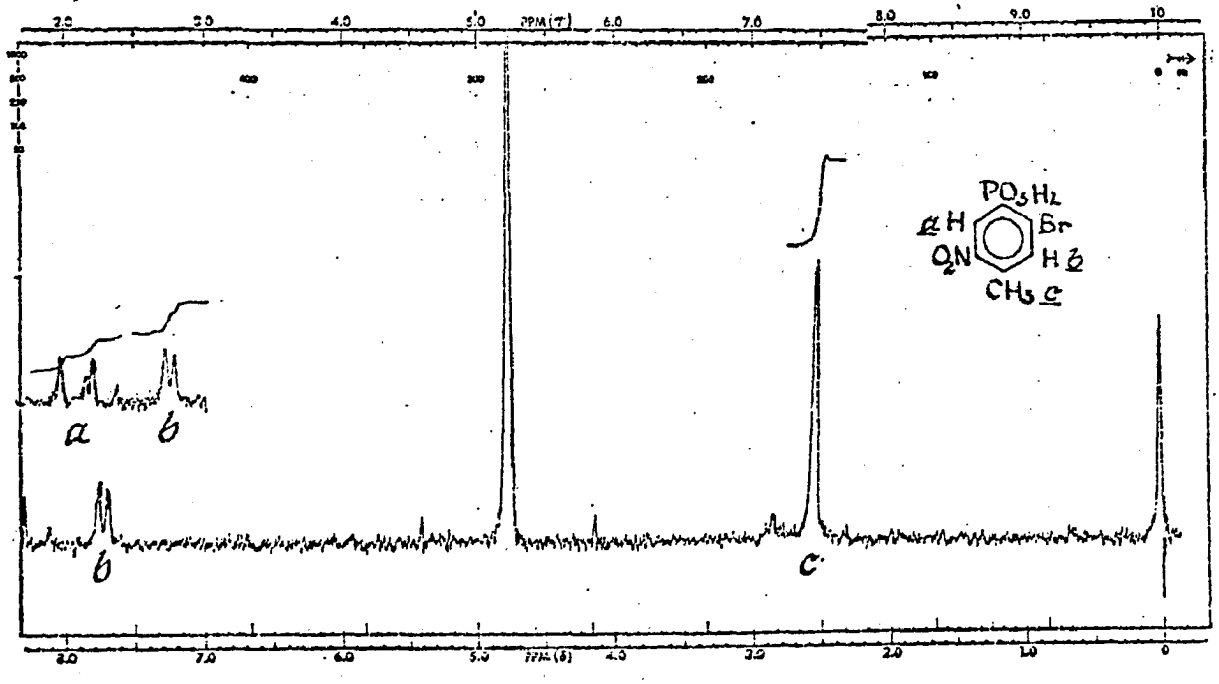
IR and NMR Spectra of





Spectrum 7a: KBr pellet



Spectrum 7b: $NaOD/D_2O$ with TSP

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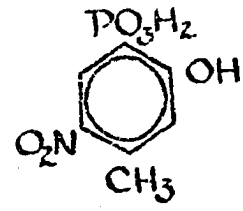
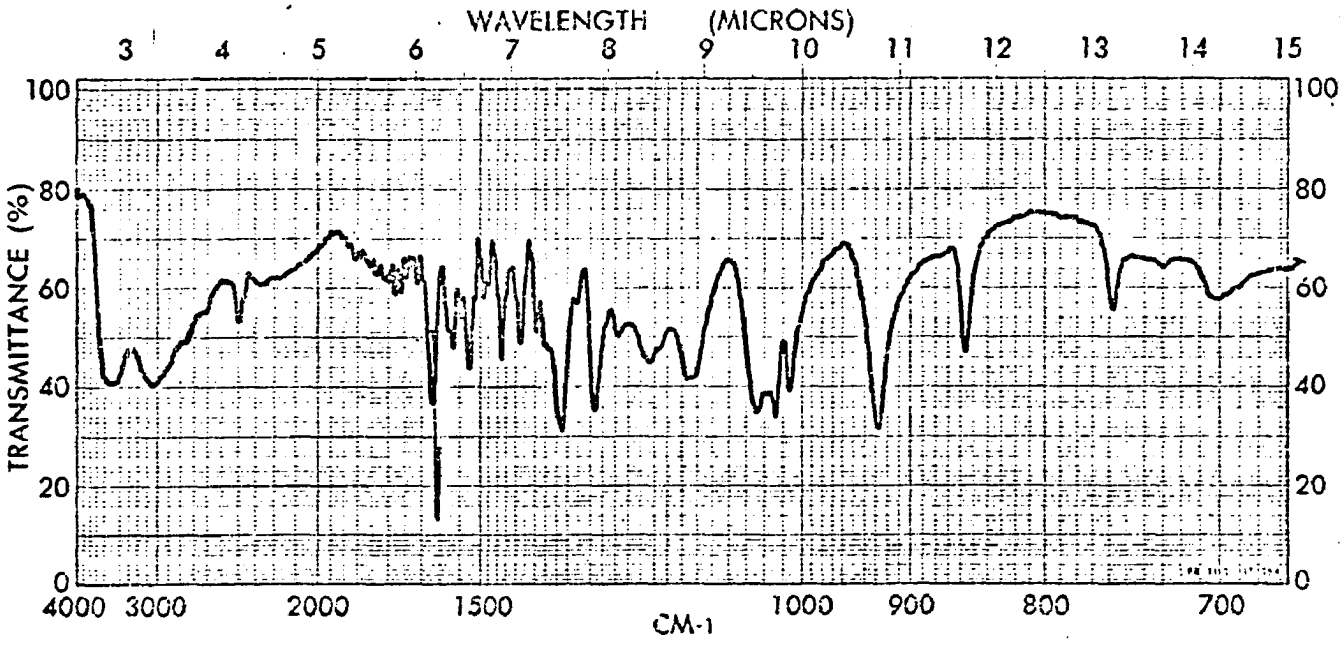
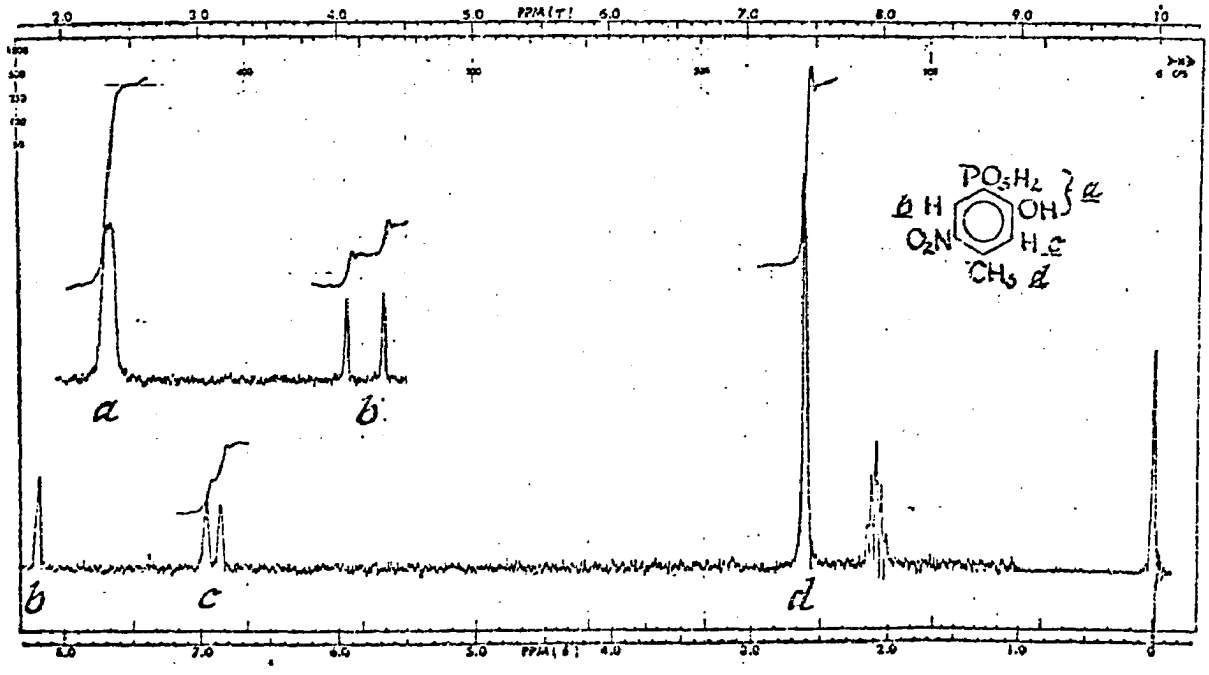
IR and NMR Spectra of 



Spectrum 8a: KBr pellet



Spectrum 8b: Acetone$_{d_6}$ with TMS, offset 175 Hz.

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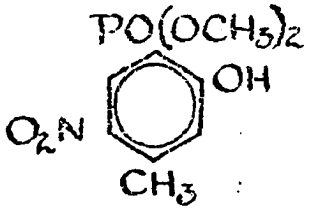
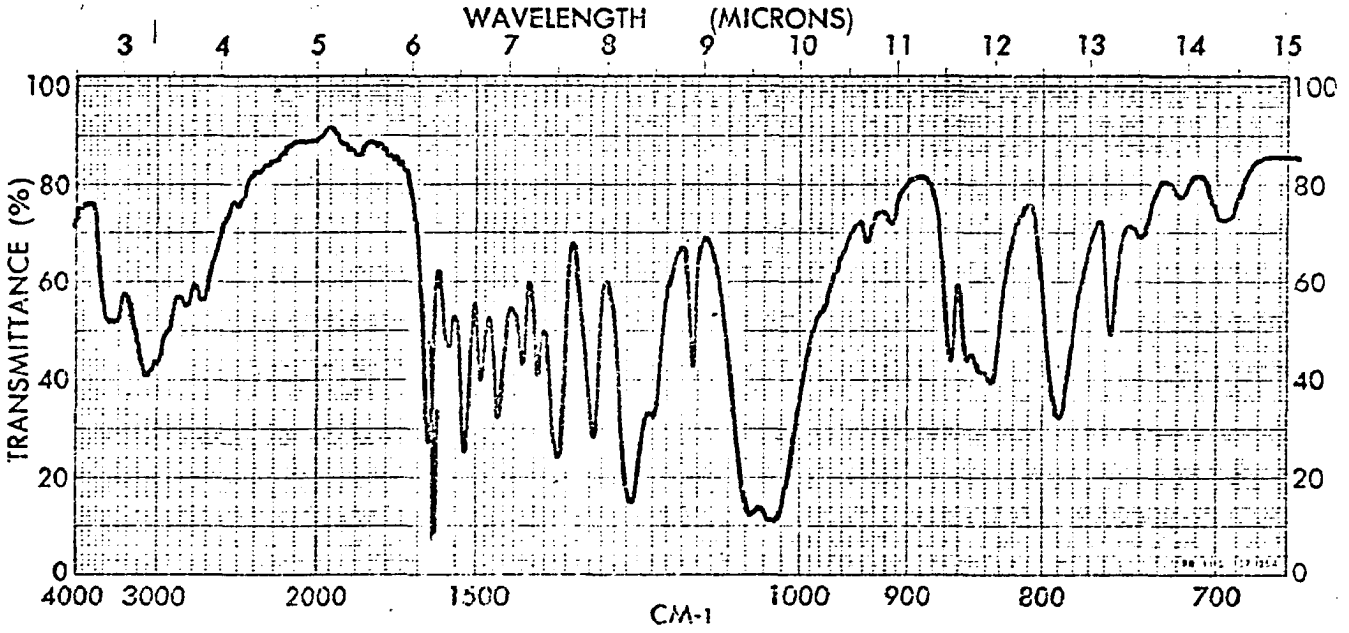
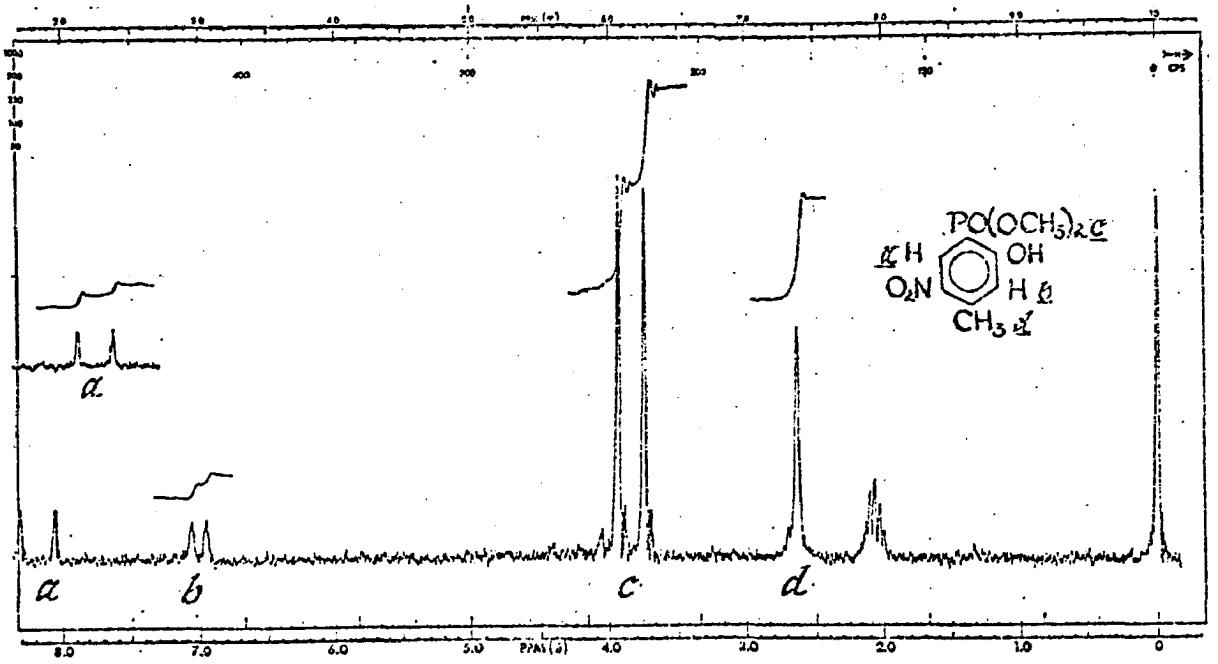
IR and NMR Spectra of 



Spectrum 9a: KBr pellet



Spectrum 9b: Acetone$_{d_6}$ with TMS, offset 25 Hz.

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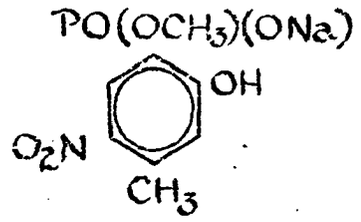
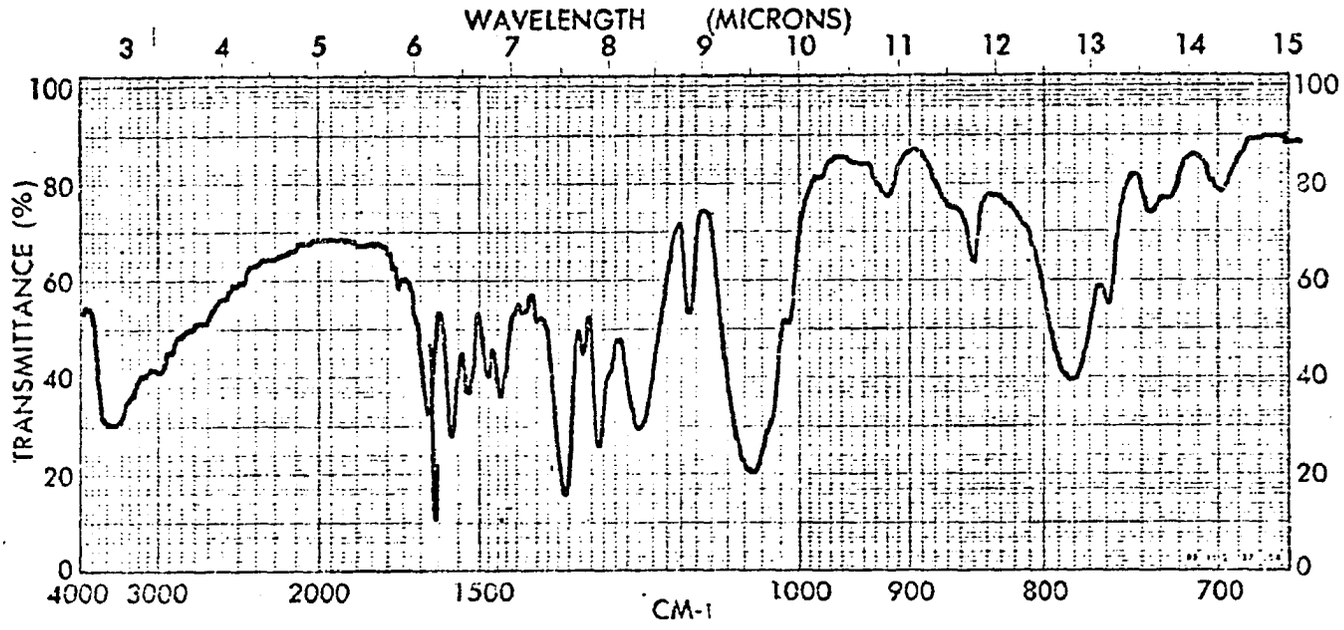
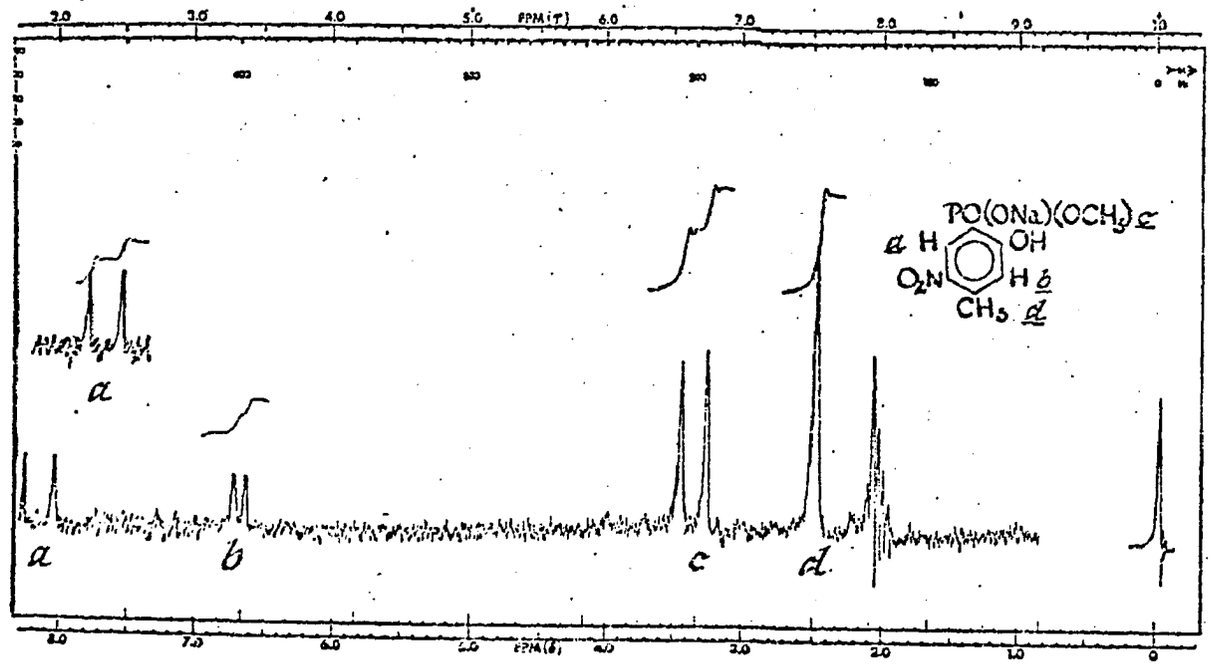
IR and NMR Spectra of 



Spectrum 10a:KBr pellet



Spectrum 10b:Acetone$_{d_6}$ with TMS

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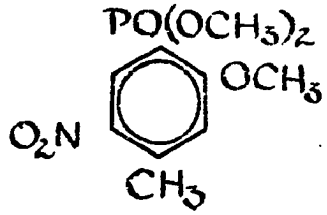
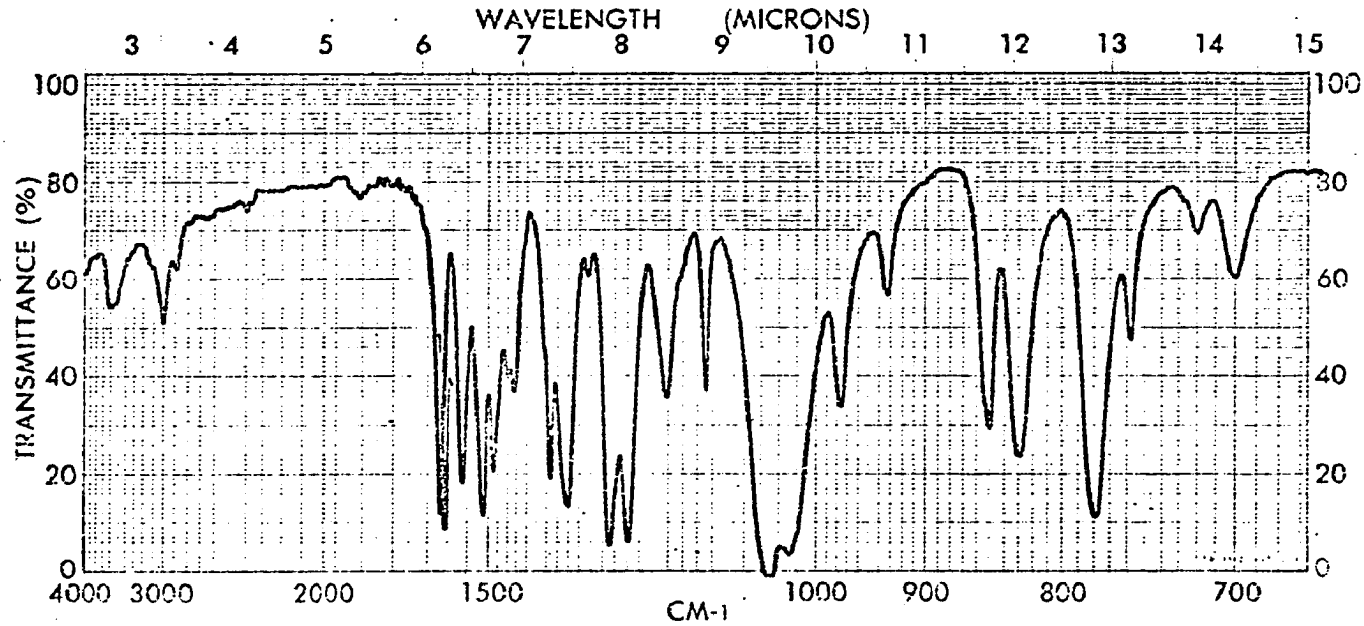
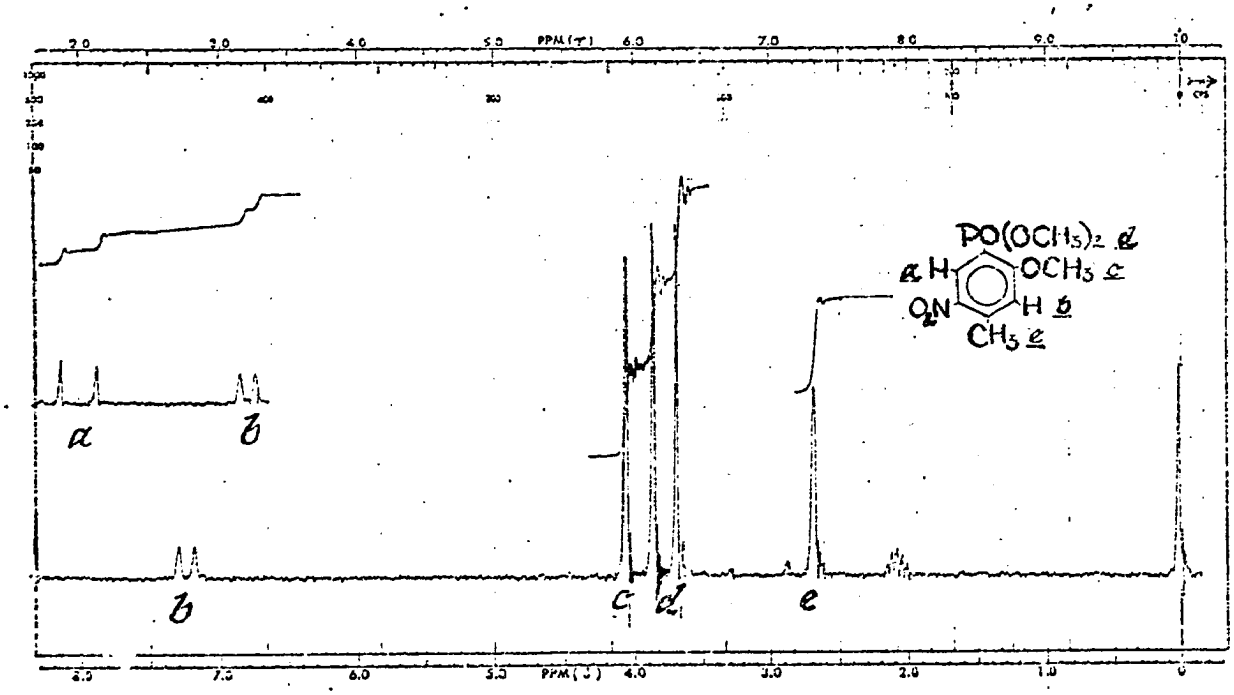
IR and NMR Spectra of 



Spectrum 11a: KBr pellet



Spectrum 11b: Acetone$_{d_6}$ with TMS, offset 30 Hz.

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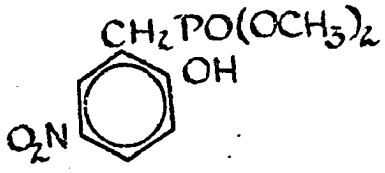
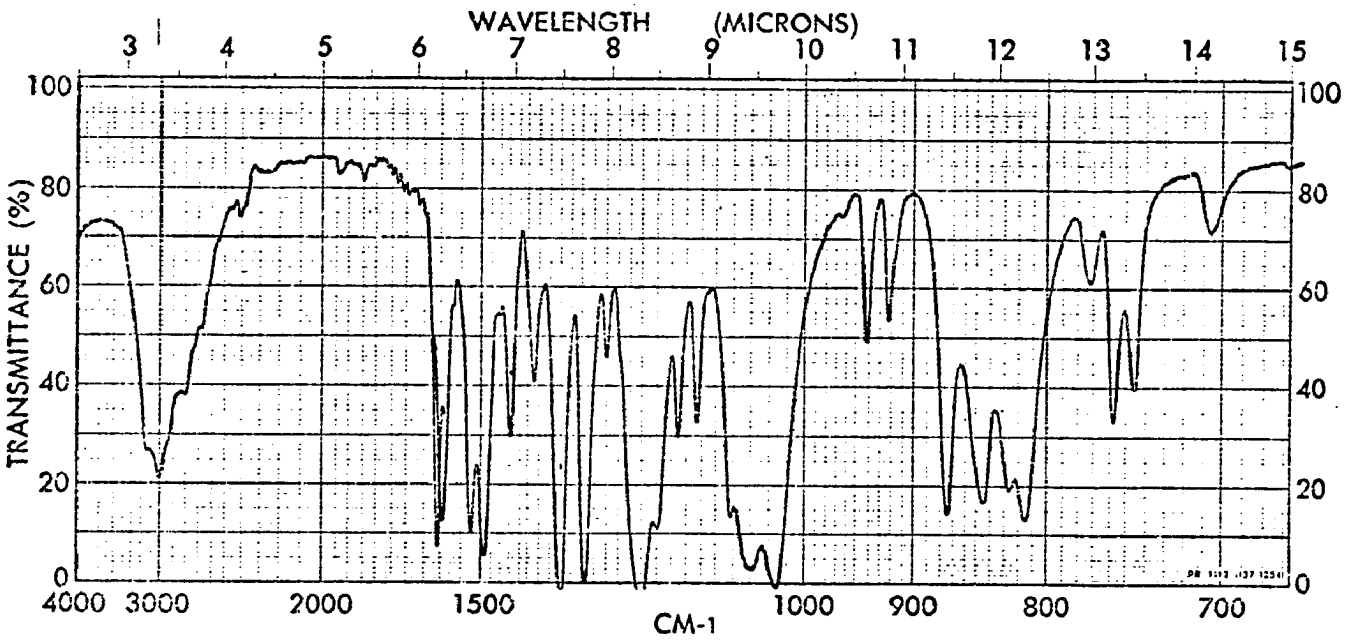
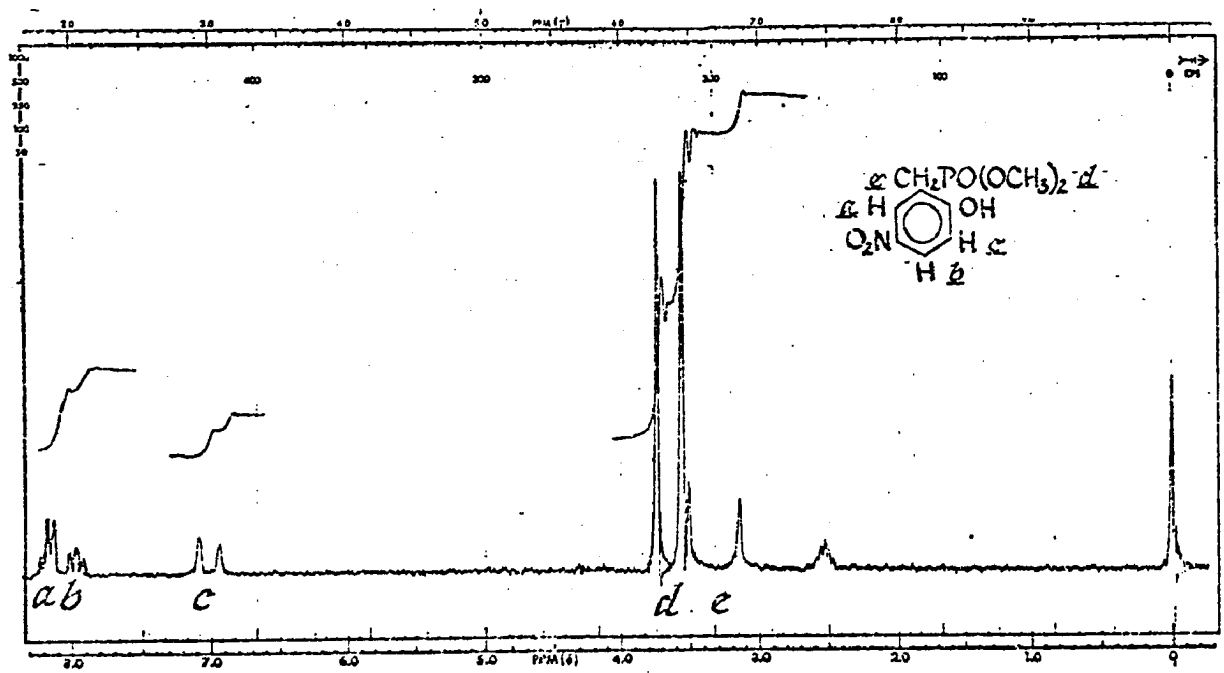
IR and NMR Spectra of 



Spectrum 12a:KBr pellet



Spectrum 12b:DMSO$_{d_6}$ with TMS

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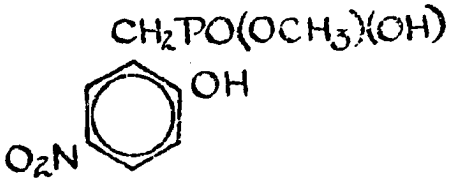
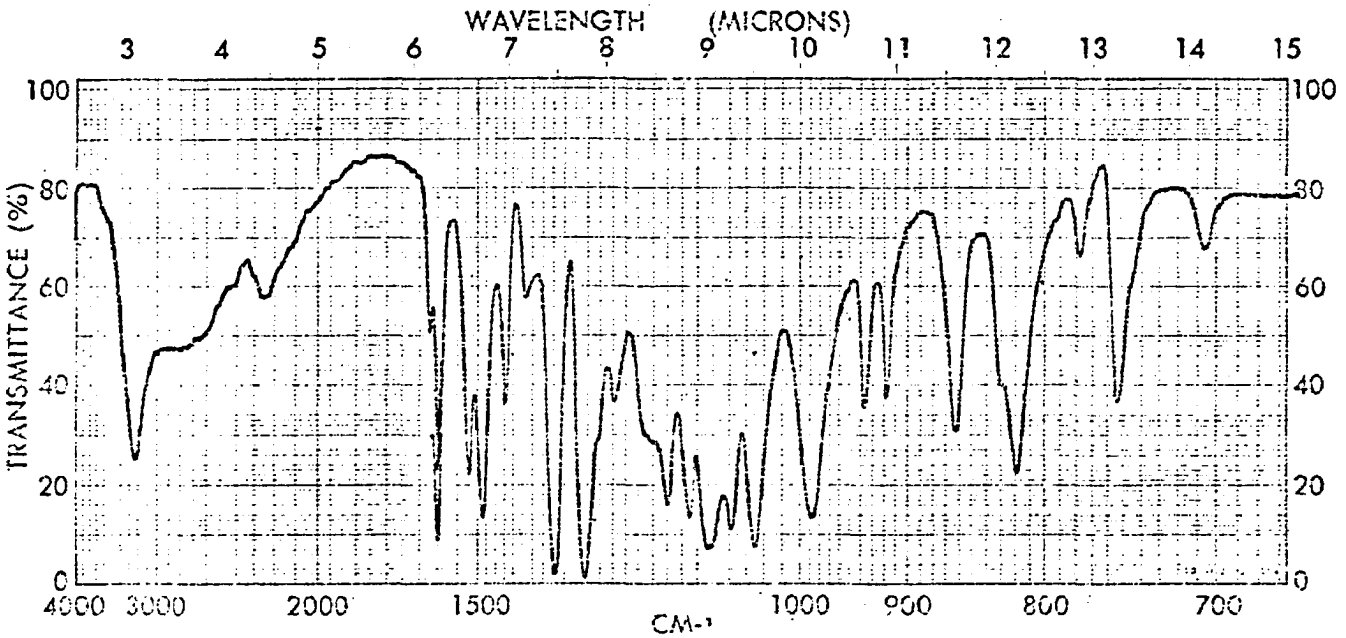
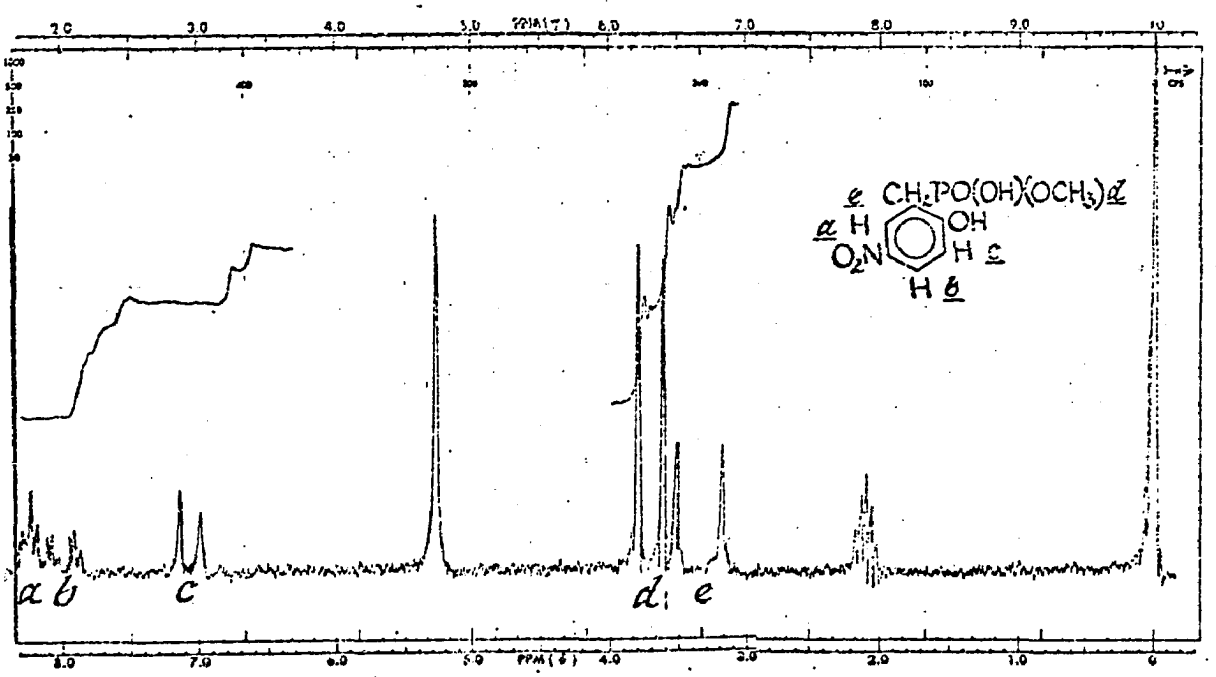
IR and NMR Spectra of 



Spectrum 13a: KBr pellet



Spectrum 13b:Acetone$_{d_6}$ and D_2O with TMS

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CHAPTER III

PHOSPHONATE DIESTERS

This chapter describes the series of experiments undertaken in the search for a molecule that would exhibit effective intramolecular acid-catalysis. The experiments are presented in the order that they were performed. When the first model, Ester I, failed to hydrolyze at a rate significantly faster than that of Ester II, Ester III was synthesized. When Ester III also failed in this respect, Ester V was prepared.

Although Ester I and Ester III never showed effective intramolecular catalysis during hydrolysis, displacement reactions on the methyl carbon of the ester moiety did proceed at rates up to 90 times faster than similar displacements on Ester II under non-aqueous conditions. Ester V, however, showed significant intramolecular catalysis in hydrolysis relative to Ester II. The catalytic factor was about 10^2 at pH 1.

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Experimental

The NMR kinetics described are an adaptation of the Dennis Method.[1] For most studies, a Varian A-60 spectrometer was used. Two scans were usually taken--one of sweep width 500 cps and one of 250 cps, each taken at 250 sec. sweep time. All integrals were scanned at 50 sec. sweep time. When the integrals did not agree, their averaged value was used.

The method used in the uv kinetic studies is described in Section E-1 of this chapter. A Cary 15 spectrophotometer was used in all runs.

For both the NMR and the uv methods, the first-order reactions were usually allowed to proceed through three half-lives (88%).

All of the kinetics described are first-order with the exception of those, in Section C, for the displacement of iodide ion on the dimethyl phosphonate esters. In determining the first-order rate constants from the NMR data, the

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[1]E.A. Dennis, Ph.D. Thesis, Harvard University, 1967.

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percentage of $[\text{Ester}]_{time}/[\text{Ester}]_o$ was plotted vs. time on semi-log paper; for the uv experiments, $|A_\infty - A_t|$ was plotted vs. time. The half-time of the reaction ($t_{\frac{1}{2}}$) was thus easily determined as seen in Figure 2, a typical first-order rate plot obtained from uv kinetics. (Appendix 2 contains a typical first-order plot from NMR kinetics.) The first-order rate constant was calculated by simply using the relationship:[2]

$$k_{obs} = \frac{\ln 2}{t_{\frac{1}{2}}\ (\text{min.})} = \frac{0.693}{t_{\frac{1}{2}}}\ \text{min.}^{-1}$$

For the few second-order reactions performed, the two reactants were in equal concentrations thus allowing for the use of the simplified rate expression: [2]

$$\frac{1}{c} - \frac{1}{c_o} = k_2 t$$

The rate constant, k_2, was obtained by plotting $1/[\text{Ester}]_{time}$ vs. time and calculating the slope of the line obtained.

---

[2]A.A. Frost, and R.G. Pearson, "Kinetics and Mechanism," 2nd Ed., John Wiley and Sons, Inc., New York, 1961, Chap. 2-3.

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The error analyses used in evaluating the precision of the data obtained are described in Appendix 2.

A Precision Scientific Company "Time-It" timer (in minutes) was used in all of the kinetic runs.

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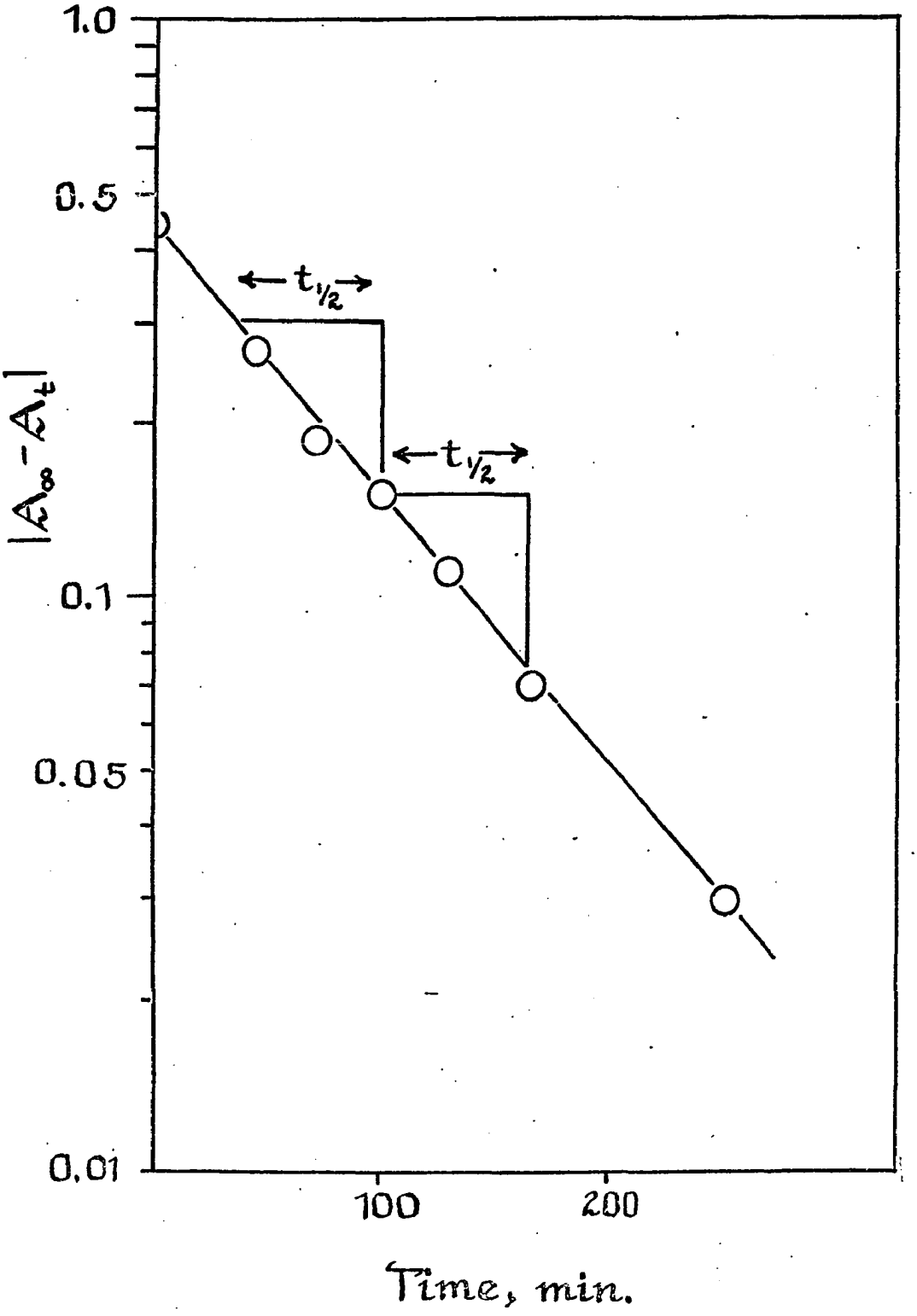

Figure 2

A first-order rate plot obtained by uv kinetics showing a determination of $t_{\frac{1}{2}}$. (From the hydrolysis of EV at pH 6.6, cacodylic acid buffer, total buffer concentration 0.136 M, T = 45°C)

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A. The Acid-Catalyzed Hydrolysis of Ester I and Ester II in 25% $DMSO_{d_6}$ - 75% D_2O

After synthesis of EI and of EII was completed, the possibility of internal acid-catalysis by the hydroxyl group of EI was investigated. $DMSO_{d_6}$ was chosen as a co-solvent because of its high boiling point (189°), its relative inexpensiveness, and its great ability to dissolve the highly water-insoluble phosphonate esters. DCl was used as a catalyst. The stability of both esters under aqueous conditions demanded relatively high temperatures (80°C) for hydrolysis to occur at a conveniently measurable rate.

1. Experimental

a. Preparation of DCl in D_2O

The DCl was prepared by the addition of 4 ml of PCl_3 to 20 ml D_2O cooled in an ice bath. Fractional distillation of the mixture gave a maximum-boiling azeotrope of 20% DCl distilling at 107°C. Upon neutralization with NaOH, a qualitative analysis of the still pot liquid using $BaCl_2$ produced a white precipitate indicative of $Ba_3(PO_4)_2$. The distillate, however, gave a negative phosphate test but a positive chloride test when treated with $AgNO_3$.

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When titrated with Fischer Reagent Grade 0.1 N NaOH solution, the distillate was found to be 6.06 N DCl. Standardized solutions of 4.16 N, 2.07 N, 1.03 N, 0.53 N, 0.43 N, 0.21 N, and 0.104 N DCl were prepared.

b. Preparation of Samples

One ml quantities of 0.2 M EI and EII solutions were prepared by weighing the esters and dissolving them to volume with 50% $DMSO_{d_6}$ - 50% D_2O. EI required some heating to effect solution. Equal amounts of ester solution and DCl in D_2O were transferred to an NMR tube using a Hamilton microliter syringe (0.10 ml). Thus, the final ester concentration was 0.10 M and the normality of the DCl was half that of its original stock solution. The total volume of sample was 0.4 ml. The NMR tubes were then sealed and placed in a thermostated oil bath.

c. Kinetic Runs

The early runs (1.0 N DCl) suffered from poor temperature control (78 $\pm$ 2°) of the oil bath but changing the temperature relay gave a more constant bath of 80.5 $\pm$ 0.5°C. To halt the reaction as well as to clean the outside of the

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NMR tubes by dissolving the adhering oil, the tubes were immersed in a flask of petroleum ether.

The progress of hydrolysis was easily obtained by NMR spectroscopy by following the decline of a doublet corresponding to the $P(O-CH_3)_2$, and the growth of the methanol peak whose identity was checked by addition of an authentic sample. Furthermore, the doublet corresponding to the $P-O-CH_3$ of the monoester was also clearly discernible and slightly upfield of the diester signal. The monoester also hydrolyzed under these conditions. A complication was furnished by the growth of a side product (less than 10%) characterized by a singlet at 2.2 δ, 0.6 ppm upfield of methanol (2.8 δ). Bubbling methyl chloride through a partially hydrolyzed sample of Ester II caused enhancement of this peak. The amount of this side product was dependent upon acid normality, being negligible for runs using only 0.05 N DCl. Methyl chloride formation can be accounted for by the displacement reaction of chloride ion on the methyl carbon of the ester. (See Section D of this chapter.)

The ratio $[Ester] / [Ester]_o$ was obtained by dividing the integrated area of the signal for the remaining diester

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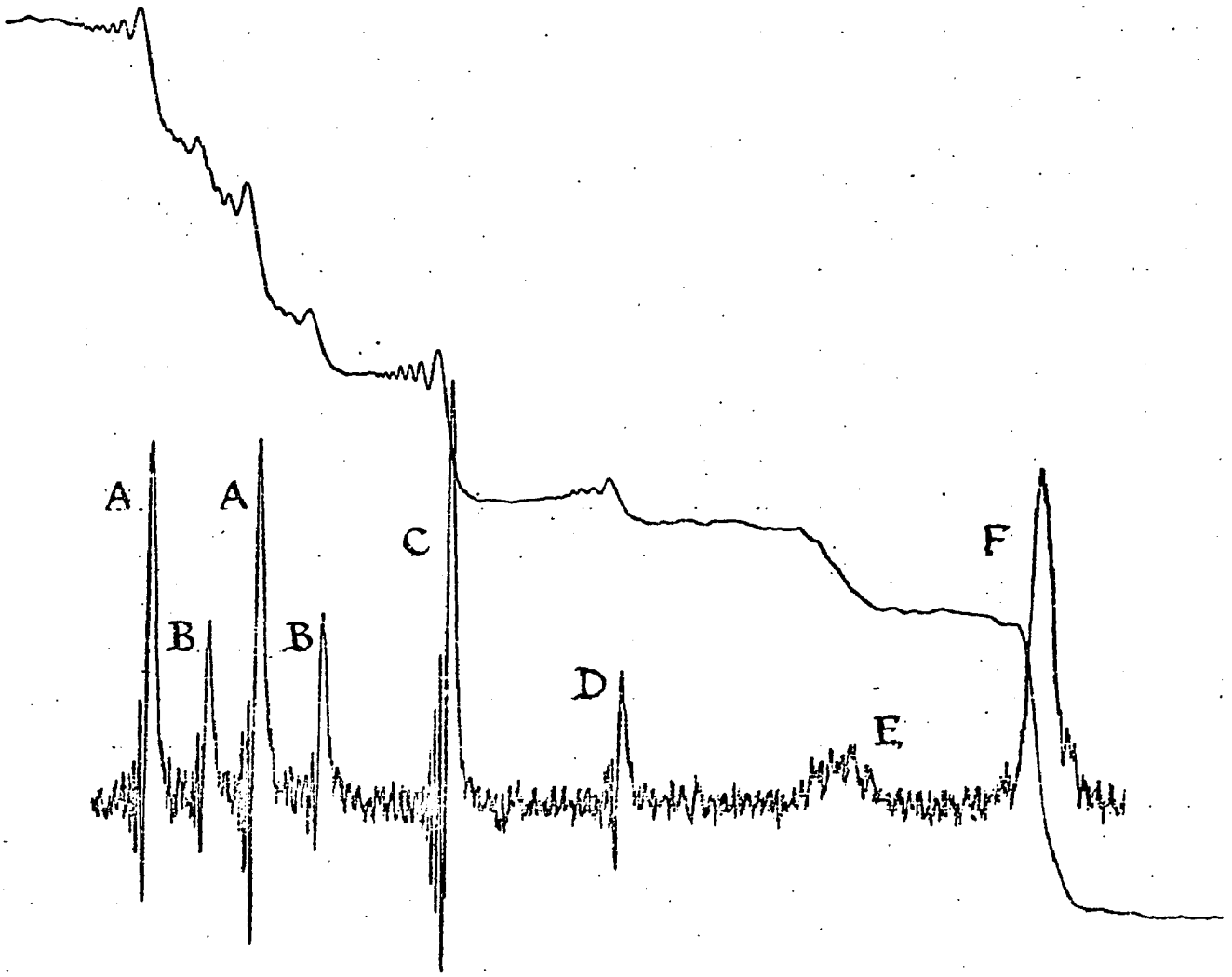

Figure 3

The NMR spectrum of partially hydrolyzed Ester I. The peaks are identified as follows: A - diester OCH_3, B - monoester OCH_3, C - methanol, D - methyl chloride, E - $DMSO_{d_6}$, F - $ArCH_3$.

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by the total integrated area for the signals for diester + monoester + methanol + methyl chloride (see Figure 3). The percentage was then plotted vs. time on semi-log paper; k_1, the first-order rate constant, was determined as described. The error analysis shown in Appendix 2 was performed.

2. Results

The rate constants obtained for Ester I and Ester II under these hydrolytic conditions are summarized in Table I. Readily apparent from these numbers is the apparent lack of the hoped-for internal acid-catalysis by the hydroxyl moiety of Ester I.

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Table I

First-Order Rate Constants for the Acid-Catalyzed Hydrolysis of EI and EII in 25% $DMSO_{d_6}$-75% D_2O ($T = 80.5 \pm 0.5^{\circ}C$)

N DCl	$k_1 \cdot 10^4$ min^{-1} (± 10%)* EI	EII	$\frac{k_{EI}}{k_{EII}}$
1.0**	7.7	5.5	1.5
0.50	3.6	2.8	1.3
0.05	1.1	0.69	1.6

* A correction for the displacement by Cl^- was not applied, but is probably about 10% for the runs in 1.0 N and 0.50 N DCl.

** $T = 78 \pm 2^{\circ}C$

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B. Dimethyl Sulfoxide-d_6 Displacement on Esters I, II, III, and IV

The following experiments were performed with the intention of showing the sought-after acid-catalysis in Ester I and Ester III. Because Ester I and Ester II in 25% $DMSO_{d_6}$-75% D_2O hydrolyze at more or less the same rate, reaction conditions were changed in order to allow the phenolic proton a greater "chance" to perform. Acid and water concentrations were reduced in the hope of uncovering a significant rate difference between the hydrolysis of the phenolic and the non-phenolic esters. Similar experiments have been performed on the acid-base catalyzed mutarotation of α-D-tetramethylglucose in benzene.[3]

Under these conditions, the reaction of EIII did exhibit a significant rate enhancement compared to the reaction of EII. As will be demonstrated, however, this enhancement was for a reaction involving displacement on the methyl ester carbon by $DMSO_{d_6}$ rather than for the hoped-for hydrolysis reaction.

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[3] C.G. Swain and J.F. Brown, J. Amer. Chem. Soc., 74, 2534 (1952).

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1. Experimental

a. Preparation of p-Toluene Sulfonic Acid$_{d_1}$ in D_2O

In order to alleviate the displacement reaction caused by the chloride ion of HCl seen in the previous experiments, a less nucleophilic acid, p-toluene sulfonic acid, (p-TSA), was used. The mono-hydrate of the acid was dissolved in D_2O and evaporated *in vacuo* to dryness. Approximately two grams of this deuterium enriched p-TSA was dissolved in 10 ml D_2O from which standardized solutions (titrated with Fischer Reagent Grade 0.10 N NaOH) of 0.98 N, 0.50 N, 0.26 N, and 0.098 N were prepared.

b. Preparation of Samples

For each phosphonate ester, two ml of 0.2 M solution was prepared by dissolving the weighed ester in DMSO$_{d_6}$ to volume. With a Hamilton microliter syringe (0.10 ml), 0.35 ml of each phosphonate ester was transferred to an NMR tube. To this was added 0.03 ml of a p-TSA solution using a 0.01 ml Hamilton syringe. TMS was added as an internal standard. The tubes were then sealed. This resulted in an ester: water ratio of 1:23, thus allowing first-order

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kinetics to be used. The final concentration of ester was 0.18 M.

c. Kinetic Runs

The silicone oil bath used in these experiments was thermostated to 110 ± 0.5°C. An ASTM Precision limited-range immersion thermometer (95-155°C, 0.2° divisions) was roughly calibrated by immersion in crushed ice and distilled water to determine the zero point, and in boiling distilled water.[4] It was found to read about 0.2° too high at the upper range. A stem correction was also applied.[4]

To see if the heating time of an NMR sample was significant, a small thermistor was slipped into an NMR tube containing 0.4 ml DMSO and immersed in the oil bath. The heating time was approximately 15 sec., an insignificant time for these reactions, as was the cooling time when the tube was immersed in a beaker of petroleum ether.

Again, the extent of reaction was determined by measuring the ratio of the integrated signal for the

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[4]F. Daniels, J.W. Williams, P. Bender, R.A. Alberty, and C.D. Cornwell, "Experimental Physical Chemistry," McGraw-Hill, New York, 1962, p. 430.

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diester doublet to the total integrated area of the signals for diester + monoester + methanol + side products (see below).

2. Results and Discussion

As determined by adding a drop of authentic sample, methanol (3.2 δ) was the major product (80% or more) of all the reactions. However, another product, (3.3 δ, singlet), which grew at a rate proportional to that of methanol, was also observed. The ultimate identity of this side product showed that the reaction of these phosphonate esters in $DMSO_{d_6}$ was one of displacement by $DMSO_{d_6}$ rather than hydrolysis. This is described below.

a. Determination of the Identity of the Side Product

Investigation of the literature revealed a small body of work examining the role of DMSO as a nucleophile on sulfonate esters and organic halides.[5]

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[5] a. S. Winstein and S.G. Smith, Tet., 3, 317 (1958). b. R.T. Major and H.J. Hess, J. Org. Chem., 23, 1563 (1958). c. R. Kuhn and H. Trischmann, Ann. Chem., 611, 117 (1958). d. H. Meerwein, V. Hederich, and K. Wunderlich, Arch. Pharm., 291, 541 (1958). e. N.J. Leonard and C.R. Johnson, J. Amer. Chem. Soc., 84, 3701 (1962).

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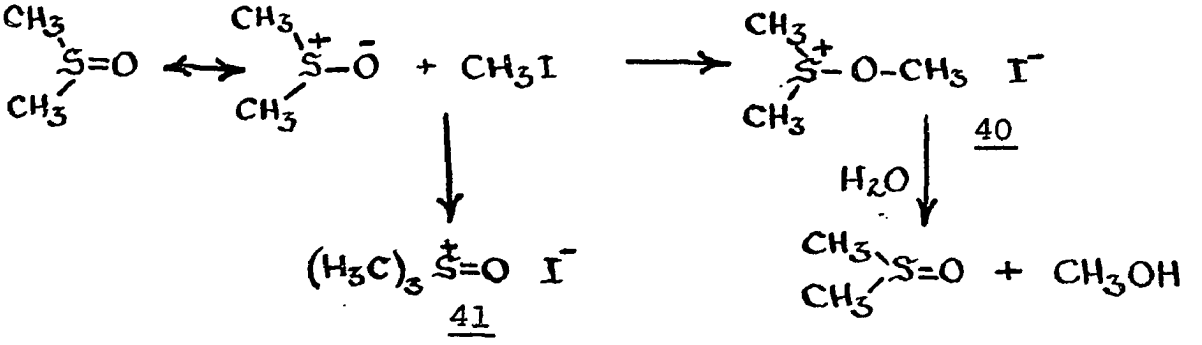
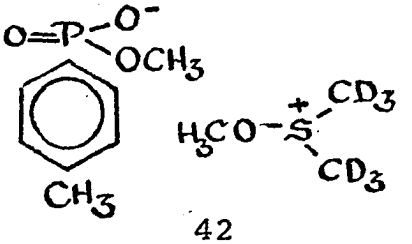
In such displacements (shown here for CH_3I), two products may be formed:



Product 40, dimethyl-methoxysulfonium iodide, is the kinetically controlled product whereas product 41, trimethylsulfoxonium iodide is thermodynamically controlled.[5a] Furthermore, product 40 is rapidly hydrolyzed by water to give DMSO and methanol.[5a] Product 41 is actually commercially available and is used in methylations.[6]

For the phosphonate esters under study, it was conceivable that similar displacements by $DMSO_{d_6}$ could occur to produce the dimethyl sulfonium salt 42 (shown here for



42

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[6] L.F. Feiser and M. Feiser, "Reagents for Organic Synthesis," Vol. I, Wiley, New York, 1967, p. 1236.

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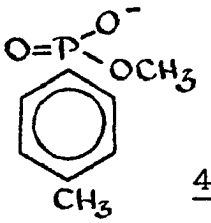
EII) which would be rapidly hydrolyzed to give the observed methanol, the chief product of these reactions.

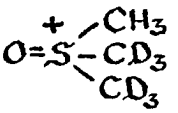
Because DMSO is known to be an oxidizing agent,[7] some thought suggested that the unidentified peak at 3.3 δ might be a methyl derivative of formic acid or formaldehyde. When, if fact, methylal $\left[(CH_3O)_2CH_2\right]$ was added, this peak was enhanced. Thus, formaldehyde was apparently formed during the ester reactions in DMSO.

Because identification of the side-product was essential to elucidating the mechanism of the reaction of these phosphonate esters in DMSO, other possibilities were also investigated.

Production of the water-stable salt 43 (the thermodynamically stable isomer of 42) might account for the side product.



43



To test this hypothesis, trimethyl sulfoxonium iodide, 41, was prepared by refluxing DMSO with CH_3I using

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[7] Ibid., p. 303.

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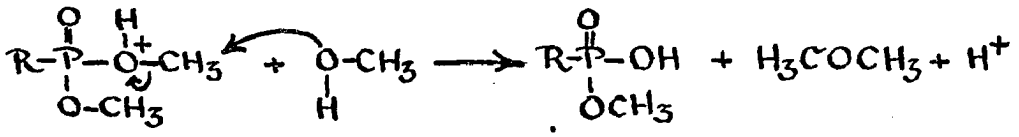
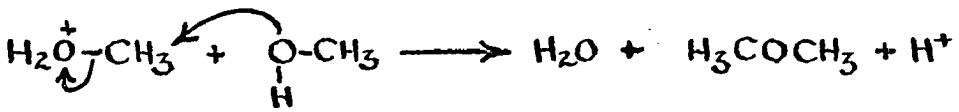
the method of Kuhn.[5c] When added to methanol in $DMSO_{d_6}$ with D_2O, compound 41 had a chemical shift of 3.9 δ, too far downfield to be the unknown side product.

The chemical shift and simplicity of the side product's NMR spectrum suggested that it could be dimethyl ether, possibly formed by either of two mechanisms:

1) 

2) 

Addition of a drop of dimethyl ether to a spent kinetic run created a new peak at 3.25 δ, between the signals from methanol and from the side product.

b. Mechanism of the Reaction of DMSO and the Phosphonate Esters

To account for the formaldehyde formation and, thus, methylal formation, the most likely mechanism requires preliminary production of a dimethyl-methoxysulfonium

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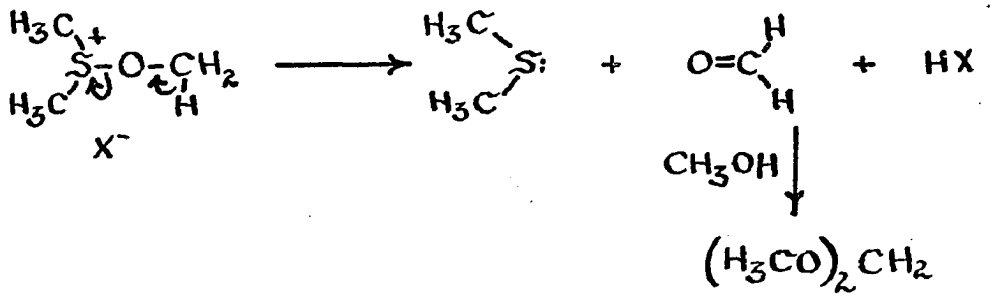
salt:[8]



To test this mechanism for formaldehyde formation from phosphonate esters, a sample of dimethyl-methoxy-sulfonium fluoborate was prepared. Water-free $AgBF_4$, synthesized by the method of Meerwein,[5d] was stirred with DMSO in anhydrous dichloroethane to which an excess of CH_3I had been added.[5d,e] An NMR sample was prepared by dissolving the recrystallized salt in $DMSO_{d_6}$, with TMS as an internal standard. The dimethyl sulfonium salt gave a spectrum corresponding to a singlet, area 1, 4.0 δ and a singlet, area 2, 3.3 δ. A few drops of D_2O were added, the tube was sealed and heated for about three min- at 110°C. The starting material had decomposed completely and a new set of NMR peaks emerged. Stepwise addition of authentic samples of the following compounds showed

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[8] Ibid., Vol. II, 1969, p. 161.

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the ultimate decomposition products of dimethyl-methoxy-sulfonium fluoborate:

1. Methanol 3.2 δ (major product)
2. $DMSO_{h_6}$ 2.6 δ (major product)
3. Methylal 3.3 δ (minor product)
4. Trimethyl sulfoxonium salt 3.9 δ (the iodide salt was added; minor product)

Dimethyl sulfide was not available at the time, but a minor peak at 2.0 δ probably corresponded to this material. In a later experiment, Ester II was reacted with $DMSO_{d_6}$-$DMSO_{h_6}$[9] -- a significant peak at 2.0 δ was produced. Addition of an authentic sample of dimethyl sulfide caused its enhancement.[10]

When the phosphonate esters were heated in sealed tubes with nearly water-free $DMSO_{d_6}$, displacement occurred at comparable rates to those observed with water present.

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[9] $DMSO_{h_6}$ was added to enhance the dimethyl sulfide peak, which under the usual conditions, is deuterated, and thus difficult to see.

[10] Further proof of the identity of dimethyl sulfide exists in analysis by odor. Opening of the sealed NMR tubes of spent reactions produced a strong stench; opening a bottle of dimethyl sulfide produced the same result.

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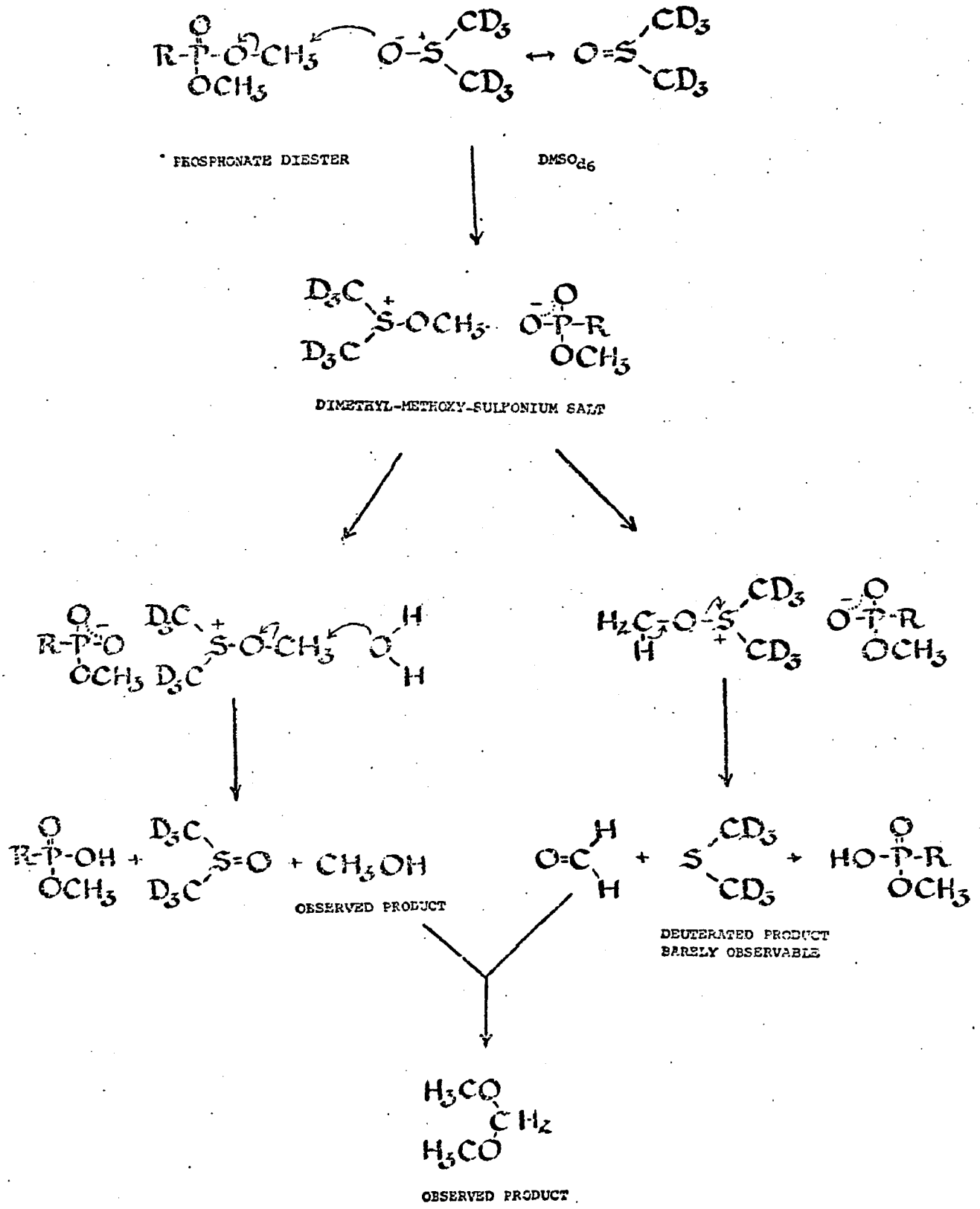

Figure 4

Scheme for the reaction of phosphonate diesters with DMSO$_{d6}$

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Furthermore, the size of the methylal peak was greatly enhanced indicating a more favorable partitioning of the dimethyl-methoxysulfonium salt to formaldehyde due to the scarcity of water.

Figure 4 indicates the probable pathway for what had been assumed to be the simple hydrolysis of the phosphonate esters. The same general scheme could probably be written for the monoesters as well.

Table II shows the rate constants for the first-order displacement by $DMSO_{d_6}$ on the phosphonate esters. The rates relative to the slowest rates (those for EII) are also indicated. Evident from Table II is the apparent minor dependence of the rate constants of Esters I and III upon the added aqueous solutions. Ester II and IV, however, show a more sizable dependence upon addition of proton donors (i.e. D_2O or p-TSA). The hydroxyl protons of EI and EIII must indeed be active as internal acid-catalysts allowing for better leaving groups, either as the phosphonate monoester or as the phosphonic acid, when nucleophilic attack by DMSO occurs. The transition state shown below may be hypothesized for EI:

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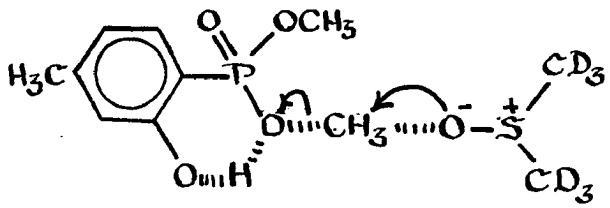

Water probably acts as a proton donor for EII and EIV in the reactions not involving p-TSA. If water could be totally eliminated from these systems, perhaps an even larger rate ratio of k_{EIII}/k_{EII} than that observed (65:1) could be attained. For the runs involving "water-free" $DMSO_{d_6}$, the NMR tubes were sealed in vacuo, but completely anhydrous conditions were still not obtained.

In the case of Ester V, when these reactions were performed, methylal formation was not observed. Apparently even with just traces of water, hydrolysis of this ester occurred much faster than displacement. This is discussed further in Section F of this chapter.

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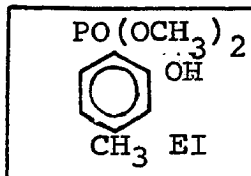
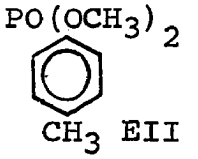
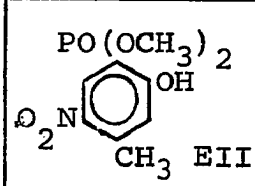
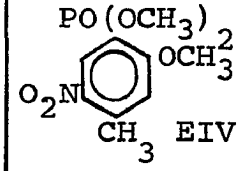
Table II

First-Order Rate Constants for $DMSO_{d_6}$ Displacement on EI, EII, EIII, and EIV

(T = 110 ± 0.5°C)

Compound		N p-Toluene Sulfonic Acid Added 0.98	0.50	0.10	0.00*	"water free"
 EI	$k_1 \cdot 10^4 min^{-1}$ **	19	15	13	14	13
	Rel. Rate ***	3	4	8	9	13
 EII	$k_1 \cdot 10^4 min^{-1}$	6.0	3.7	1.7	1.6	1.0
	Rel. Rate	1	1	1	1	1
 EIII	$k_1 \cdot 10^4 min^{-1}$	62	53	55	53	65
	Rel. Rate	10	14	32	33	65
 EIV	$k_1 \cdot 10^4 min^{-1}$	10	7.3	5.5	4.9	3.5
	Rel. Rate	1.7	2	3	3	3.5

*D_2O without p-TSA added

**Rate constants ± 10%

***Relative rates are those compared to that of EII

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C. Iodide Displacement on Ester I, II, III, and V

When treated with iodide ions, the dimethyl phosphonate esters I, II, III, and V were found to undergo nucleophilic displacement at temperatures lower than those required for displacement by DMSO. Preliminary studies made use of potassium iodide (0.2 M) and the ester (0.2 M) dissolved in $DMSO_{d_6}$. The samples were heated at 60°C, a temperature too low for significant occurrence of displacement by DMSO on the phosphonate esters. Early results showed EIII to react about 100 times faster than EII. The data could not be analyzed accurately, however, due to undesirable side reactions involving oxidation of the iodide by DMSO as well as displacement on the product methyl iodide by DMSO.

A mixed solvent system of 75% $acetone_{d_6}$ - 25% D_2O was used next. At concentrations of 0.25 M ester and 0.25 M KI at 60°C, CH_3I was the only product formed. However, the rate ratio of k_{EIII}/k_{EII} dropped to 16:1.

Fortunately, sodium iodide is quite soluble in acetone as are the phosphonate esters. This combination produced displacement reactions that occurred at observable rates at room temperature.

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1. Experimental

a. Preparation of Samples

Acetone$_{d_6}$ solutions of 0.4 M EI, EII, EIII, and EV were prepared. To dissolve EV in the acetone, a few drops of DMSO$_{d_6}$ were required to effect solution. Due to its hygroscopic nature, the sodium iodide was dried before use; a 0.4 M NaI in acetone$_{d_6}$ solution was prepared.

A 0.2 ml sample of ester and 0.2 ml of NaI solution were transferred to an NMR tube using a Hamilton microliter syringe. The final concentration for both ester and iodide was 0.2 M. TMS was added as an internal standard.

b. Kinetic Runs

The tubes were sealed with an NMR cap covered with a layer of parafilm, and placed in a thermostated 30°C water bath (Haake). For transporting the tube containing Ester III to the Varian A-60, a dewar containing 30°C water was used; all times were measured continuously.

With equal amounts of the two reactants, the simplest second-order rate plot, $1/[\text{Ester}]_t$ vs. time, was used. The error analysis used is described in Appendix 2.

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The products of the iodide displacement on EI, EIII, and EV, (the monosodium salts of the monomethyl esters), remained dissolved in the acetone. The fraction of unreacted ester, (x), was measured, as before, by dividing the integrated signal of remaining diester by the total of the integrated signals of diester + monoester + methyl iodide. Using (x) and knowing that $[\text{Ester}]_o$ was 0.2 M, the quantity $1/[\text{Ester}]_t$ (where $[\text{Ester}]_t = [\text{Ester}]_o \cdot (x)$) was easily determined.

However, MEII, the product of displacement on EII, was completely insoluble in acetone. The fraction of unreacted EII was thus found by dividing the integrated signal of remaining diester by the total integral of diester + 2(methyl iodide).

In the case of Ester V, even small traces of water present in the acetone$_{d_6}$ caused hydrolysis to occur simultaneously with displacement by iodide. Methanol was observed to form (checked by addition of an authentic sample) at a rate less than that for methyl iodide. To avoid complicated calculations, only the initial points of the reaction, where $[\text{Ester V}] \cong [I^-]$, were used to determine the rate constant k_2 for EV.

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With the longer reaction times, slight yellowing of the solutions of EII and EV indicated that some oxidation of iodide had occurred.

2. Results

Table III shows the rate constants for the second-order displacement of iodide ion on the phosphonate esters. The rates relative to the slowest rate (that of EII) are also indicated.

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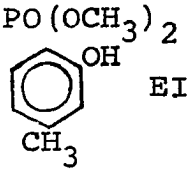
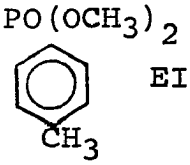
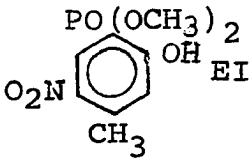
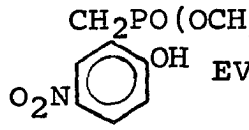
Table III

Second-Order Rate Constants for Iodide Displacement on EI, EII, EIII, and EV in Acetone$_{d_6}$ (T = 30 $\pm$ 0.1°C)

Ester	k·10^4 l/mole-min.	k_E/k_{EII}
 EI	59 $\pm$ 9	8.1
 EII	7.3 $\pm$ 1.0	1.0
 EIII	650 $\pm$ 50	89
 EV	17 $\pm$ 5	2.3

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D. The Hydrolysis of Ester II and Ester III in Acetone$_{d_6}$

The experiment described in this section was undertaken with the same goal in mind as that described in Section B, the DMSO$_{d_6}$ experiments. Acetone $_{d_6}$ was chosen for its non-nucleophilicity, its effective solvation of the phosphonate esters, its relative cheapness, and its inability to undergo hydrolysis (unlike CD_3CN or DMF$_{d_7}$).

Experimental and Results

With a Hamilton syringe, 0.4 ml of 0.2 M acetone$_{d_6}$ solutions of EII and EIII were transferred to NMR tubes. To these was added 0.05 ml of 0.4 M p-TSA in D_2O. The tubes were sealed and immersed in an approximately 70°C oil bath. The rate of hydrolysis for both esters was extremely slow under these conditions, first-order rate constants being expressable in days^{-1}. Methanol and the derivative monoesters appeared as the products.

After partial hydrolysis, the following rate constants were calculated:

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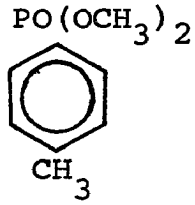
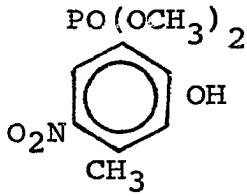


EII $k_1 = (4.6 \pm 0.9) \times 10^{-3}$ days^{-1}

(after 10% hydrolysis)



EIII $k_1 = (3.1 \pm 0.6) \times 10^{-2}$ days^{-1}

(after 50% hydrolysis)

so $k_{EIII} / k_{EII} \simeq 7$

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E. The Hydrolysis of Ester II and Ester V-- Determination of pH-Rate Profiles

Unlike all of the previously prepared phosphonate diesters, Ester V was found to be extremely susceptible to hydrolysis, as revealed by preliminary NMR experiments. Apparently, the hydroxyl moiety of this molecule was providing effective intramolecular catalysis of some sort. To establish the nature of this internal catalysis, be it the sought-after acid-catalysis provided by the hydroxyl proton, or a nucleophilic catalysis provided by the phenolic oxygen, the pH dependence of the hydrolysis was studied. Ester II was again used as a standard for determining the effectiveness of the catalysis; a few crude experiments were also performed on Ester III to support mechanistic arguments presented in the Discussion (Section F).

1. Experimental

The insolubility of both Esters II and V in water ruled out the possibility of using NMR kinetics as an easy method for establishing the pH dependence of the two esters' rates of hydrolysis. A uv method was therefore selected for its

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relative ease and for its requirement of only minute quantities of solute.

a. Preparation of Stock Solutions

Ten ml of 1.5 x 10^{-2} M solution of EII was prepared by dissolving 30.6 mg of the diester in about 1 ml of 95% ethanol. This was diluted to volume with deionized water. The larger extinction coefficient of EV permitted the use of a more dilute solution (8 x 10^{-4} M) of this material. In a 25 ml volumetric flask, 5.2 mg of EV was dissolved in 2.5 ml 95% ethanol and diluted to volume with deionized water. The stock solutions were stoppered, sealed with parafilm, and stored in a freezer at about -30°C until used.

b. Preparation of Buffers

Many of the constant ionic strength buffers (I = 0.1) used in these experiments were prepared according to the directions in the "Biochemists' Handbook."[11] Buffers of α-picoline, 2,6-lutidine, and imidazole were prepared by mixing 20 ml of a 1 M aqueous solution of the buffer with

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[11]C. Long., ed., "Biochemists' Handbook," Van Nostrand Co., Inc., 1961, pp. 29-42.

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10 ml of 1 N HCl (Fisher Certified). The various pyridine buffers were prepared by mixing five parts of a 1 M pyridine solution with one part of 1 N HCl. For the more dilute buffers prepared, the ionic strength was maintained at 0.1 by addition of KCl.

The pH of each buffer was checked with a Radiometer glass electrode pH meter with expandable scale. Commercially available buffer solutions of pH 4.0, 7.0, and 10.0 (Fisher Certified) were used for standardization.

The three buffers prepared in D_2O (cacodylic acid and potassium phosphate, monobasic) were prepared in the same way. Neither reagent was treated with D_2O and evaporated to give a deuterium enriched chemical. The pD of these buffers were measured with the glass electrode and the usual correction applied where:

$$pD = pH \text{ (meter reading)} + 0.4^{12}$$

Table IV contains a complete list of the buffers used in the experiments.

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[12] P.K. Glasoe and F.A. Long, J. Phys. Chem., 64, 188 (1960).

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c. Kinetic Runs

Runs at 94.3°C

Separate sealed ampoules were used to determine the rate constants at 94.3°C. One ml of stock solution was pipetted into a 10 ml volumetric flask and diluted to volume with a buffer solution. The final concentration of EII was thus 1.5×10^{-3} M and that of EV was 8×10^{-5} M. Each buffered solution was then divided into ten one ml aliquots; each aliquot was sealed in a 10x75 mm Exax soft glass test tube.

The labelled sealed tubes were placed in a silicone oil bath thermostated at 94.3 $\pm$ 0.5°C. A Fisher Scientific thermometer, 0-110° range, 0.1° divisions, was crudely calibrated as described in Section B of this chapter and the corrections applied.

At various time intervals, the tubes were pulled from the bath and immersed in a beaker of petroleum ether. For the fast reacting Ester V, the beaker of petroleum ether

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was chilled on ice. Compared to the long reaction times of Ester II, the heating time for the samples was insignificant; for Ester V, however, heating time contributed to the error in the results. The tubes of quenched solutions were inverted several times to wash condensed solvent from the test tube walls and were stored in the freezer. Each tube was opened and the pH of the contents was measured. No correction for the actual pH at 94.3°C was applied.

For a given kinetic run, each sample was scanned to give an entire spectrum and all scans were superimposed on the same chart paper. The scans for Ester II ran from 350 nm to 240 nm; for Ester V, the scans were from 550 nm to about 300 nm. Rather poor isosbestic points were usually obtained by this method.

When, by uv kinetics, the rate of hydrolysis of Ester II did not substantially decrease with increasing pH, the possibility of a displacement reaction by the buffer on the methyl ester carbon was raised. A uv sample in H_2O without added salts was prepared and the kinetics run in the usual way.

Some NMR experiments using p-toluenesulfonic acid

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as catalyst were also performed. One ml of 0.75 M EII was prepared by dissolving 0.15 gm EII in acetone$_{d_6}$. A 0.10 ml aliquot of this stock solution was diluted with 0.40 ml of each of the following D_2O solutions: 0.98 N p-TSA, 0.50 N p-TSA, 0.10 N p-TSA, D_2O, and 1.0 N NaOD. The final concentration of EII was thus 0.15 M; the solvent composition was 20% acetone$_{d_6}$-80% D_2O. With the exception of the sample of NaOD, the pD's were measured, the NMR tubes sealed and placed in the 94.3°C bath. The hydrolysis of the NaOD sample proceeded readily at room temperature so that the kinetics for this solution were run in the NMR probe.

Two NMR samples of EIII were also prepared in the above manner. The acetone solution of EIII was diluted with 0.98 N p-TSA and with D_2O.

Runs at 45.0°C

The pH-rate profile of Ester V's hydrolysis was re-determined at 45.0°C, the rate at 94.3°C being inconveniently fast for accurate measurement.

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Using the buffer solutions, 0.2 ml aliquots of the stock solution of EV was diluted to 2 ml. The pH of each resultant solution was measured before its transfer to a uv cuvette.[13] The filled cuvette was stoppered and its top wrapped in parafilm to retard evaporation. The cuvette was placed in a 45.0°C Lauda K-2/R water bath and allowed to equilibrate for two to three minutes before the timer was started. After various intervals of time, the cuvette was removed from the water bath, wiped dry, placed in the Cary 15, and the spectrum scanned. The cell block of the Cary was approximately the same temperature as the water bath, therefore, times were measured continuously. Excellent isosbestic points were obtained by this method. Figure 5 is an example of the scans of a typical run. At the end of each run, the pH was again measured at 25°C. No correction for the actual pH at 45.0°C was applied.

Hydrolysis of Ester V at pH's 1 to 4 and at pH 10 proceeded slowly and produced only a very small change in absorbance. To check the results obtained by the above method, an alternative uv method was used. A 10^{-2} M stock solution

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[13]For the samples prepared by dilution with 1.0 N NaOH (Fisher Certified) and 0.5 N NaOH, the pH's were calculated approximately rather than measured.

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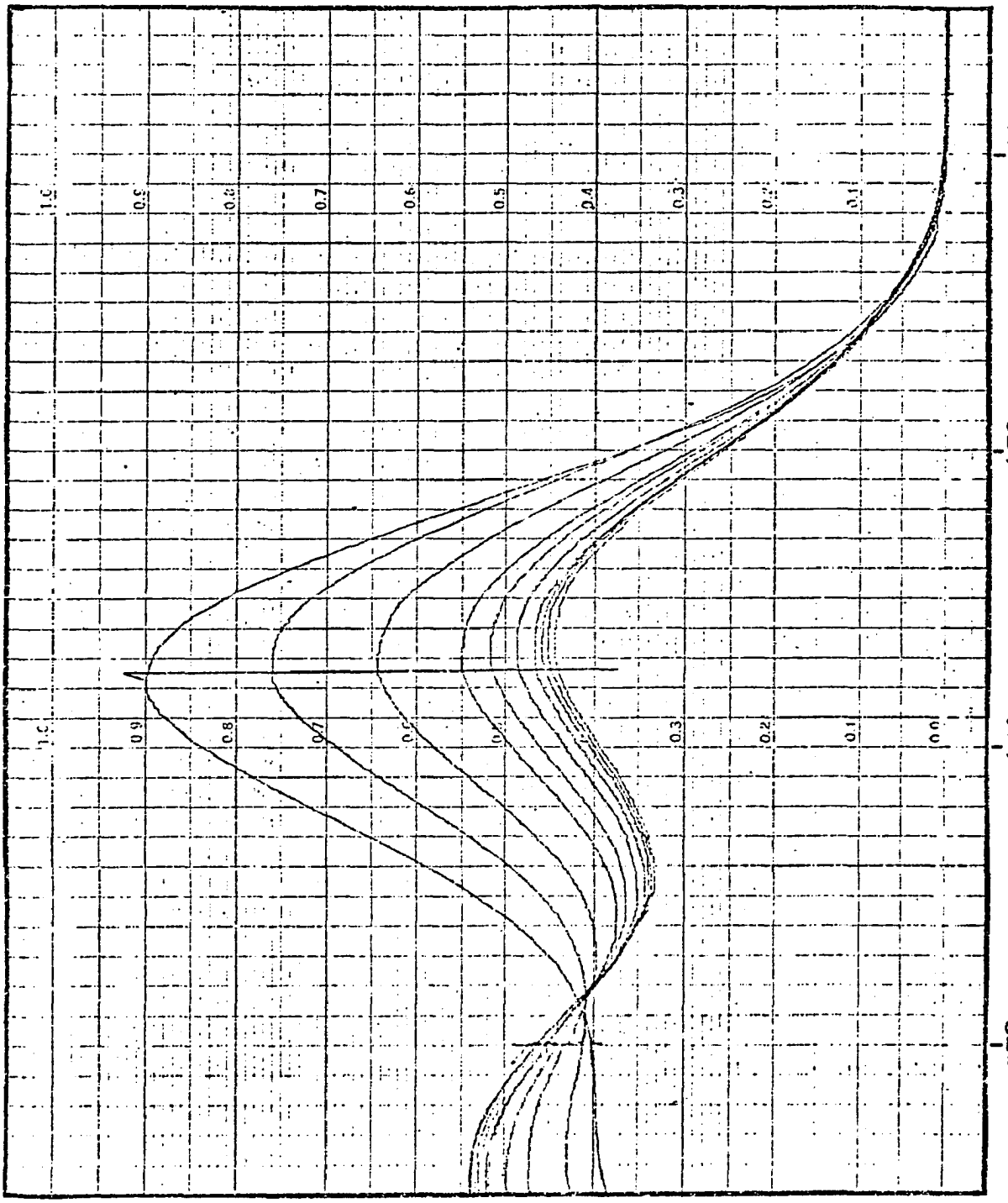

Figure 5

A set of Cary 15 scans taken during the hydrolysis of Ester V
(pH 6.9 , T = 45.0°C)

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of EV was prepared by dissolving 12.9 mg of EV in 1 ml 95% ethanol and diluting to 5 ml with deionized water. One ml of this solution was then diluted to 10 ml with buffers of approximate pH 1.0 and 3.0. The final concentration of EV was thus 10^{-3} M. For the runs at pH's 4 and 10, ca. 5 mg of EV was dissolved in 1 ml ethanol and diluted to 25 ml with the buffer. The pH's were measured. The volumetric flasks were then stoppered, sealed with parafilm, and suspended in the 45.0°C bath for about thirty minutes prior to starting the timer. From time to time, a 0.5 ml aliquot of solution was withdrawn from the flask and diluted to 5.0 ml with 1 M tris buffer (pH 8.0); immediate yellowing of solution resulted. For the runs performed at pH 10.0, the aliquots were diluted with 0.05 M phosphate buffer (pH 7.0). As the hydrolysis proceeded, the change in absorbance of the diluted aliquots was substantial. The spectrum of the diluted aliquot was recorded. The pH's of the stock solutions from which the aliquots were withdrawn were checked after the run was completed.

d. Products of Hydrolysis

The uv spectra of reacted EV corresponded to those of MEV at the same pH. Crude NMR experiments performed at alkaline pH in both H_2O and D_2O revealed, by addition of authentic sample, that methanol and MEV were the products of the reaction in basic medium.

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The uv spectra of hydrolyzed Ester II were like those of MEII at the same pH. However, the uv spectrum of MEII is not substantially different from that of its hydrolysis product, p-toluene phosphonic acid. At low pH's,Ester II most likely hydrolyzed completely to the phosphonic acid rather than stopping at Monoester II. This conclusion was borne out by the NMR studies conducted in 20% acetone$_{d_6}$ - 80% D_2O. In these studies, only a small amount of Monoester II accumulated (checked by addition of authentic sample). The methanol peak was also apparent by NMR and checked by authentic sample.

e. Determination of the Rate Constants by UV Kinetics

The rate of hydrolysis of the phosphonate diester, EV, could be easily followed by uv at pH's > 5 <9. At these pH's, the phenolate absorption of EV at 410 nm had a larger extinction coefficient than that of Monoester V. The steady decline in the absorbance gave a good measure of the the extent of reaction (see Figure 5). At pH's <5, the reaction was marked only by a shift of λ_{max} from 320 nm to 325 nm. Reaction progress was checked at 360 nm. At

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pH's >9, the spectrum's λ_{max} shifted from 410 nm to 430 nm; reaction progress was followed at 460 nm.

For Ester II, the extinction coefficient of the entire spectrum declined. To obtain the kinetic data, the absorbance of a peak located at 273 nm was followed.

To obtain a first-order rate constant from uv kinetics, $\ln \left| A_{\infty} - A_t \right|$ was plotted vs. time, where A_{∞} = absorbance at the infinity point and A_t = absorbance at a given time. An approximate A_{∞} was determined in the following manner: absorbance at a selected wavelength was plotted vs. time on graph paper. With the aid of a French curve, a smooth exponential curve was fitted through the points and the infinity point chosen as that absorbance where the curve flattened out (see Figure 6). This method worked well for all runs but that of Ester V at pH 1. At this pH, the hydrolysis of MEV was only three times slower than that of EV. At all other pH's, MEV hydrolyzed at least ten times slower than EV.[14] Using the initial points of the absorbance vs. time plot at this pH, and by comparison with the A_{∞} at pH 3, an approximate A_{∞} could be established.

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[14] From a comparison of the rate constants of EV and of MEV at 94.3°C.

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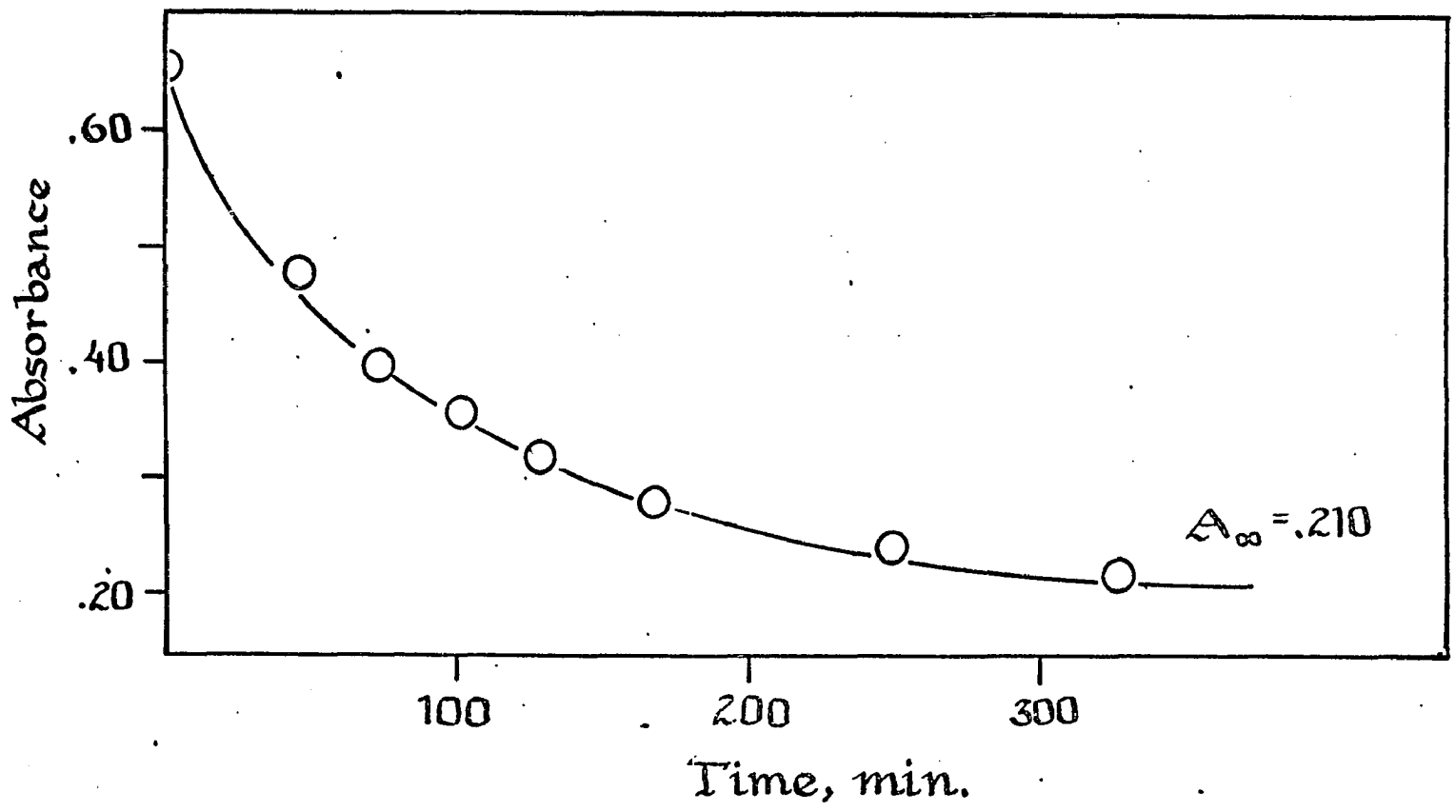

Figure 6

A plot of absorbance vs. time; the value of A_∞ is indicated. (From the hydrolysis of EV at pH 6.6, cacodylic acid buffer, total buffer concentration 0.136 M, T = 45°C)

A semi-log plot of $|A_\infty - A_t|$ vs. time was prepared (see Figure 2), minor changes in the value of A_∞ were made in order to better fit the final kinetic points. Having established the "best" A_∞ value and hence the best line, $t_{\frac{1}{2}}$ was determined and k_1 calculated. Most of the semi-log plots were linear through at least two to three half-lives.

Preliminary kinetic runs revealed that, in the neutral pH's, the reaction rates were dependent on the buffer concentrations. To obtain the buffer-independent rate constants for the hydrolysis of EV, two or three runs at a constant pH but at different buffer concentrations were performed. The observed rate constants from a given set of constant pH runs were plotted vs. the concentration of the acid form of the buffer. The intercept on the y-axis of the resultant line (buffer concentration equal to zero) was assumed to be the buffer-independent rate constant. These rate constants were used in the determination of the pH-rate profile of Ester V. The extrapolation method is illustrated in Figure 7.

The second-order rate constants, $k_{2_{HB}}$, for the buffer catalysis could also be obtained graphically. As will be shown later, a plot of $k_{obs}\left[\frac{[H^+]}{K_a} + 1\right]$ min^{-1} vs. concentration of the acid form of the buffer has a resultant slope equal

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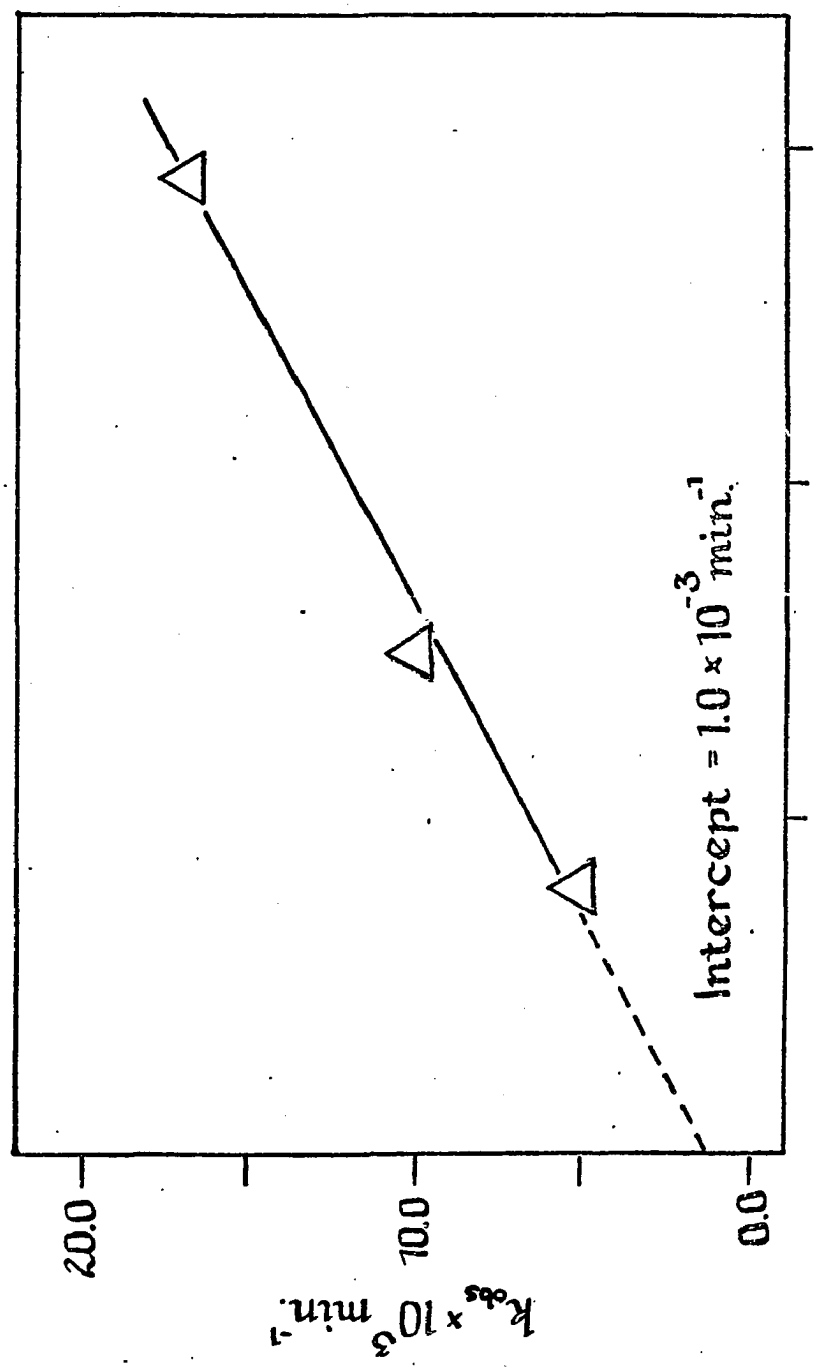

Figure 7

A plot showing the method used to obtain the buffer-independent rate constant (taken from runs in cacodylic acid buffer, pH 6.0)

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to $k_{2_{HB}}$. For the runs in D_2O, the value calculated for $\left[\frac{[D^+]}{K_a} + 1\right]$ was corrected for pD in the usual manner.[12] The determined pK_a of EV could also be corrected:

$$pK = pK_{DA} - pK_{HA}$$
$$= 0.48 \text{ for p-nitrophenol}^{15}$$

Figure 8 shows this method when applied to the runs in phosphate buffers at pH's 7.0 and 7.9 and at pD 7.5. Alternatively, the values of $k_{2_{HB}}$ could be calculated for each run using the equation for k_{obs} and the values for k_o and K_a given in Section F-6 of this chapter.

f. Determination of Reaction Order

To check that the hydrolysis of EV was, in fact, first-order in ester, a dilution experiment was performed. One ml of the 8×10^{-4} M stock solution of Ester V was diluted to 10 ml with deionized water. One ml of this solution was then diluted with a buffer (resultant pH 6.9). The final concentration, therefore, equaled 8×10^{-6} M--one tenth that of the original.

The kinetics were run at 45°C using the cuvette method. The Cary 15 0.1 slide wire was implemented.

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[15] A.O. McDougall and F.A. Long, J. Phys. Chem., 66, 429 (1962).

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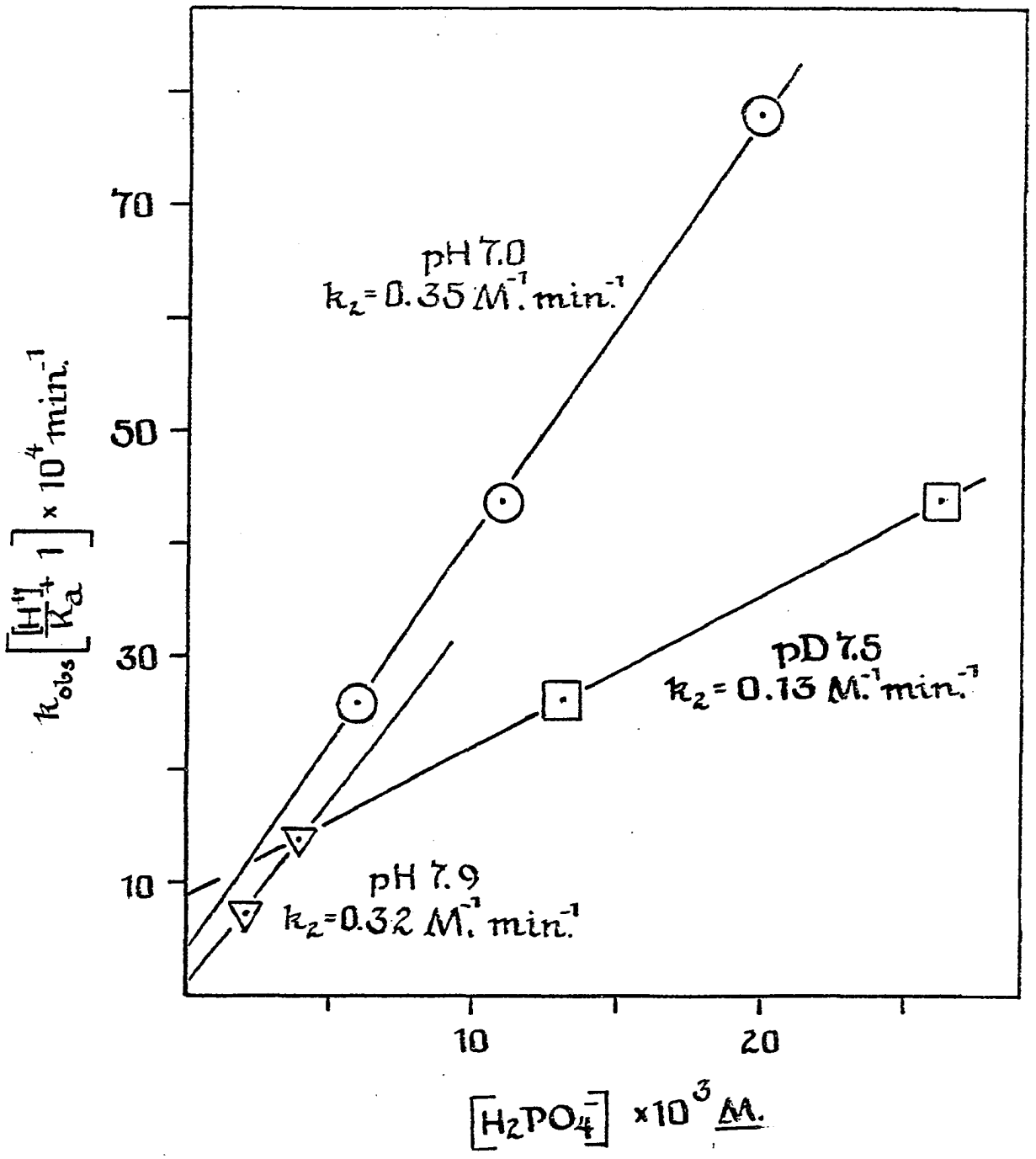

Figure 8

A plot showing the method used to obtain the buffer-dependent rate constants, k_{2HB}

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Despite the substantial back-ground noise, it was readily apparent that hydrolysis proceeded with the same rate constant as that previously determined.

As noted earlier in this section, the experiments at pH's 1 and 3 were repeated by an aliquot method using 10^{-3} M EV solution. The rate constants determined in those experiments also agreed well with the previously determined values.

g. Determination of the pK_a

The pK_a of Ester V's hydroxyl group was determined at room temperature using an adaptation of the Tobey method.[16] A 0.2 ml aliquot of a 10^{-3} M stock solution (prepared in the manner described earlier) was pipetted into a 2 ml volumetric flask and diluted to volume with buffers of pH values near the assumed pK_a (ionic strength = 0.1) and with 0.10 N HCl and 0.10 N NaOH (Fisher Certified). The pH's of the buffered samples were measured. The spectrum of each sample was scanned. By this method, the pK_a of

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[16] S. Tobey, J. Chem. Ed., 35, 514 (1958).

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Ester V was determined to be 6.72 ± 0.07.

The 0.10 N HCl and 0.10 N NaOH solutions of EV were diluted to ½ and ¼ of the original concentrations. A plot of absorbance vs. concentration was linear, demonstrating that Beer's Law was applicable.

2. Results

The observed rate constants for the hydrolysis of EV in buffers of ionic strength 0.1 at 45°C are shown in Table IV. Also shown in Table IV are the buffer compositions. Table V contains the rate constants used in the pH-rate profile for Ester V (see Figure 9). Table VI shows the values for k_{2HB} for the buffers and Figure 10 is a Brønsted plot of these values. The statistical correction for polybasic acids was applied.[17] Table VII gives a few rate constants obtained for the hydrolysis of Ester V at 94.3°C.

The rate constants obtained for the hydrolysis of Ester II in buffered solutions at 94.3°C are given in

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[17]L.P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., New York, 1970, p. 319.

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Table VIII. The rate constants for the NMR experiments performed on EII and EIII at 94.3°C are shown in Tables IX and X. The pH-rate profile for the hydrolysis of Ester II is shown in Figure 11.

The mechanistic interpretations and the calculations for the rate profiles are discussed in Section F of this chapter.

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Table IV

Observed Rate Constants in the Hydrolysis of Ester V in Buffers of Ionic Strength 0.1

(T = 45.0°C)

Buffer	pH	Total Buffer Conc. M	[HB] M*	$k_{obs} \cdot 10^3 min.^{-1}$
Hydrochloric Acid	1.1	0.10	0.0	1.8
	1.1	0.10	0.0	1.6
Formic Acid-Sodium Formate	2.9	0.57	0.47	1.9
	3.0	0.57	0.47	1.9
Acetic Acid-Sodium Acetate	4.0	0.56	0.46	2.1
	4.0**	0.29	0.24	1.8
	5.0	0.15	0.05	2.9
Pyridinium Hydrochloride-Pyridine	5.8	0.50	0.10	14
	5.8**	0.25	0.05	7.5
	5.8**	0.125	0.025	4.6
Cacodylic Acid-Sodium Cacodylate	6.0	0.244	0.144	17
	6.0	0.244	0.144	17
	6.0**	0.124	0.074	10
	6.0**	0.064	0.039	5.0
	6.6	0.136	0.036	10.5
	7.0	0.144	0.014	7.3
	6.6(pD)	0.244	0.144	9.0
α-Picolinium Hydrochloride-α-Picoline	6.0	0.20	0.10	4.3

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Buffer	pH	Total Buffer Conc. M	[HB] M*	$k_{obs} \cdot 10^3 min.^{-1}$
Potassium Dihydrogen Phosphate-Disodium Hydrogen Phosphate	6.0	0.08	0.07	6.3
	6.0	0.08	0.07	6.2
	6.0	0.08	0.07	6.0
	6.5	0.06	0.04	7.5
	6.6	0.06	0.035	7.7
	6.9**	0.024	0.012	3.4
	7.0	0.047	0.02	5.0
	7.0**	0.024	0.011	2.8
	7.0**	0.013	0.006	1.7
	7.1	0.046	0.019	5.5
	7.3	0.042	0.013	3.6
	7.4	0.04	0.01	2.9
	7.4	0.04	0.01	3.5
	7.9	0.036	0.004	1.3
	7.9**	0.018	0.002	0.72
	7.5(pD)	0.05	0.026	2.8
	7.5(pD)**	0.025	0.013	1.7
2,6-Lutidinium Hydrochloride-2,6-Lutidine	6.8	0.20	0.10	1.7
	6.8	0.20	0.10	1.9
	6.8**	0.10	0.05	1.1
Tris(hydroxymethyl)-aminomethane Hydrochloride-Tris(hydroxymethyl)aminomethane	7.0	0.107	0.10	1.3
Imidazolium Hydrochloride-Imidazole	7.3	0.20	0.08 (I≠0.1)	6.3
Glycine-Sodium Glycinate	10.0	0.152	0.052	0.35
	10.0**	0.076	0.026	0.30
	10.0**	0.038	0.013	0.025

*Concentration of the acid form of the buffer
**KCl added to make μ = 0.1

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Table V

Rate Constants for the Hydrolysis of Ester V in Buffers of Ionic Strength 0.1

($T = 45.0^{\circ}C$)

Buffer	pH	$k \cdot 10^3 min.^{-1}$
HCl	1.1	1.8
HCl	1.1	1.6
Acetic Acid-Sodium Acetate	4.0	1.5*
Pyridinium Hydrochloride-Pyridine	5.8	1.4*
Cacodylic Acid-Sodium Cacodylate	6.0	1.0*
2,6-Lutidinium Hydrochloride-2,6-Lutidine	6.8	0.45*
Potassium Dihydrogen Phosphate-Disodium Hydrogen Phosphate	7.0	0.32*
Potassium Dihydrogen Phosphate-Disodium Hydrogen Phosphate	7.9	0.2*
Glycine-Glycine Hydrochloride	10.0	0.2*
NaOH	ca 13.6	8.2
NaOH	ca 13.9	25.0

*Extrapolated rate constants; all other rate constants were determined directly.

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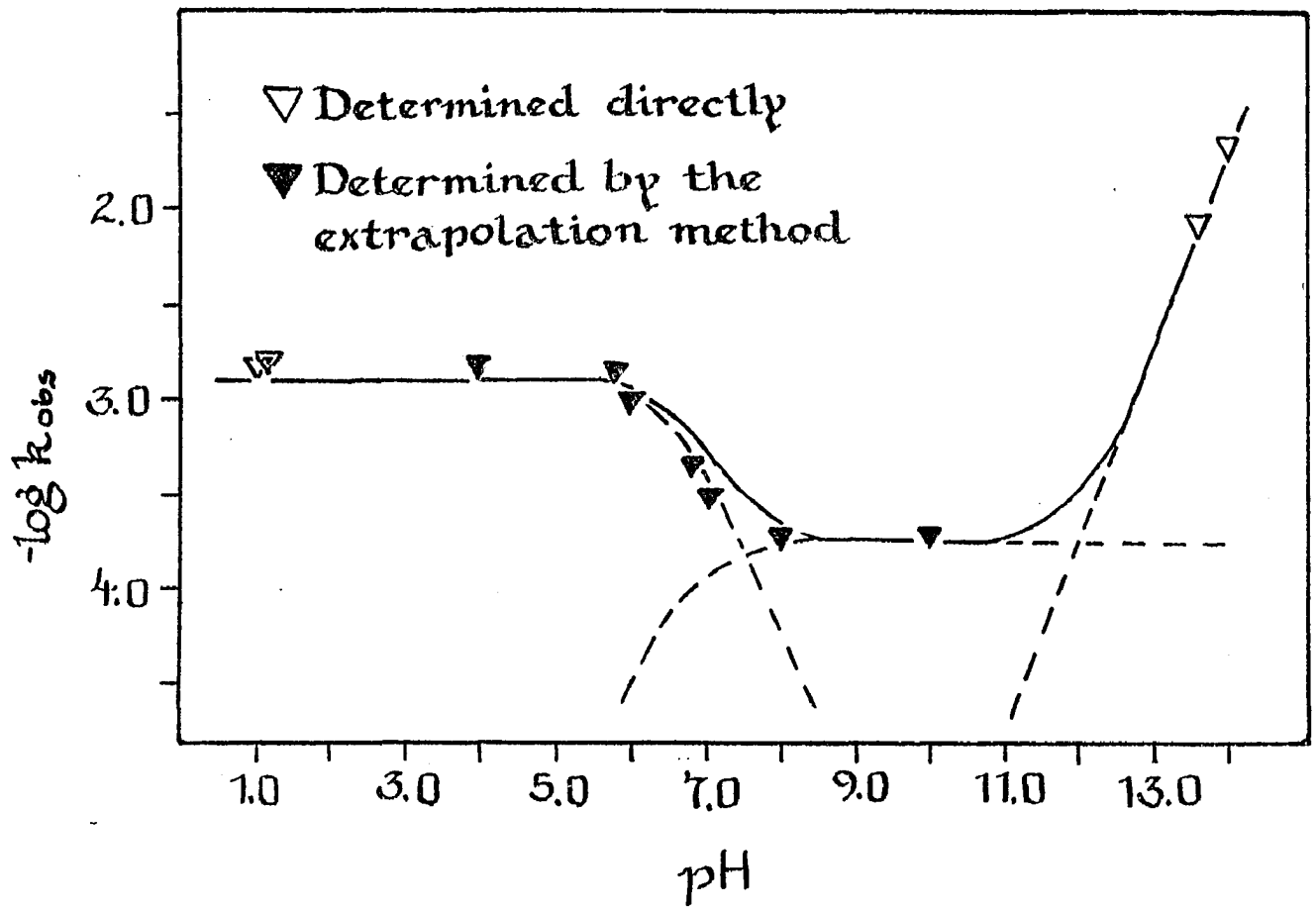

Figure 9

The pH-rate profile for the hydrolysis of Ester V at 45.0°C. The solid and dashed curves are explained in Section F-6.

Table VI

Second-Order Rate Constants for Buffer Catalysis in the Hydrolysis of Ester V

(T = 45.0°C)

Buffer	pK_a	Statistically Corrected pK_a	k_{2HB} $M^{-1}min.^{-1}$
Acetic Acid	4.8[18]		7.0
Pyridinium Ion	5.2[18]		2.3
α-Picolinium Ion	6.0[18]		0.19*
Cacodylic Acid	6.3[19a]		0.70
2,6-Lutidinium Ion	6.7[19b]		0.025
Imidazolium Ion	6.9[18]	7.2	0.09*
Dihydrogen Phosphate	7.2[18]	7.5	0.33
Tris(hydroxymethyl)-aminomethane Ion	8.1[18]	8.6	0.014*
Glycine	9.6[18]	10.1	2.5×10^{-3}
Water	15.7[20]	16.0	3.6×10^{-6}

* k_{2HB} calculated from a single point by assuming $k_o = 1.3\times10^{-3}min.^{-1}$; all other rate constants determined graphically

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[18]Merck Index, 8th Ed., Merck and Co., Inc., Rahway, N.J., 1968.

[19]a. G.Kortum, W. Vogel, K. Andrussow,"Dissociation Constants of Organic Acis in Aqueous Solution," Butterworths, London, 1961. b. D.D. Perrin, "Dissociation Constants of Organic Bases in Aqueous Solution," Butterworths, London, 1965.

[20]W.P. Jencks, "Catalysis in Chemistry and Enzymology," p. 172.

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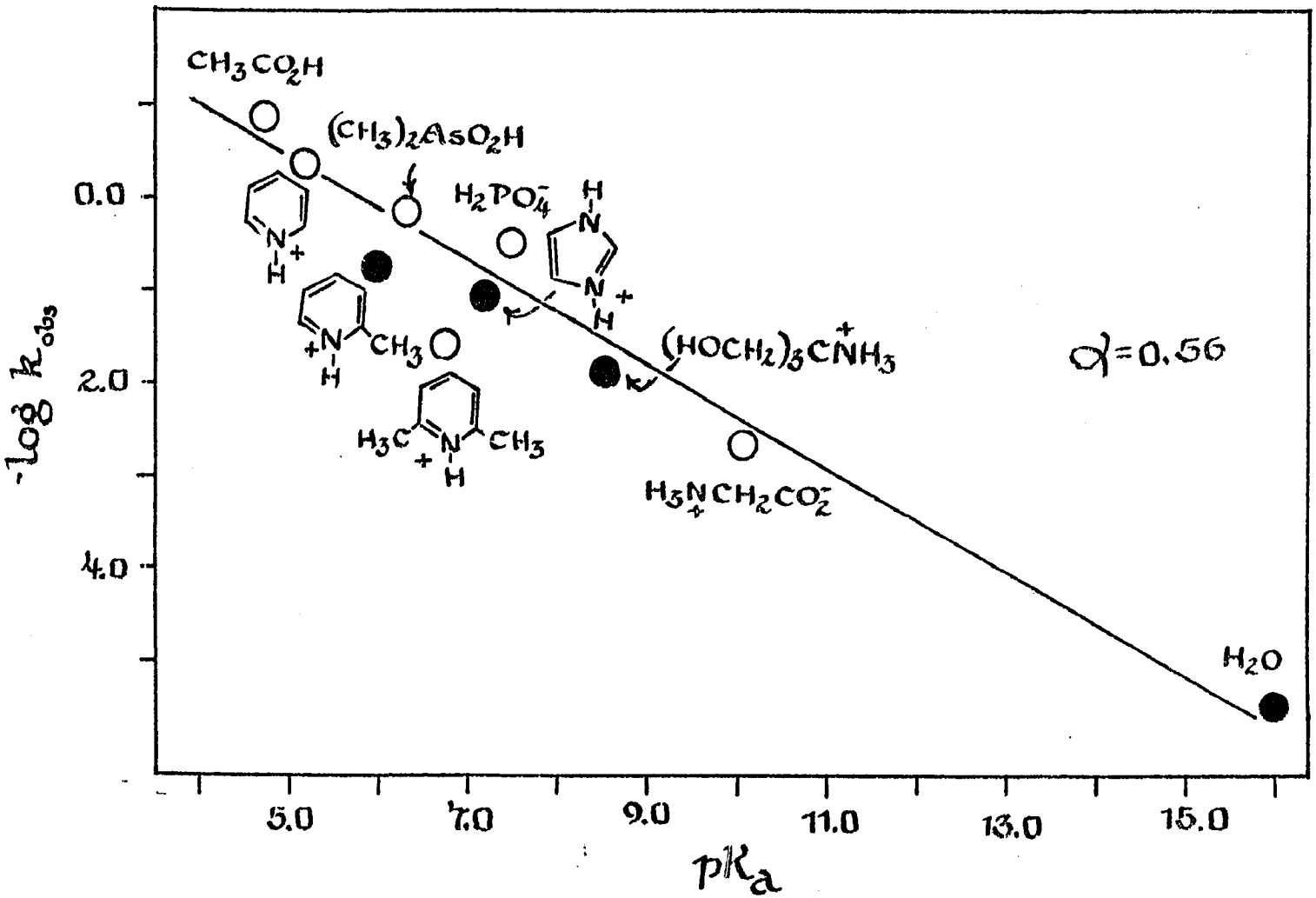

Figure 10

A Brønsted plot of the buffers used in the hydrolysis of Ester V. ○ Graphically determined k_{2HB}; ● calculated k_{2HB} from assumed rate, k_o.

Table VII

The Hydrolysis of Ester V in Buffers of Ionic Strength 0.1

($T = 94.3 \pm 0.5^{\circ}C$)

Buffer	pH	$k \cdot 10^2$ min.$^{-1}$
HCl, 0.10 M	1.2	6.6
Formate, 0.57 M	3.0	5.0
Acetate, 0.15 M	5.0	11
Phosphate, 0.08 M	5.9	14
Phosphate, 0.06 M	6.9	12
Tris, 0.17 M	7.7	7.7

Rate constants determined by uv kinetics using the ampoule method; an error of about ± 20% should be allowed.

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Table VIII

The Hydrolysis of Ester II in Buffers of Ionic Strength 0.1

($T = 94.3 \pm 0.5^{\circ}C$)

Buffer	pH	$k \cdot 10^4$ min.$^{-1}$
HCl, 0.1 M	1.1	5.1
HCl, 0.1 M	1.3	4.3
Formate, 0.57 M	3.0	3.5
Acetate, 0.15 M	5.0	4.6
H_2O ($I \neq 0.1$)	ca 6.5	3.6

Rate constants allowed an error of ± 15%.

Table IX

The Hydrolysis of Ester II in 20% Acetone$_{d_6}$ - 80% D_2O

($T = 94.3 \pm 0.5^{\circ}C$)

Conditions	pD	$k \cdot 10^4$ min.$^{-1}$ *
0.8 N p-TSA	0.4	3.6
0.4 N p-TSA	0.8	2.0
0.08 N p-TSA	1.2	1.2
D_2O	ca 2.5	0.96**
0.8 N NaOD	ca 14	700

*Rate constants determined by NMR kinetics, an error of ± 15% allowed.

**Estimated first-order rate constant (NaOD $\cong$ 7 fold excess), T = NMR probe temperature, ca 30°C

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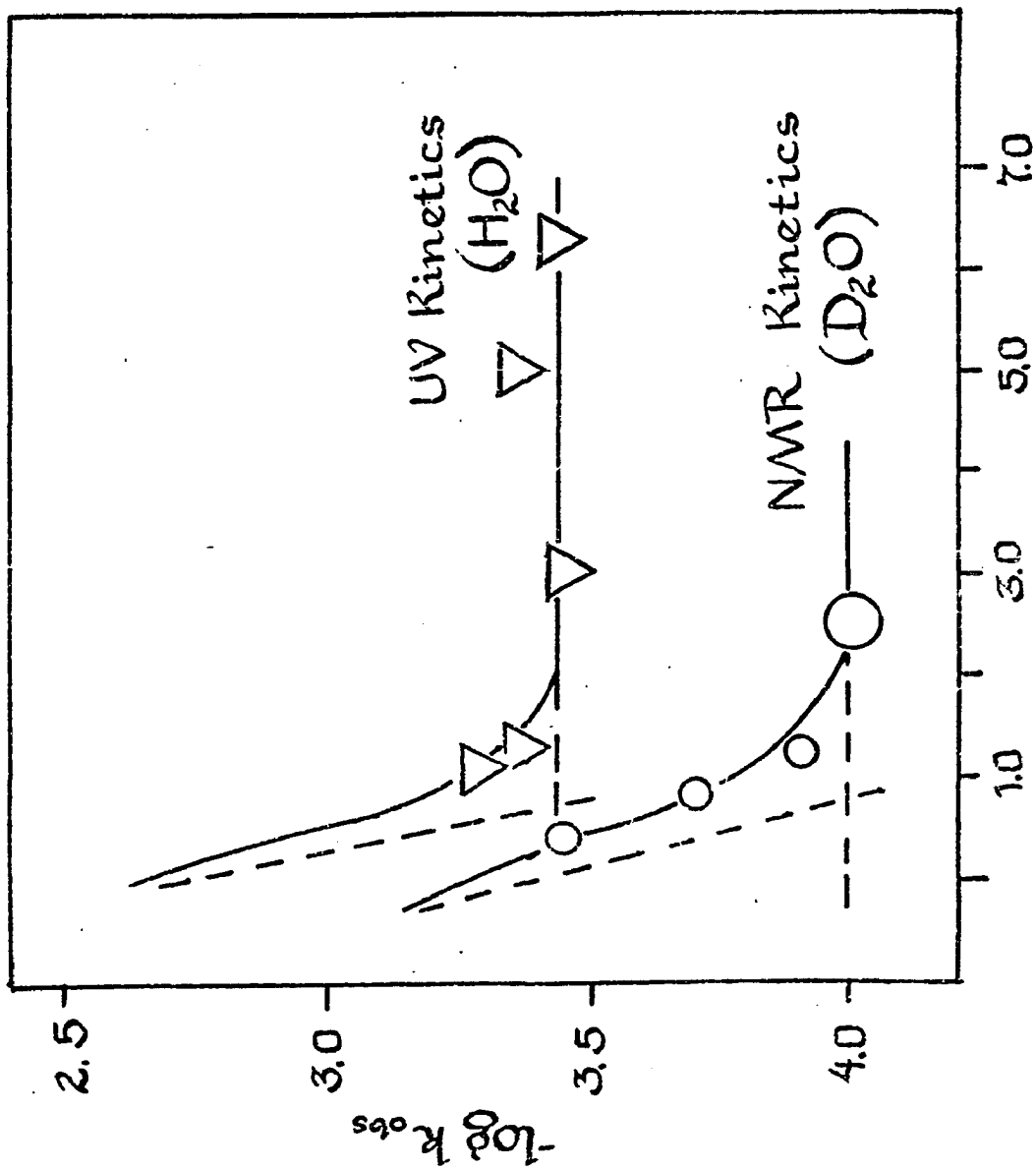

Figure 11

The pH-rate profile for the hydrolysis of Ester II at 94.3°C.

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Table X

The Hydrolysis of Ester III in 20% Acetone$_{d_6}$ - 80% D_2O

(T = 94.3 ± 0.5°C)

Conditions	pD	$k \cdot 10^4$ min.$^{-1}$	k_{EIII}/k_{EII}
0.8 N p-TSA	0.3	10	3
D_2O	ca 3	5.4	5

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F. Discussion

This discussion is concerned primarily with investigating the mechanism of Ester V's hydrolysis and hence, its source of intramolecular catalysis. The structurally similar but unreactive Ester III is assigned a comparative role. Chapter I describes some of the varied proposals that have been made to explain rate enhancement in similar molecules. Some of these mechanisms are re-introduced here and their applicability to the present study examined. The following arguments which concern the role of intramolecular acid-catalysis as a source of significant (i.e.> 10 fold) rate enhancement rely heavily upon the ability of a molecule to effectively participate in intramolecular hydrogen bonding. The drawings of Ester III and of Ester V used to illustrate the arguments attempt to be faithful to the actual Dreiding models examined.

1. A Brief Review of Hydrogen Bonding

The properties of the hydrogen bond are discussed at great length by Pimentel and McClellan.[21] The importance of

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[21] G.C. Pimentel and A.L. McClellan, "The Hydrogen Bond," W.H. Freeman and Co., San Francisco, 1960.

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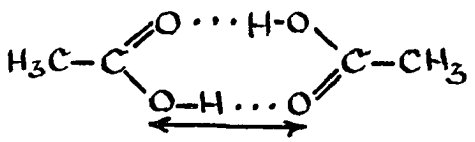
hydrogen bonds in catalysis is related by Jencks.[22] Some facts concerning H-bonding (from reference 16) that are relevant to this work are presented below.

A "short" hydrogen bond, that is, one in which the hydrogen is symetrically located between the two electronegative atoms, has an O-H···O distance less than 2.44 Å in length. An example of a very strong hydrogen bond is the $[F\text{-}H\text{-}F]^-$ ion in which the F-F distance is 2.26 Å. The hydrogen bonds of dimeric acetic acid are longer:



2.76 Å

Pimentel suggests that the limit for O-H···O hydrogen bond interaction is about 3.2 Å.

Although a linear arrangement of the three atoms participating in the H-bond is energetically most favorable, deviations from linearity are usually the case--e.g., the crystal structure of $CuCl_2.2H_2O$ exhibits a deviation of 15°. In intramolecular H-bonds, of interest in the present

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[22] W.P. Jencks, "Catalysis in Chemistry and Enzymology," Chapter 6.

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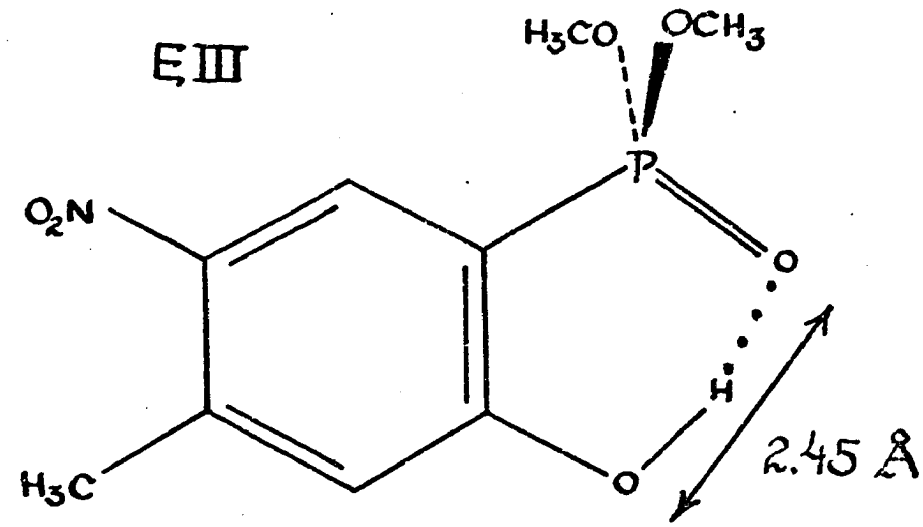

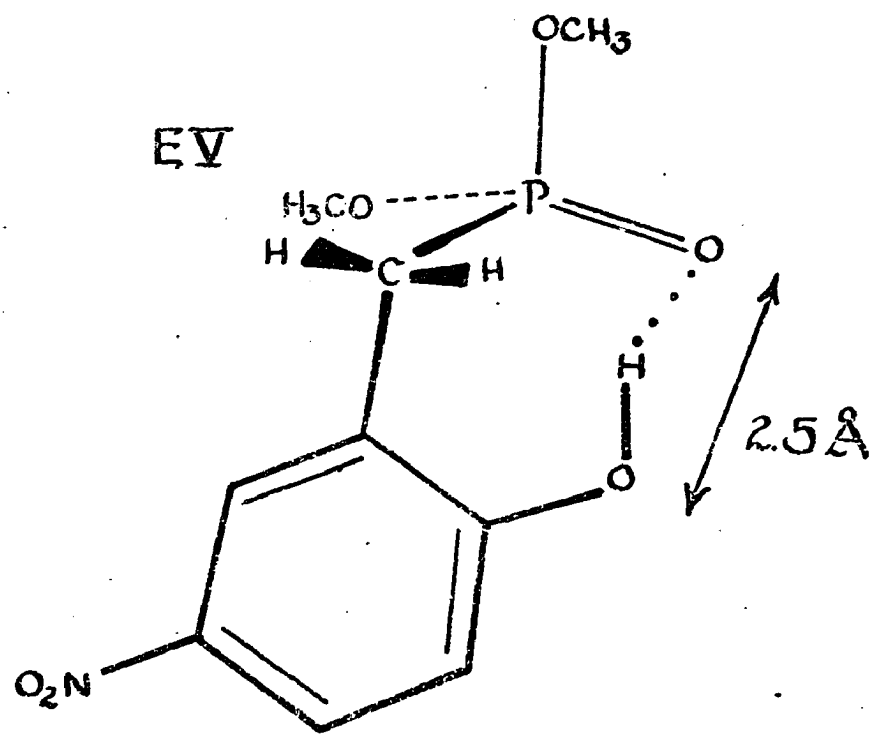

Figure 12

EIII and EV in hydrogen-bonded conformers. The measured (from Dreiding models) O-H...O distance is indicated.

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work, deviations from linearity are usually necessary in order to avoid other sources of strain.

A simple experiment for determining if an H-bond is intra- or intermolecular consists of running IR spectra at different concentrations of absorbing material. Both EIII and EV showed intramolecular hydrogen bonding--the absorbance at the frequencies assigned to their respective hydrogen bonded O-H stretches did not, upon dilution of the sample, diminish relative to the other absorbances of the spectrum. Figure 12, drawn from Dreiding models, attempts to illustrate the probable H-bonds. It should be noted that the various rotations available to Ester V allow hydrogen bond O-H···O distances as small as 1 Å (a very uncomfortable situation). EV is illustrated here in its conformer that gives an O-H···O distance approximately equal to that of EIII in its most favorable arrangement.

2. Mechanism for Hydrolysis under Acidic Conditions

Under acidic conditions, Ester V hydrolyzed 10^2 times faster than Ester II (T = 94.3°C). The hydrolysis of Ester III, however, did not show any significant enhance-

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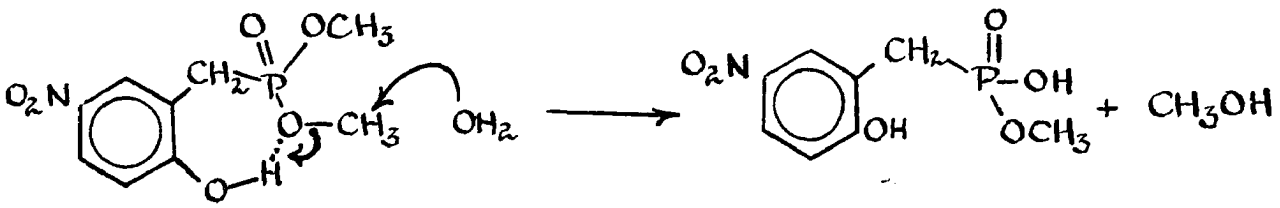
ment of rate when compared to that of Ester II. At pH's less than 4, Ester V is present to the extent of more than 99% as the neutral species. Examination of Table IV and Figure 7 shows that the rate of hydrolysis of EV is relatively constant between the pH's of 1 and 5. Therefore, the reaction is not acid-catalyzed.

A number of mechanisms has been proposed to explain effective intramolecular catalysis (see Section A, Chapter I). Four mechanisms are explored below in an attempt to determine which mechanism fits the existant data the best.

i) Intramolecular acid-catalyzed $S_N 2(C)$ attack by water:



The $S_N2(C)$ mechanism has been suggested in the acid-catalyzed hydrolysis of simple dimethyl phosphonate esters (see reference 9, Chapter I). The above mechanism was suggested and subsequently discarded by Cadogan and co-workers as a source of catalysis in the rapid hydrolysis

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of the oximes 9 (see reference 8, Chapter I). Examination of Dreiding models shows that EIII and EV are both capable of effectively hydrogen bonding to produce a good leaving group (a "protonated" phosphonate monoester) and thus allow facile $S_N2(C)$ displacement (see Figure 13). Despite this, the $S_N2(C)$ mechanism may be dismissed in the rapid hydrolysis of Ester V.

In simple displacement reactions, EIII underwent an S_N2 reaction with iodide ion 90 times faster than EII; EI underwent this reaction almost ten times faster than EII. EV, however, showed only a two-fold rate enhancement suggesting that the conformer illustrated in Figure 13 is not highly favorable. Furthermore, in the reactions with $DMSO_{d_6}$, EV showed no methylal formation and presumably did not react with DMSO as did the other phosphonate esters. Clearly, therefore, if the $S_N2(C)$ mechanism accompanied with intramolecular acid-catalysis was a truly effective hydrolytic mechanism, then EIII should have shown the same (if not a greater) ease of hydrolysis as EV. Conversely, EV should have reacted much more readily than EII with iodide ions.

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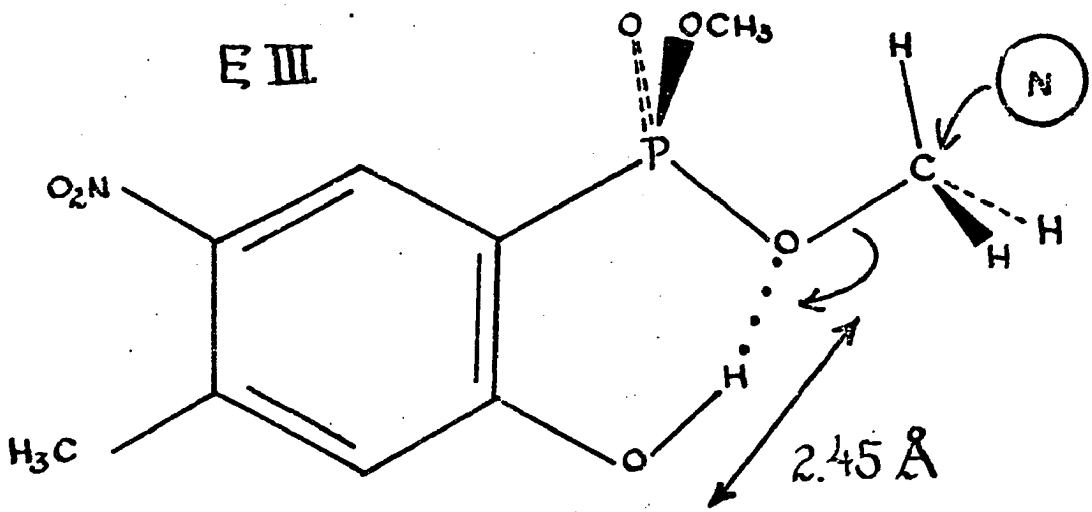

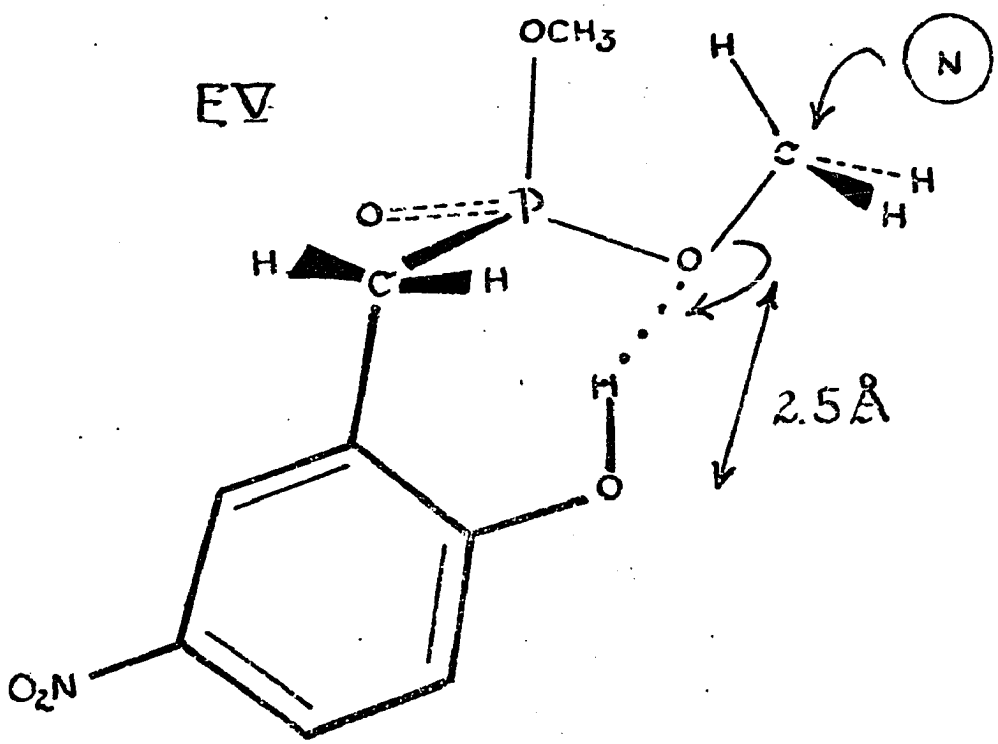

Figure 13

EIII and EV in conformers which effectively internally catalyze $S_N2(C)$ displacements

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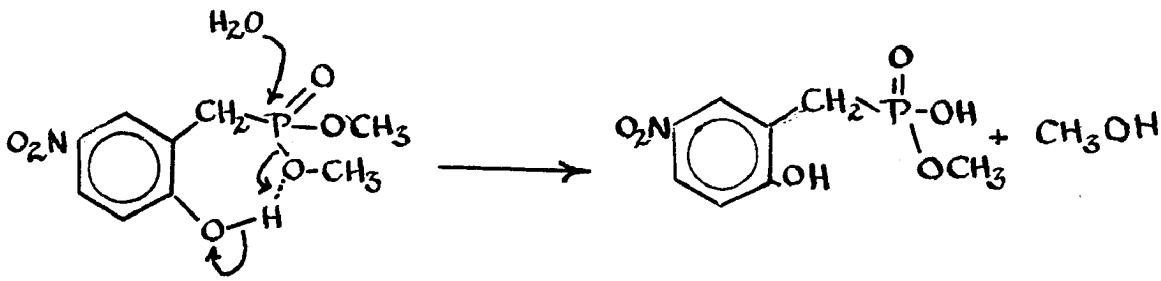
ii) Intramolecular acid-catalyzed $S_N2(P)$ attack by water:



This mechanism was favored by Cadogan (ref. 8, Chap.I) and by Gordon (ref. 13, Chap. I) to explain catalysis in their respective phosphonate esters 9 and 12. Molecular models reveal little difference between EIII and EV; if internal acid-catalysis were important in an $S_N2(P)$ displacement, then EIII and EV should have reacted at similar rates. As shown in Figure 11, both EIII and EV appear capable of hydrogen bonding the leaving group ($O-CH_3$). Furthermore, neither molecule appears to be sterically hindered for a back-side attack. Although the actual shape and dimensions of the supposed transition state for an $S_N2(P)$ reaction are not known, examination of models using a penta-coordinated phosphorus is instructive. Figure 14 fails to show that the supposed transition state for an $S_N2(P)$ attack on the EIII phosphorus would be any less

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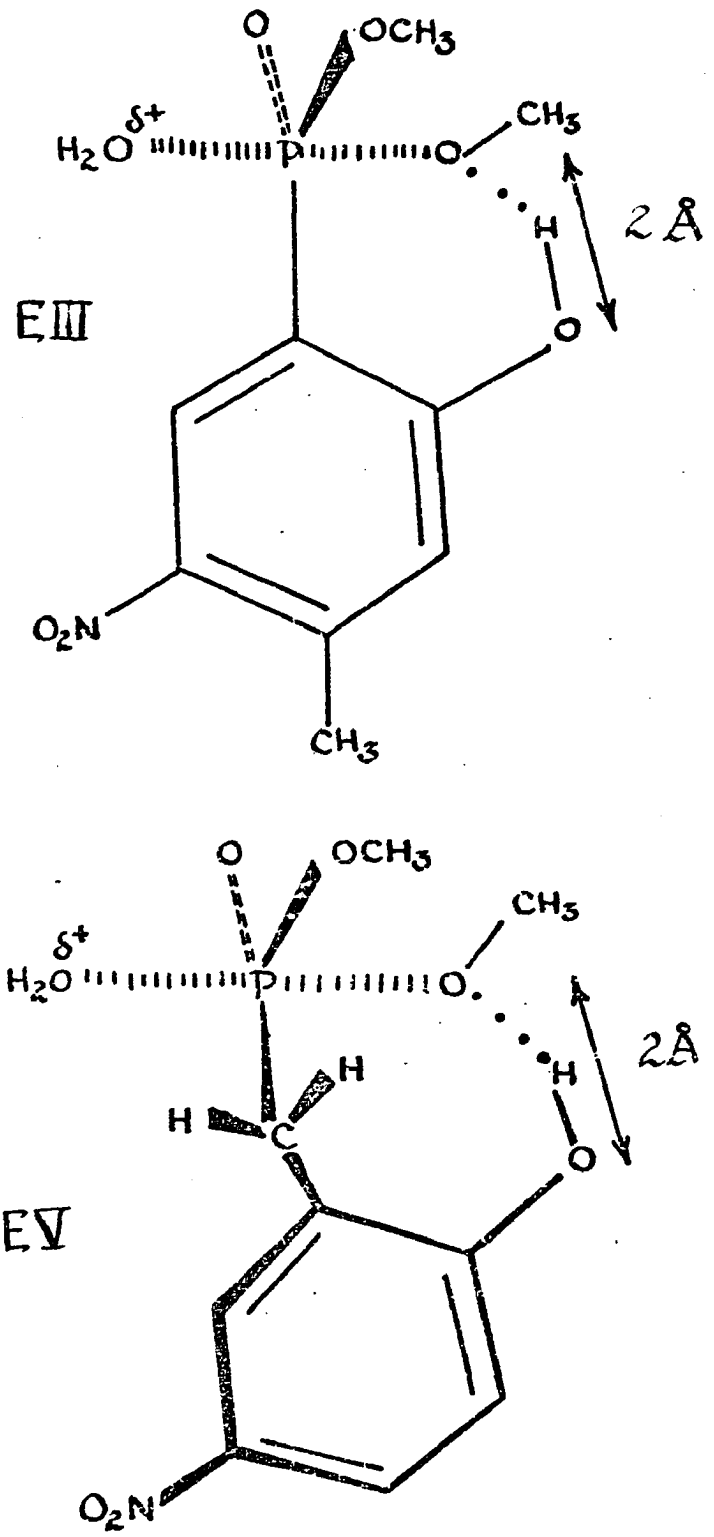

Figure 14

Hydrogen bonding in the proposed transition state for $S_N2(P)$ displacement in EIII and EV

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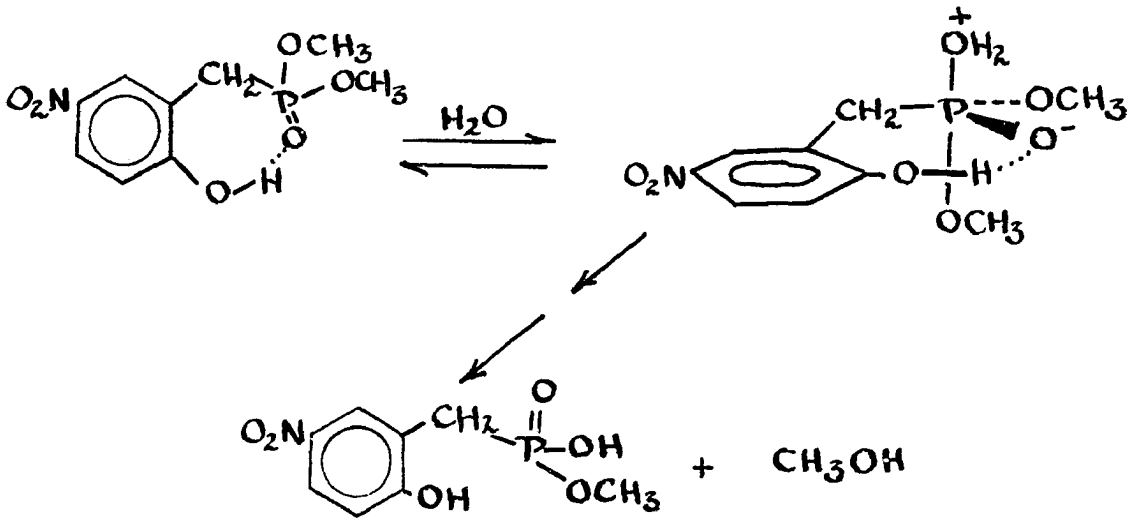
effectively internally acid-catalyzed than that of EV.

iii) Intramolecular Acid-Catalyzed Addition-Elimination



In simple dialkyl phosphonate esters, C-O rather than P-O cleavage is observed, thus making this mechanism improbable (ref. 9, Chap. I). However, in the present case, the possibility of hydrogen bonding to the phosphoryl oxygen (see Figure 12) could render the phosphorus more electrophilic and hence susceptible to nucleophilic attack by water. Looking at the penta-coordinated intermediates (see Figure 15) that would result, EV is, of course, by suitable rotation, able to form an effective H-bond. EIII, however, may also maintain its hydrogen bond with its phosphoryl oxygen-- the O-H···O distance is 2.8 Å;

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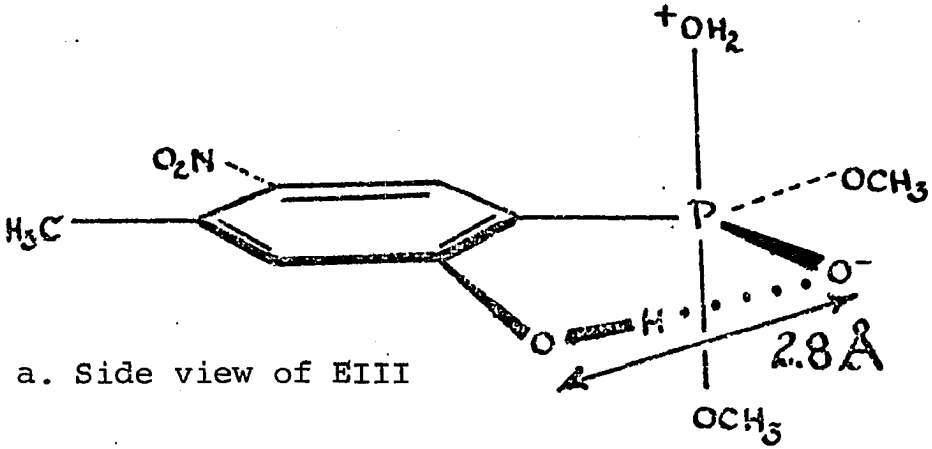
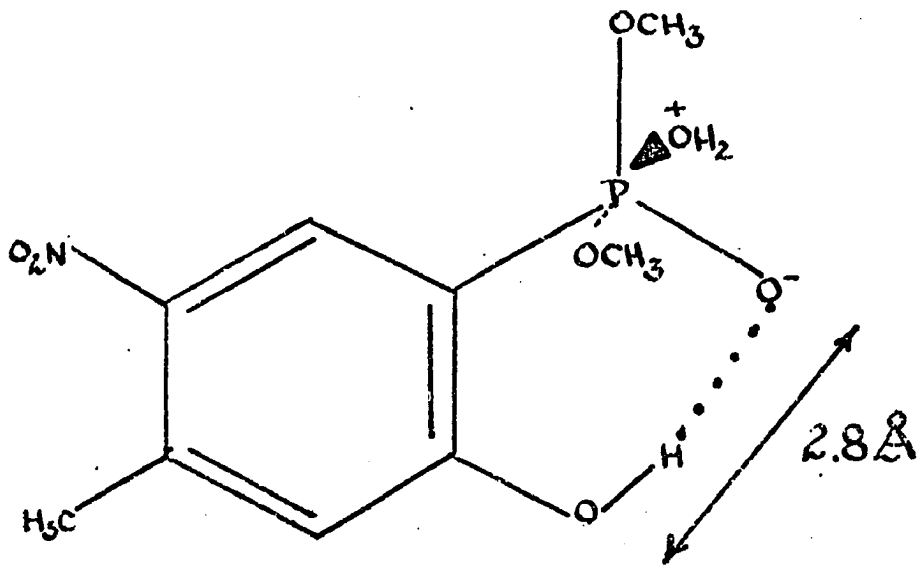
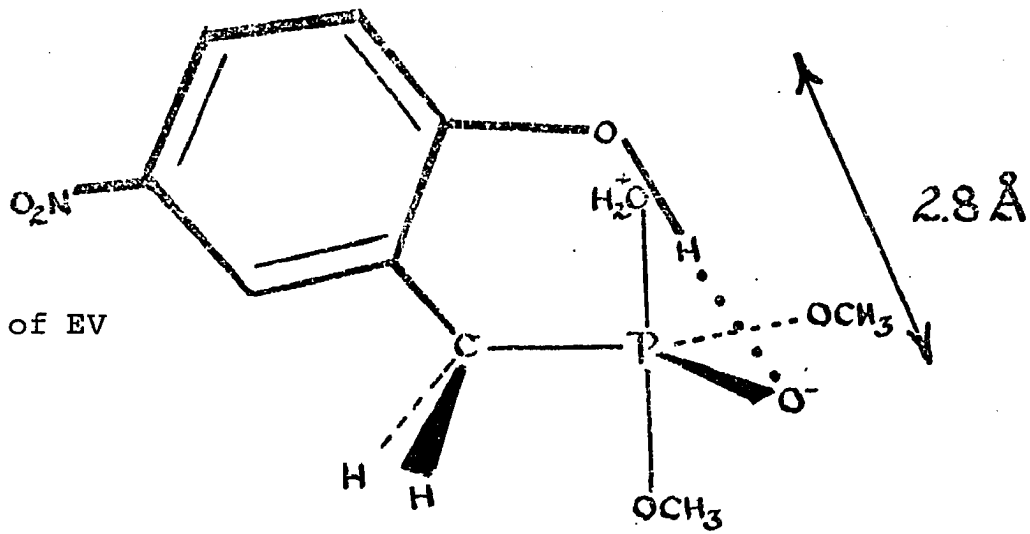


a. Side view of EIII



b. Top view of EIII



c. Side view of EV

Figure 15

Hydrogen bonding in penta-coordinated intermediates of EIII and of EV

furthermore, only a small deviation from linearity exists. Again, one must conclude that if this mechanism were operative, then EIII should also show an enhanced rate of hydrolysis.

The above discussion has attempted to provide arguments against the effectiveness of intamolecular acid-catalysis in the hydrolysis of phosphonate diesters. Examination of Dreiding models supports this investigator's belief that Ester III should have shown equally enhanced rates of hydrolysis as Ester V if any of the above mechanisms were, in fact, operative. Other mechanisms invoking water-mediated reactions of the type supported by Lieske and co-workers (ref. 11, Chap. I) seem equally unreasonable. Intramolecular acid-catalysis seems, therefore, to be effective in accelerating the rate of phosphonate hydrolysis by less than an order of magnitude and cannot account for the accelerations of 10^7 that have been reported.

In keeping with the results of Kirby, Simons, Blackburn (references 3, 6, and 14 of Chapter I) and others, the following mechanism seems most likely.

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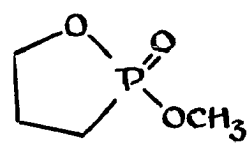
iv) Intramolecular nucleophilic attack by oxygen (see Figure 16)

This mechanism is not available to Ester III due to the enormous strain resulting from formation of an intermediate with a four-membered ring (structure 16). The intermediates shown in Figure 16 are easily constructed with Dreiding models, having very little strain. The cyclic phosphonate, c-EV, on the other hand, is strained and might be expected to undergo rapid ring opening, therefore serving as a proper intermediate (see Figure 17). The phosphonate anhydride 15 underwent hydrolysis with ring opening 26 times faster than the phosphonate diester 12 at pH 2 (ref. 14, Chap.I). Similarly, the methyl phostonate 44 hydrolyzed with greater than 99% ring-opening in acid at 25°C with a rate about ten times greater than EV hydrolysis at 45°C.[23]



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[23] a. E.A. Dennis and F.H. Westheimer, J. Amer. Chem. Soc., 88, 3432 (1966). b. E.A. Dennis, Ph.D. Thesis, Harvard University, 1967.

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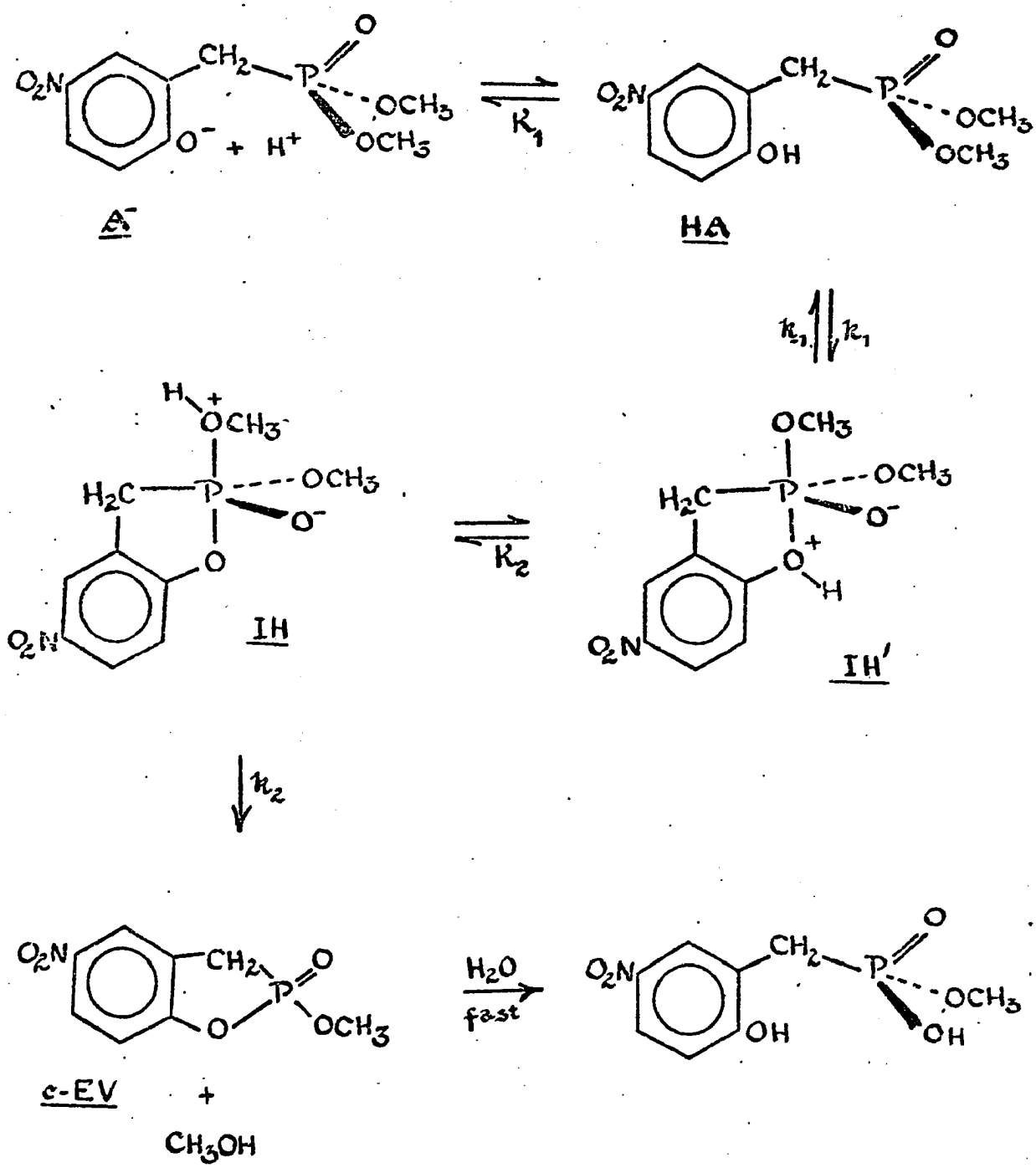

Figure 16

Proposed mechanism for the hydrolysis of EV under acidic conditions

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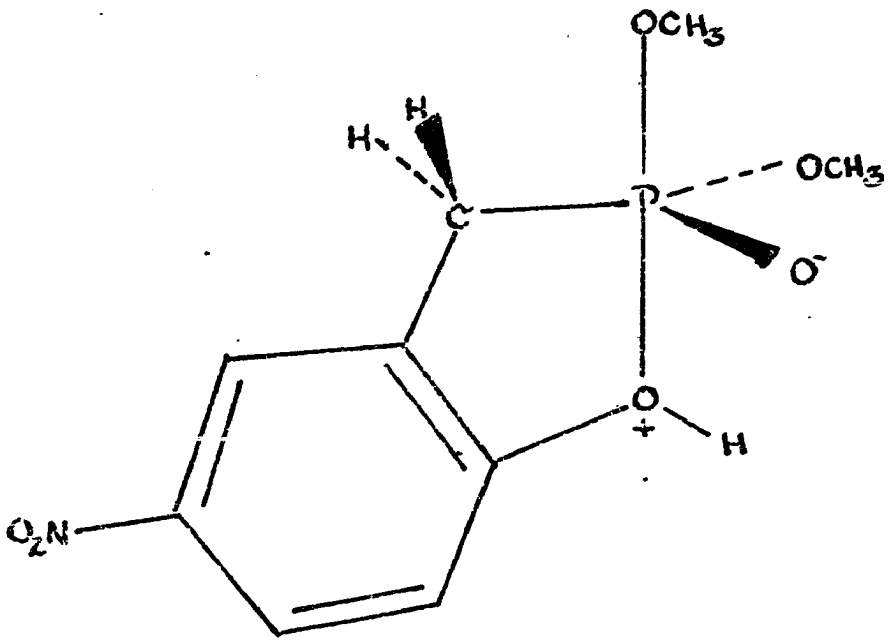
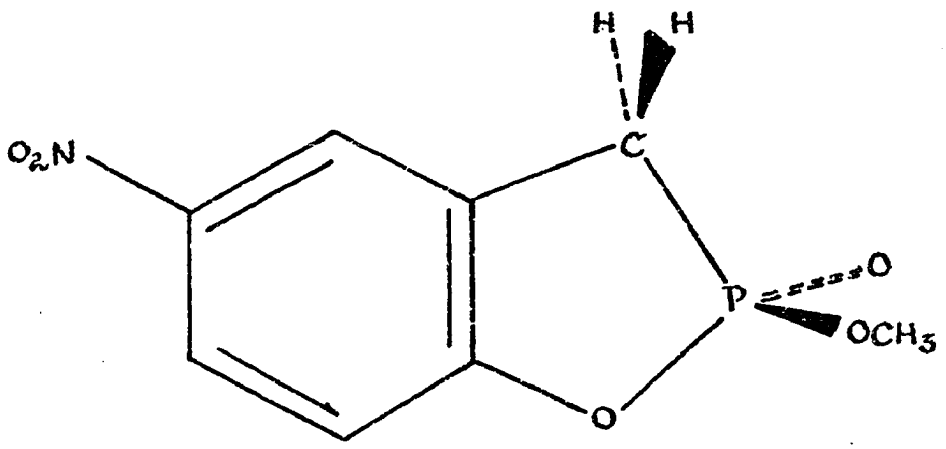
Figure 17



a. The unstrained cyclic penta-coordinated intermediate of of EV hydrolysis



b. The strained cyclic phosphonate, c-EV

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The mechanism described in Figure 16 is closely related to that described by Blackburn[24] for the hydrolysis of diethyl 2-carboxyphenylphosphonate, compound 12. Furthermore, the pH-rate profile determined by uv kinetics for the hydrolysis of EV in this work has the same kinetic expression as that determined for 12 by pH-stat titration. As proposed by Blackburn, two mechanisms that are kinetically indistinguishable from that of Figure 16 are illustrated in Figure 18--pathway "a" involves preliminary protonation of the phosphoryl oxygen by the phenolic moiety whereas pathway "b" involves a concerted, 4-center attack with proton transfer.

The mechanism shown in Figure 16 gives the following rate expression:

$$\text{Rate} = \frac{k_o [S]}{1 + K_1/[H^+]}$$

where $k_o = \dfrac{k_1 k_2}{k_2 + \dfrac{k_{-1}}{K_2}}$ and $[S] = [HA] + [A^-]$.

This expression is derived in Appendix 1.

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[24]G.M. Blackburn and M.J. Brown, J. Amer. Chem. Soc., 91, 525 (1969).

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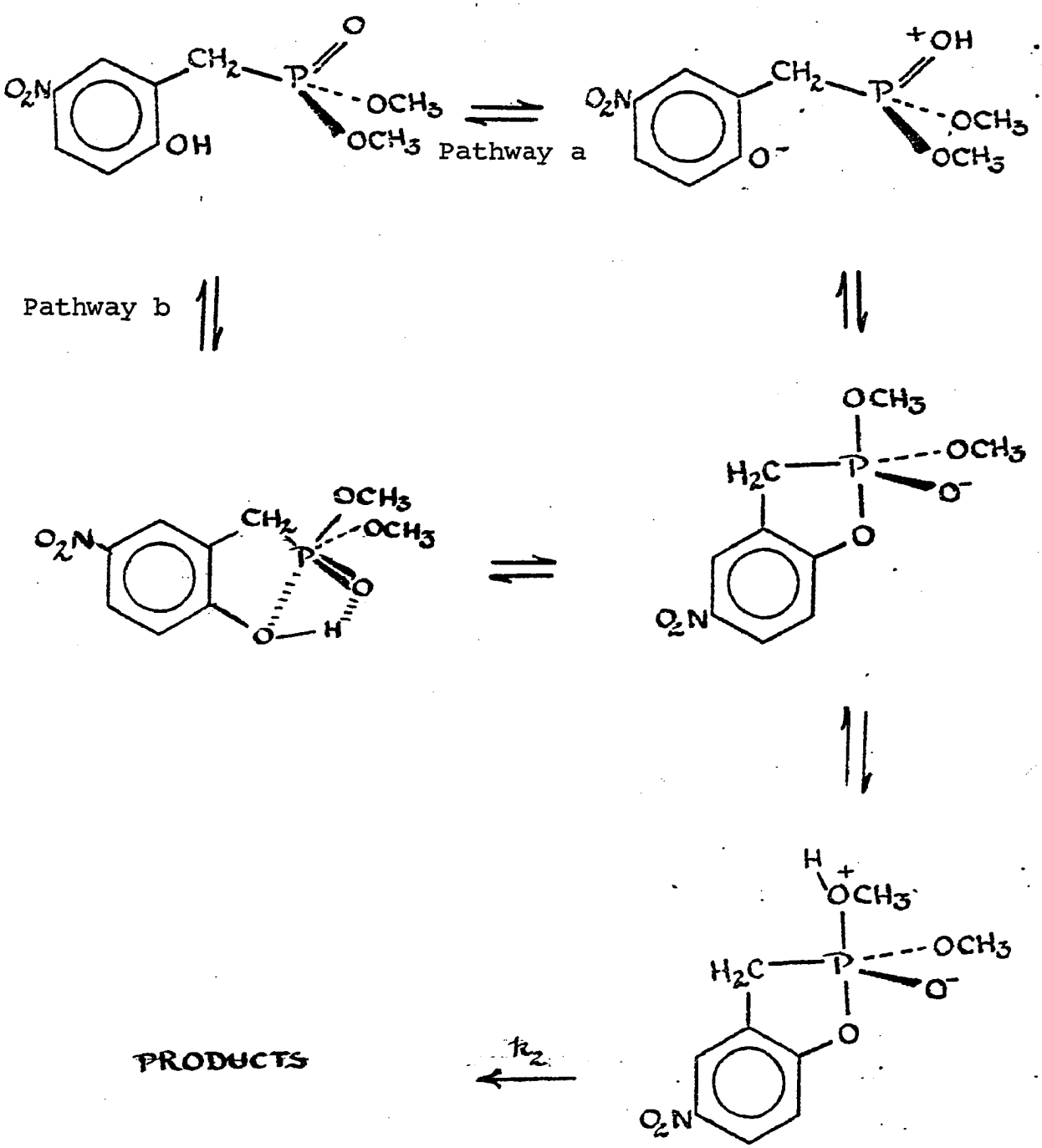

Figure 18

Alternative mechanisms for the hydrolysis of Ester V under acid conditions

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3. Mechanism for Hydrolysis under Neutral Conditions—Buffer Catalysis

In neutral solutions, the rate of hydrolysis of Ester V was found to be strongly dependent on buffer identity and concentration. As described in Section E of this chapter, the second-order rate constants for the buffer-dependent reaction could be easily determined and are given in Table VI. Reaction mechanisms that include participation of buffers usually depict the buffer as a general acid or general base. An excellent account of the phenomenon of general acid and general base catalysis is given by Jencks.[25]

That general acid (or general base) catalysis is involved is supported by the solvent isotope effect observed in phosphate buffers. Figure 8 shows that k_{H_2O}/k_{D_2O} is about 2.8.

The Brønsted plot of the k_{2HB} values for the various buffers used (see Figure 10) gives a line with slope $\alpha = 0.56$. The largest negative deviation from the Brønsted line was for 2,6-lutidinium ion (a factor of 10.5) and dihydrogen phosphate showed a positive deviation by a factor of three.

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[25]W.P. Jencks, "Catalysis in Chemistry and Enzymology," Chapter 3.

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Large negative (as well as positive) deviations are not unusual.[26] For ammonium-type salts and for those buffers with hydroxyl groups, such deviations are attributed to solvation effects.[27] Furthermore, the relative ineffectiveness of sterically hindered buffers such as 2,6-lutidine has been previously demonstrated by Covitz[28] in the general acid catalysis of the inversion of menthone and in the general base catalysis of the hydrolysis of methyl ethylene phosphate and the mutarotation of glucose. In the latter case, 2,6-lutidine was 25 times less effective than predicted by the Brønsted plot.

One may postulate a variety of mechanisms, all kinetically indistinguishable, to describe the role of buffers in aiding the hydrolysis of Ester V. The one described here is most attractive due to its obvious resemblance to that scheme proposed for the hydrolysis of EV under acid conditions. In this scheme, shown in Figure 19, the buffer

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[26]R.P. Bell and W.C.E. Higginson, Proc. Roy. Soc. (London), A197, 141 (1949).

[27]R.G. Kallen and W.P. Jencks, J. Biol. Chem., 241, 5864 (1966).

[28]F. Covitz and F.H. Westheimer, J. Amer. Chem. Soc., 85, 1773 (1963).

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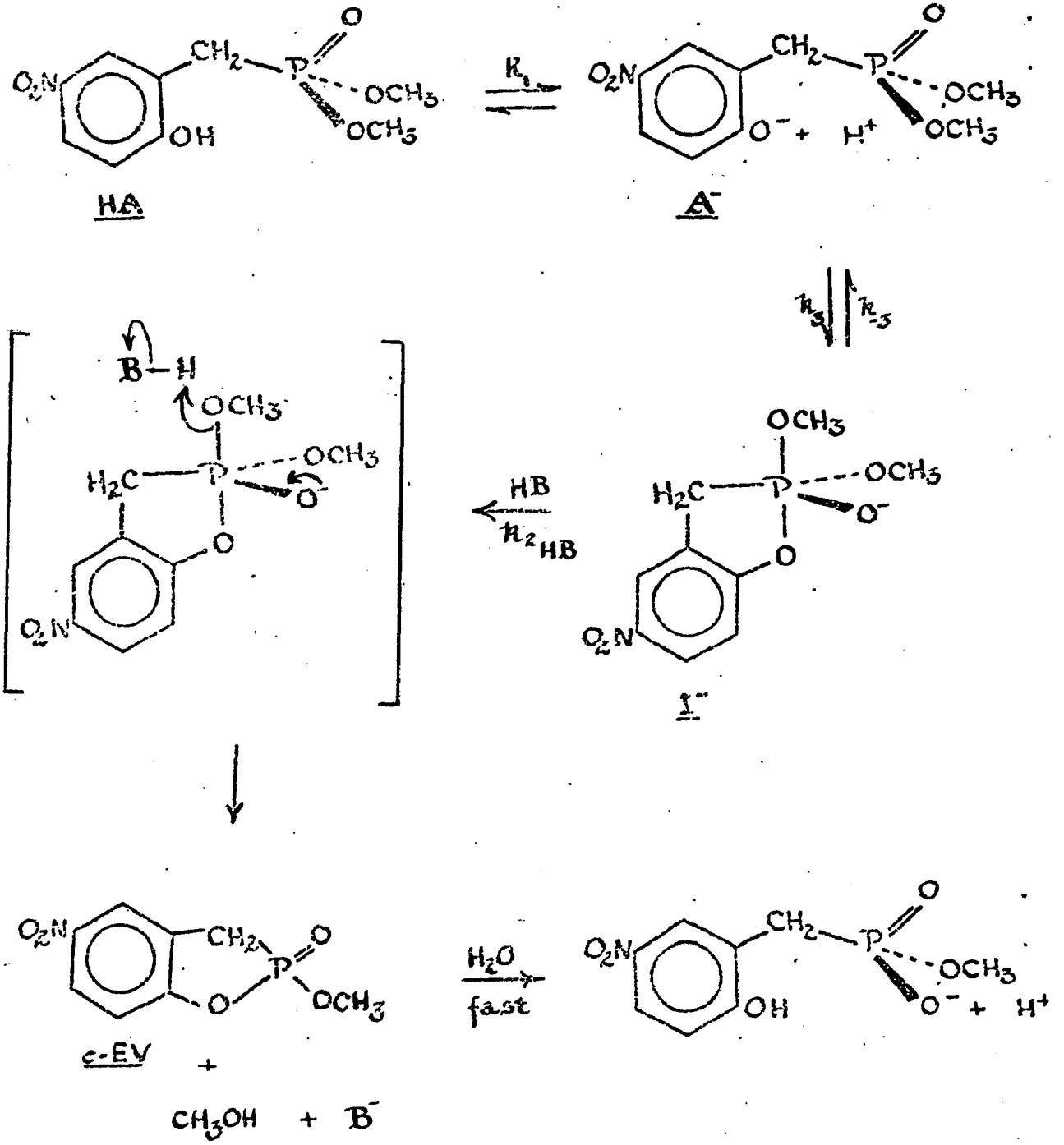

Figure 19

Proposed mechanism for the hydrolysis of EV under neutral conditions with general acid catalysis

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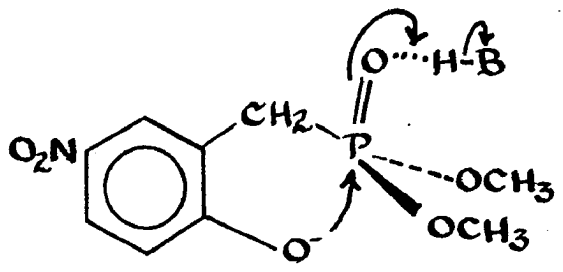
behaves as a general acid in reacting with ionized Ester V. Closely related to this mechanism is one in which the general acid protonates the phosphoryl oxygen:



Under these mechanisms for general acid catalysis, the reaction of ionized EV with water is easily accounted for. In this case, water also acts as a general acid but at a rate much lower than those of the buffers used in these experiments.

Other mechanisms may of course be postulated for these buffer-dependent reactions. The possibility of external nucleophilic attack by the base form of the buffer is one of these. Although the deviations of the sterically hindered buffers, 2,6-lutidine and α-picoline, are perhaps an indication of nucleophilic attack, this explanation seems unlikely. Furthermore, the deviations of these buffers are no larger than those observed by Bell and by Covitz. A possible experiment to resolve this question would involve the use of ^{18}O

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labelled phosphate buffer in the hydrolysis of EV. By the mechanism proposed by Samuel and Silver[29] for the phosphate catalyzed hydrolysis of tetraethyl pyrophosphate, the labelled oxygen would appear in MEV, the product of the hydrolysis of EV.

The rate expression which includes participation of the buffer as shown in Figure 19 is simply:

$$\text{Rate} = \frac{k_{2_{HB}} [HB][S]}{\frac{[H^+]}{K_a} + 1}$$

This expression is derived in Appendix 1.

4. Mechanism for Hydrolysis under Basic Conditions

Saponification of dialkyl phosphonate esters to the monoalkyl ester is known to occur with P-O cleavage. The rate of saponification is substantially reduced when the central phosphorus is sterically hindered. Thus the favored mechanism is a simple $S_N2(P)$ reaction (see ref. 9, Chapter I).

A crude experiment[30] performed on Ester III revealed

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[29]D. Samuel and B. Silver, J. Chem. Soc., 4321 (1961).

[30]An unweighed sample of EIII was dissolved in 1.0 N NaOD. The hydrolysis was followed at NMR probe temperature (ca 30°C).

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that its rate of hydrolysis under very alkaline conditions was approximately that of EV's. Under the same conditions, EII hydrolyzed an estimated ten times faster than EIII and EV.

It appears, therefore, that the $S_N2(P)$ reaction mechanism holds for the present phosphonate esters as well. The ten-fold rate difference is explicable on electrostatic principles--the phenolate ion of EIII and EV repulses hydroxide ion, making its approach to the phosphorus more difficult.

The rate expression for EV hydrolysis by this mechanism is simply:

$$\text{Rate} = \frac{k_{OH}\,[OH^-][S]}{\frac{[H^+]}{K_1} + 1}$$

5. Mechanism for Hydrolysis of EII

Although this work is little concerned with the mechanism of simple phosphonate ester hydrolysis, a few words will be said about it.

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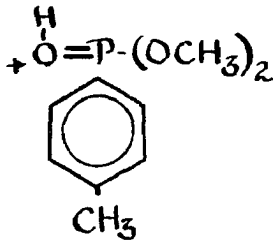
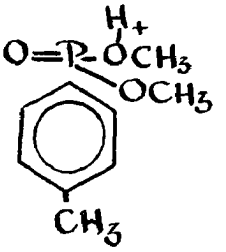
Under strongly acidic conditions, EII exhibits acid-catalysis (see Figure 11). The rate of hydrolysis of EII in 0.8 N p-TSA is comparable to that of dimethyl methylphosphonate, $\left[H_3C\text{-}PO(OCH_3)_2\right]$, under similar conditions.[31] C-O bond cleavage has been observed under such conditions and an $S_N2(C)$ attack by water has been the mechanism of choice. Either of the protonated species shown below would render the phosphonate a better leaving group:





Under mildly acidic to neutral conditions, the rate of hydrolysis appears to be independent of pH. A solvent isotope effect $k_{H_2O}/\ k_{D_2O} \cong 4$ was observed. Such a positive isotope effect suggests general-base catalysis where water may act as the base.[32] Either an $S_N2(C)$ or an $S_N2(P)$ mechanism might be operating:

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[31] R.F. Hudson and L. Keay, J. Chem. Soc., 1956, 2463.

[32] A.J. Kirby and S.G. Warren, "The Organic Chemistry of Phosphorus," p. 306.

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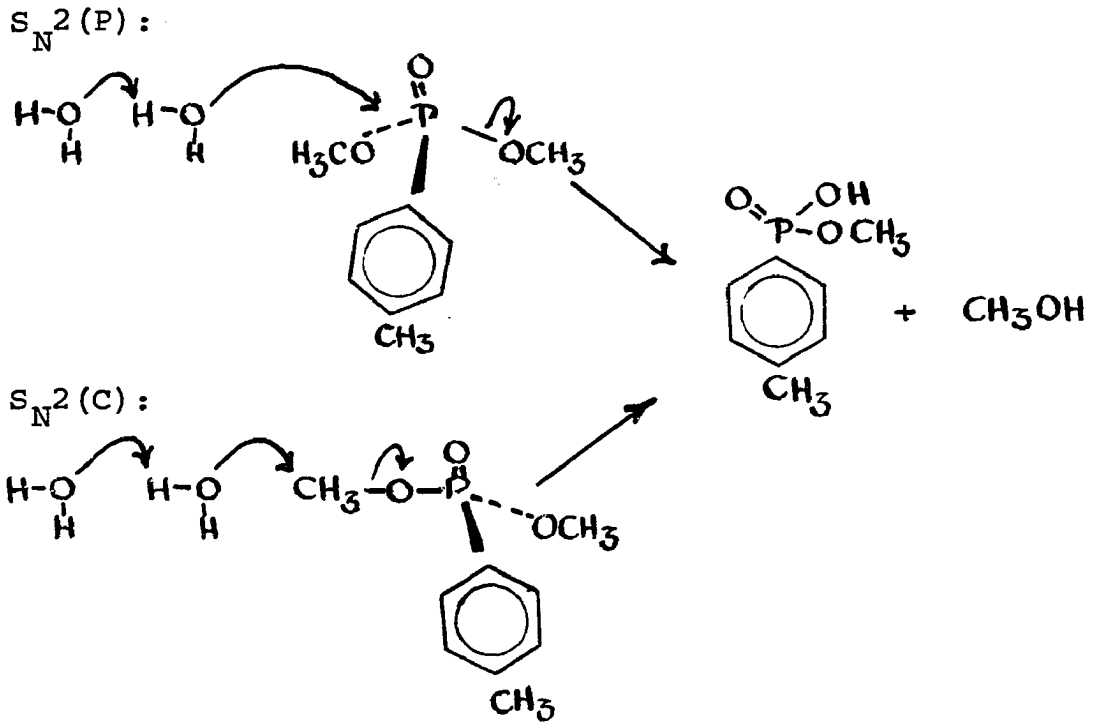

In alkali, the $S_N2(P)$ mechanism is favored, as discussed in the previous section.

6. Calculation of pH-Rate Profiles

Figures 9 and 11 represent the calculated pH-rate profiles for EV and EII, respectively. The dashed lines represent the contribution of each term of the rate expression (see below), the solid lines represent the sum of all contributions. The experimental points are indicated with ▽ (uv kinetics) and ◯ (NMR kinetics). The NMR points taken from runs in D_2O are plotted as pD.

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a. Rate Profile for EII

The rate expression for the hydrolysis of Ester II under acidic and neutral conditions is

$$\text{Rate} = k_{H^+}[H^+][S] + k_{H_2O}[S]$$

so

$$k_{obs} = k_{H^+}[H^+] + k_{H_2O}$$

For the H_2O reactions, the following rate constants were used:

$$k_{H^+} = 2 \times 10^{-3} \text{ min.}^{-1}$$

$$k_{H_2O} = 3.6 \times 10^{-4} \text{ min.}^{-1}$$

For the D_2O reactions, the constants used were:

$$k_{D^+} = 6.5 \times 10^{-4} \text{ min.}^{-1}$$

$$k_{D_2O} = 10^{-4} \text{ min.}^{-1}$$

b. Rate Profile for EV

The rate expression for Ester V hydrolysis from pH 1-14 is

$$\text{Rate} = k_o[HA] + \sum \frac{k_{2HB}[HB][S]}{\frac{[H^+]}{K_1} + 1} + \frac{k_{OH}[OH^-][S]}{\frac{[H^+]}{K} + 1}$$

As shown in Appendix 1, this becomes

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$$\text{Rate} = \frac{k_o[S]}{\frac{K_1}{[H^+]} + 1} + \sum \frac{k_{2HB}[HB][S]}{\frac{[H^+]}{K_1} + 1} + \frac{k_{OH}[OH^-][S]}{\frac{[H^+]}{K_1} + 1}$$

so that

$$k_{obs} = \frac{k_o}{\frac{K_1}{[H^+]} + 1} + \sum \frac{k_{2HB}[HB]}{\frac{[H^+]}{K_1} + 1} + \frac{k_{OH}[OH^-]}{\frac{[H^+]}{K_1} + 1}$$

By extrapolating [HB] to zero as described in Section E of this chapter but retaining the rate due to water as a general acid, the buffer-independent rate expression becomes simply:

$$k_{obs} = \frac{k_o}{\frac{K_1}{[H^+]} + 1} + \frac{k_{H_2O}}{\frac{[H^+]}{K_1} + 1} + \frac{k_{OH}[OH^-]}{\frac{[H^+]}{K_1} + 1}$$

This expression was used to calculate the pH-rate profile given in Figure 9. The following values were used in calculating k_{obs}:

$K_1 = 2 \times 10^{-7}$ M

$k_o = 1.3 \times 10^{-3}$ min.$^{-1}$

$k_{H_2O} = 1.8 \times 10^{-4}$ min.$^{-1}$

$k_{OH} = 2 \times 10^{-2}$ M^{-1} min.$^{-1}$

The values of k_{2HB} determined for the various buffers used in this work are given in Table VI.

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7. Thermodynamic Activation Parameters (E_a and $\Delta S^{\ddagger}$)

The energy of activation, E_a, and the entropy of activation, $\Delta S^{\ddagger}$, of EV hydrolysis were calculated using the treatment of Schaleger and Long.[33] Using rate constants obtained at two different temperatures, E_a is easily calculated from:

$$E_a = \frac{RT_1T_2 \ln k_2 / k_1}{T_2 - T_1}$$

Because the enthalpy of activation, $\Delta H^{\ddagger}$, only differs from E_a by $-RT$,[34]

$$k_{obs} = \frac{kTe}{h}^{(\Delta S^{\ddagger}/R)(-E_a/RT)}$$

$$\Delta S^{\ddagger} = R \ln k_{obs} - R \ln \frac{kT}{h} + \frac{E_a}{T}$$

For the pH "independent" region of EV hydrolysis, (pH 1-4), $k_1 = 1.7 \times 10^{-3}$ min.$^{-1}$ at $T_1 = 45.0^{\circ}C$; $k_2 = 6 \times 10^{-2}$ min.$^{-1}$ at $T_2 = 94.3^{\circ}C$, so

E_a = 17 kcal/mole

$\Delta S^{\ddagger}$ = –26 eu

---

[33] L.L. Schaleger and F.A. Long, in "Adv. in Phys. Org. Chem.," Vol. 1, Academic Press, Inc., New York, 1963, p.1.

[34] A.A. Frost and R. Pearson, "Kinetics and Mechanism," 2nd Ed., p.100.

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At pH 6.9 using phosphate buffer ([HB] ca 0.06 M), $k_1 = 9.8 \times 10^{-3}$ min.$^{-1}$ at $T_1 = 30^{o}$[35], $k_2 = 2.2 \times 10^{-2}$min^{-1} at $T_2 = 45^{o}$, $k_3 = 1.4 \times 10^{-1}$ min.$^{-1}$ at $T_3 = 94^{o}$, so

$$E_a \cong 10 \text{ kcal/mole}$$

$$\Delta S^{\ddagger} \cong -44 \text{ eu}$$

As seen in Table XI, the $\Delta S^{\ddagger}$ for the hydrolysis of

Table XI

Compound	Reference		$\Delta S^{\ddagger}$ (eu)
2	3b	Chap. I	-15
5	5b	Chap. I	-16
9	8c	Chap. I	-19
11	12	Chap. I	-30
EV(acid soln)	this work		-26
EV(neutral soln)	this work		-44

Ester V in acid solution (the buffer-independent reaction) is very nearly equal to those observed by other workers. Mechanisms of the type expounded in this work were preferred in many of the above cases despite the negative entropies of activation calculated. Good explanations for these negative

[35]This was the only rate constant obtained at 30°C, being determined by uv kinetics using the cuvette method.

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values have not yet been proposed, but rigidity of the penta-coordinated transition state is assumed.

The very negative $\Delta S^{\ddagger}$ observed in the neutral solution of EV and phosphate buffer strongly indicates the bimolecularity of the reaction and further supports the scheme given in Figure 19.

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CHAPTER IV

PHOSPHONATE MONOESTERS

This chapter briefly examines the possibility of a "metaphosphate"-like intermediate being involved in the hydrolysis of the ortho-hydroxy monoesters, MEI and MEIII (see Section B-1, Chapter I). As will be shown in Section A, there was no proof for the existence of this type of intermediate.

A brief investigation was also directed at finding an effective metal ion catalyst to promote the rate of hydrolysis of MEI relative to that of MEII. The data from this study are negligible and must be labeled "inconclusive."

Once again, however, the interesting chemistry centers around the benzyl phosphonate, MEV. Relative to Monoester II, it is estimated to undergo hydrolysis 10^5 times faster at pH 7. It also exhibited the ability to continue to hydrolyze in the alkaline region. Its pH-rate dependence was determined in some detail.

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Experimental

NMR kinetics were used predominantly in these studies. The few uv experiments involving MEV hydrolysis in buffered solutions made use of the ampoule method described in Section E-1c, Chapter III.

The first-order kinetic treatments and error analyses are the same as those used for the phosphonate diesters. A Precision Scientific Co. "Time-It" timer was again employed.

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A. The Hydrolysis of Monoesters I, II, and III at 84.5°C

By measuring the pK_a's of Monoesters I and III and by applying the method of Butler (ref. 11, Chap. II), it was determined that, if the mechanism of hydrolysis of MEI and MEIII were to occur through a "metaphosphate"-type intermediate, then the rate maximum should occur at a pH between 5 and 9 for MEI and between 4 and 7 for MEIII. MEII was hydrolyzed to obtain comparative rates. This section describes the preliminary NMR kinetics performed on these monoesters.

1. Experimental

a. Determination of the pK_a's

The pK_a's of Monoesters I and III were determined by the method described in Section E-1g, Chapter III. The values obtained were pK_a(MEI) = 11.8 $\pm$ 0.4 and pK_a(MEIII) = 8.30 $\pm$ 0.05. Beer's Law was demonstrated to hold for these compounds.

b. Preparation of Samples and Kinetic Runs

The NMR samples, 0.25 M in MEI, MEII, and MEIII, were prepared by weighing out a calculated amount of the

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monoesters' sodium salts and dissolving the material in the desired buffer. The buffers in D_2O were prepared according to the "Handbook of Chemistry and Physics."[1] The pH of each sample was measured, and the usual correction for pD applied (ref. 13, Chap. III). The NMR tubes were sealed and placed in an oil bath at 84.5 $\pm$ 0.5°C. The pH of most samples was not measured following hydrolysis, but for those samples whose hydrolyses approached completion, the pH probably decreased by at least one unit.

The extent of hydrolysis was easily determined by dividing the integral for the signal of unreacted monoester by the sum of the integrated signals of monoester + methanol. In the case of MEI, the samples discolored slightly and the spectra were complicated by the growth of a doublet 0.25 ppm downfield from the original $P-O-CH_3$ doublet. Addition of EI to the NMR tube showed that it was not the side product. The side product remains unidentified, but the discoloration suggests

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[1] R.C. Weast, Ed., "Handbook of Chemistry and Physics," 47th Ed., The Chemical Rubber Co., Cleveland, 1964, p. D-73.

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that oxidation of the phenol may have occurred.

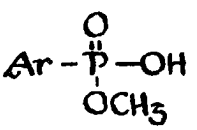
2. Results

The crudely determined first-order rate constants are given in Table X. Rather than finding the hoped-for acceleration in the rates of hydrolysis of MEI and MEIII at pH 4 or 5, these esters actually hydrolyzed less readily than MEII.

3. Discussion

Table X shows that the hydrolysis of Monoesters I, II, and III is acid-catalyzed. Were the spontaneous "monomeric metaphosphate"-type reaction discussed in Section B-1, Chapter I, operative, it should have been most favorable for MEIII due to resonance stabilization of the intermediate; a rate maximum at pH 4-5 would be expected. Evidence for such a mechanism is obviously lacking.

The acid-catalysis observed points to the monoalkyl-monoacid 46 as the reactive species in solution. A



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reasonable reaction mechanism would parallel that discussed in Section F-5, Chapter III, on the hydrolysis of Ester II. The slightly decreased rates of MEI and MEIII hydrolysis relative to that of MEII are not really compatible with the $S_N2(C)$ mechanism, however. An examination of molecular models (compare Figures 9 and 12) shows that some degree of hydrogen bonding is lost on going from a tetrahedral to a trigonal bipyramidal phosphorus. Therefore, a mechanism involving a re-hybridization of phosphorus in the transition state (such as an $S_N2(P)$ reaction) may actually be invoked. ^{18}O tracer studies could help resolve this question.

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Table XII

First-Order Rate Constants for the Hydrolysis of MEI, MEII, and MEIII

($T = 84.5 \pm 0.5^{\circ}C$)

k_1 $min.^{-1}$

Conditions	MEI	MEII	MEIII
DCl buffer pD ca. 1.3	$2x10^{-4}$	$2.2x10^{-4}$	ca $2x10^{-4}$
Potassium biphthalate-DCl pD ca. 4.0		$6x10^{-6}$ (65% hydrolysis after 130 days)	$1.5x10^{-6}$ (23% hydrolysis after 130 days)
Acetic acid-DCl pD ca. 5.1	Not measurable after 130 days	$7.7x10^{-7}$ (11% hydrolysis after 130 days)	

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B. The Hydrolysis of Monoester II and Monoester V at 94.3°C

Preliminary NMR investigations on the behavior of Monoester V in aqueous solution showed it to undergo rapid hydrolysis relative to the other monoesters prepared. As with its parent compound, Ester V, the pH-rate dependence of the hydrolysis was determined. MEII was used as a comparative standard.

1. Experimental

a. Determination of pK_a

The pK_a of the phenolic function of MEV was determined as described in Section E, Chapter III. Its value was found to be 7.55 $\pm$ 0.04. Beer's Law was again found to hold for this material.

b. Preparation of Samples and Kinetic Runs

For use in uv kinetics, a 10^{-3} M stock solution of MEV was prepared by dissolving 6.7 mg of its pure sodium salt in 25 ml deionized water. As described in Section E-1c, Chapter III, sealed ampoules were prepared. The ampoules were placed in a 94.3°C oil bath.

The NMR kinetics for both MEII and MEV were per-

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formed by mixing 0.1 ml of an approximately 0.5 M stock solution of their sodium salts with 0.4 ml of a buffer solution. The sodium salt of MEV contained its hydrolysis product, 2-hydroxy-5-nitrobenzylphosphonic acid, as a contaminant (ca 30% by NMR).[2] The deuterated buffers were of ionic strength 0.1 and were prepared according to directions in the "Biochemists' Handbook" (ref. 11, Chap. III). The pH was measured before and after each run and corrections for the actual pD applied. The sealed NMR tubes were placed in the 94.3°C bath.

Because of the large solute concentrations required for NMR studies, it could not be expected that the buffers would be entirely effective in maintaining a constant pD. To minimize the change in pD, the NMR experiments were only allowed to proceed through less than one half-life (usually about 33% hydrolysis was allowed to occur). In most cases, the pD did not change by more than 0.1 to 0.2 pD units. The identity of methanol

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[2] In a few later experiments, the monoester-monoacid of MEV was used. Solution was achieved by dissolving the material in dil. NaOD.

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as a product was checked by addition of an authentic sample.

The uv data was treated in the manner described in Section E, Chapter III. The NMR data was also treated in the usual manner.

2. Results

The rate constants obtained for the hydrolysis of MEV and MEII are noted in Tables XIII and XIV. The pD-rate profiles for the hydrolysis of Monoester V and II are shown in Figures 20 and 21.

3. Discussion

The following discussion attempts to formulate mechanisms for the hydrolysis of Monoester V in the pD range 1 to 14. Unlike the phosphonate diesters, intramolecular catalysis in the hydrolysis of phosphonate monoesters has drawn little attention (ref. 14, Chap. I). Some of the arguments presented below are suggested by work that has been performed on dialkyl phosphates.

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Table XIII

The Hydrolysis of Monoester V in Buffered Solutions

(T = 94.3 ± 0.5°C)

Buffer	pD	$k \cdot 10^3$ min.$^{-1}$*
DCl, 0.10 M	1.5	20
Formate, 0.57 M	3.4	4.6
Acetate, 0.15 M**	5.0(pH)	3.8
Acetate, 0.22 M	5.3	3,2
Phosphate, 0.08 M**	6.0(pH)	2.5
Phosphate, 0.05 M	6.4	2.3
Phosphate, 0.05 M**	7.0(pH)	2.2
ND_3, 0.17 M	7.3	1.9
Acetate, 0.15 M***	7.3	2.5
Tris, 0.17 M**	7.8(pH)	2.7
ND_3	8.4	1.8
ND_3	9.3	1.6
ND_3, 0.5 M	9.9	1.1
NaOD	ca 12	0.52
NaOD, 0.10 M	ca 13	0.43

*All rate constants but those indicated were determined by NMR kinetics using initial rates; an error of ± 15% is allowed.

**Determined by uv kinetics using the ampoule method.

***This sample was prepared by using a solution of MEV-A dissolved in NaOD and diluted with acetate buffer.

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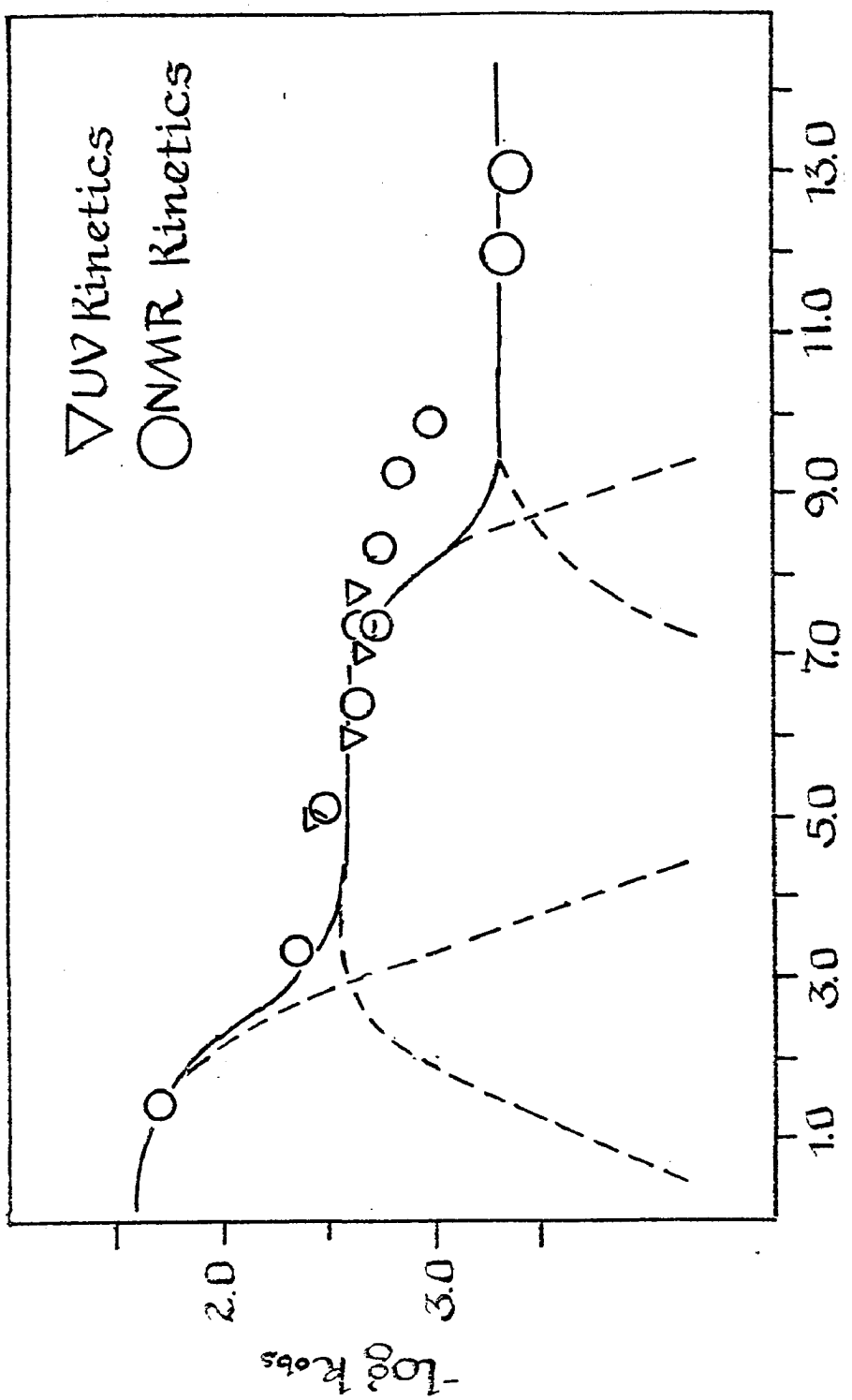

Figure 20

The pD-rate profile for the hydrolysis of Monoester V at 94.3°C

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Table XIV

The Hydrolysis of Monoester II in Buffered Solutions

(T = 94.3 ± 0.05°C)

Buffer	pD	$k \cdot 10^5$ min.$^{-1}$*
DCl, 0.10 M	1.4	81
DCl-KCl, 0.10 M	2.1	29
Formate, 0.57 M	3.4	3.8
Acetate, 0.15 M	5.2	ca 0.10

* An error of ± 15% is allowed.

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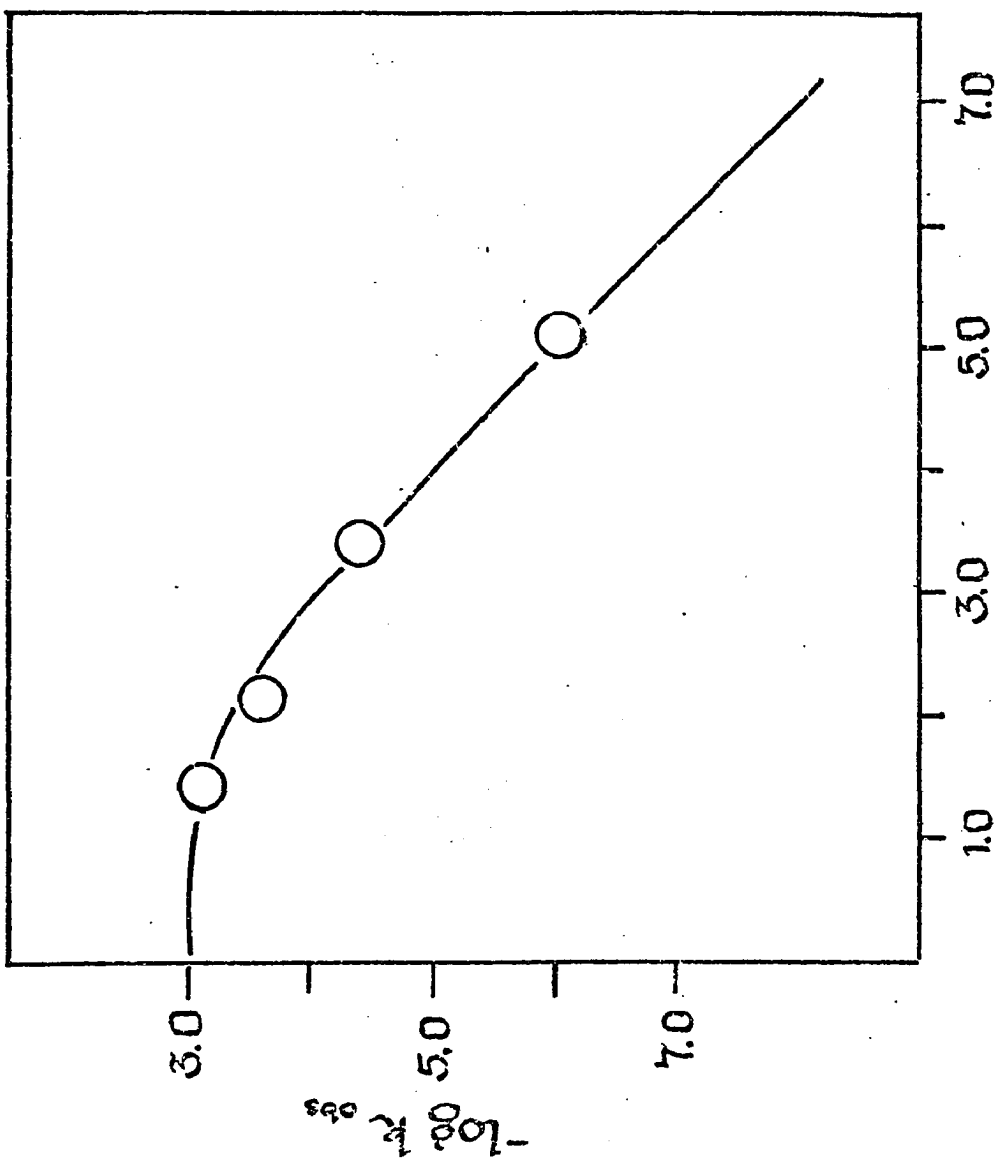

Figure 21

The pD-rate profile for the hydrolysis of Monoester II at 94.3°C

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a. Mechanism for Hydrolysis under Acidic Conditions

The reactive phosphonate in acid solution must be the neutral monoester-monoacid of MEV. The most reasonable mechanism (see Figure 22) under these conditions is one analogous to that proposed in Section F-2, Chapter III for the hydrolysis of Ester V in acidic medium. In fact, the rate of hydrolysis of MEV is nearly equal to that of EV at 94.3°C.

b. Mechanism under Neutral Conditions

In the pD range of 4 to 7.3, the hydrolysis of MEV was observed to continue at a constant rate. The possibility of buffer catalysis as seen in the hydrolysis of EV was not thoroughly investigated for MEV. There are, however, indications that it is absent in this pD range. Examination of Table XIII reveals that phosphate buffer concentration was varied (as was the pD) without change in rate. Furthermore, both the acetate buffer (completely ionized at pD 7.3) and the ND_3 buffer (completely protonated at pD 7.3) did not give different results for the rate of hydrolysis of MEV. No solvent isotope effect was noted on changing from

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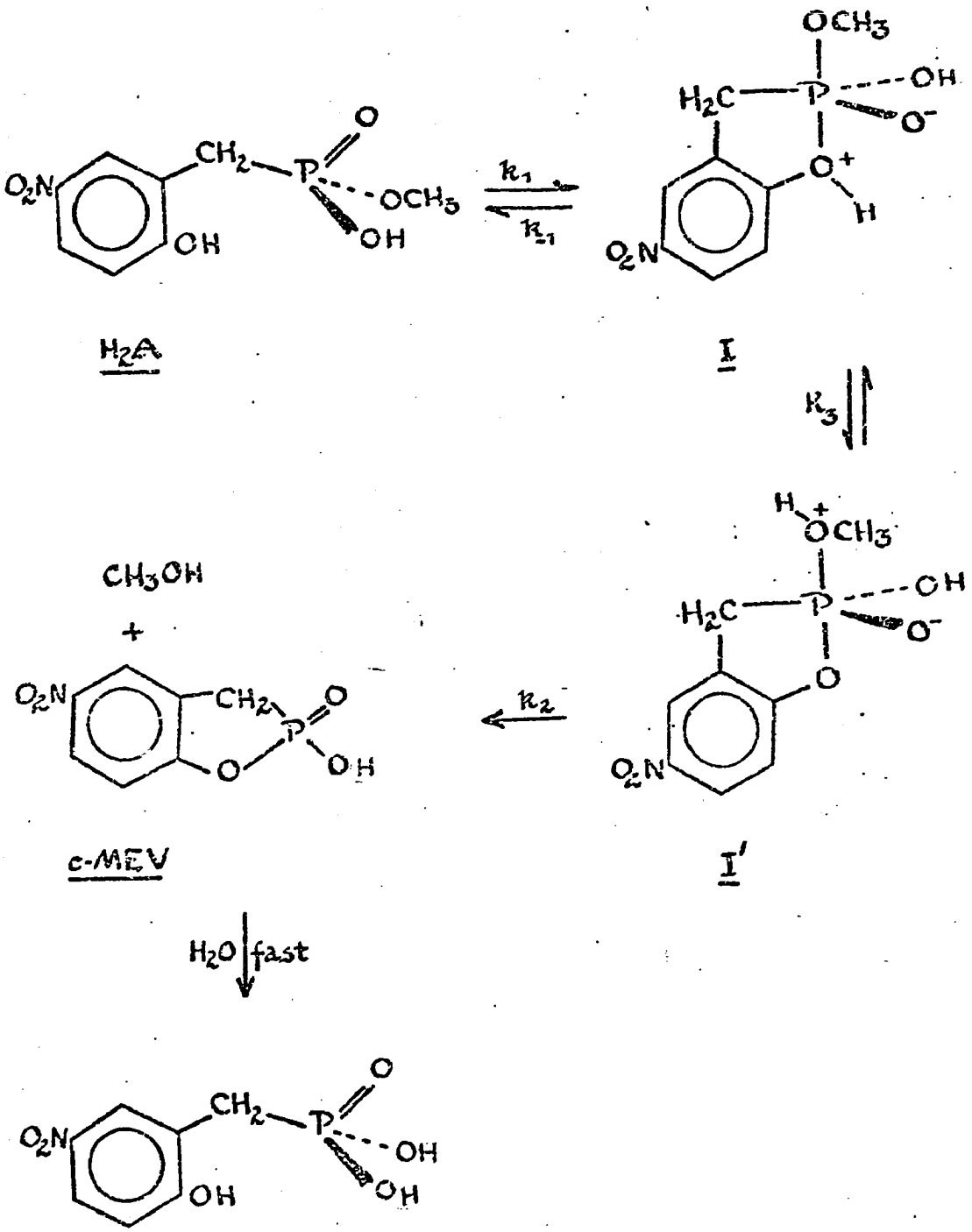

Figure 22

Proposed mechanism for MEV hydrolysis under acidic conditions

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H_2O (uv experiments) to D_2O (NMR experiments). It appears, therefore, that general acid catalysis is not involved in the hydrolysis of MEV in the pD range 4 to 7.3. The possibility of buffer catalysis should, however, be more thoroughly investigated.

One may again propose an intramolecular attack by phenol on phosphorous (see Figure 23). This differs from the previous mechanism shown in Figure 22 only in that the phosphonic acid moiety is now ionized.

The rate expression as derived in Appendix 1 is

$$\text{Rate} = \frac{k_{HA}[S]}{1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}}$$

where $k_{HA} = \frac{k_3 k_4}{\frac{k_3}{k_4} + k_4}$

c. Mechanism for Hydrolysis under Basic Conditions

Examination of Table XIII reveals that the rate of hydrolysis of MEV does not drop sharply, as one might expect on electrostatic grounds, as the hydroxyl moiety becomes ionized. Most monoalkyl phosphonates are stable to hydrolysis

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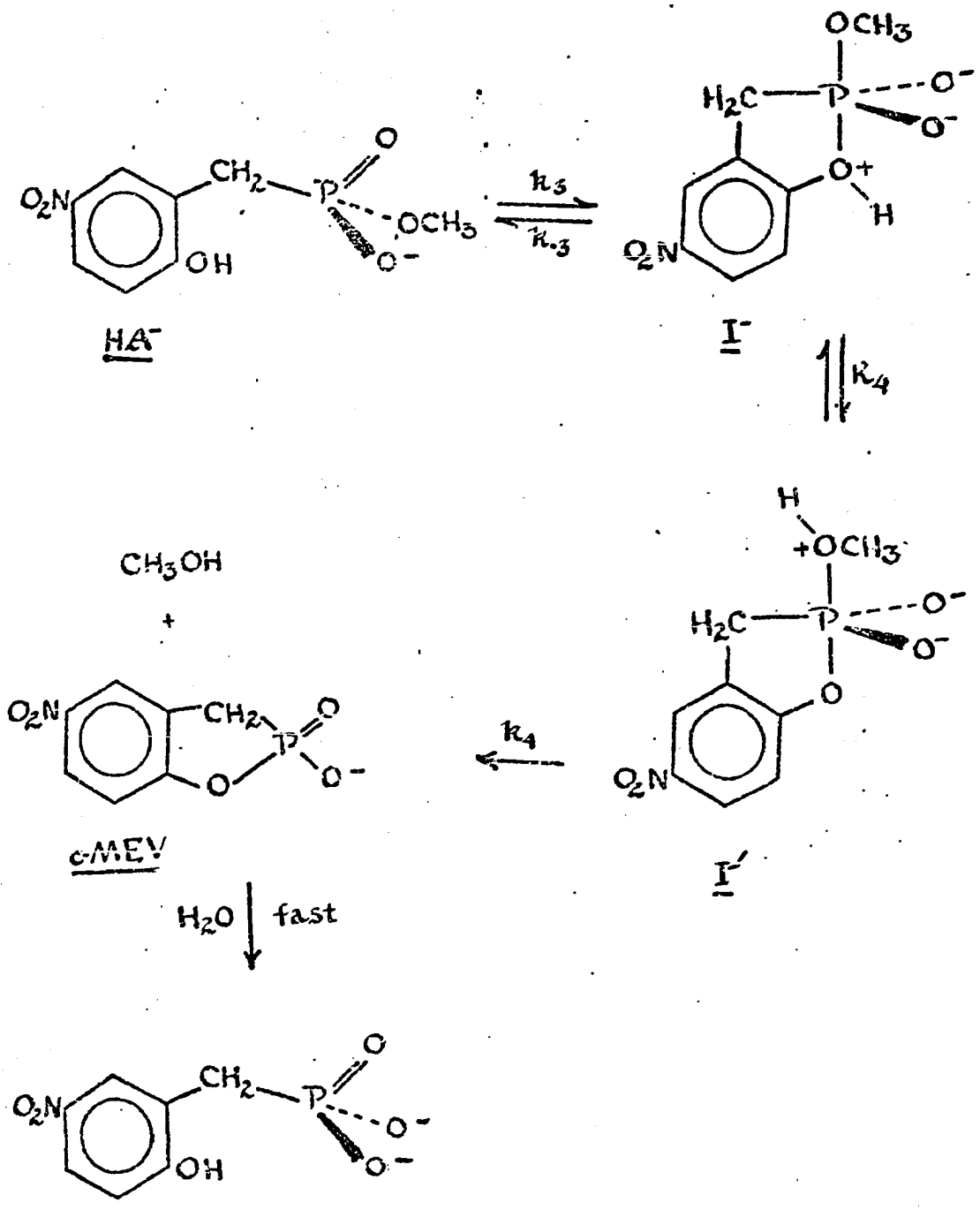

Figure 23

Proposed mechanism for the hydrolysis of MEV under mildly acidic conditions

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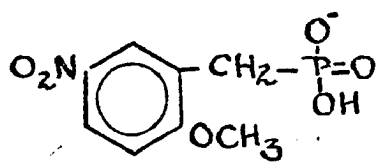
under alkaline conditions.[3] At pH 10, MEII showed no signs of hydrolysis after six days at 94.3°C; in 1 N NaOD, no saponification of MEII and MEIII was apparent after 2 days at 94.3°. Surprisingly, however, the dianion of MEV continued to hydrolyzed at readily measurable rates.

The possibility of an $S_N2(C)$ displacement by phenoxide to yield 48 was ruled out:

1. the NMR singlet observed to grow as hydrolysis proceeded was enhanced by addition of methanol.
2. the Ar-O-CH_3 absorption of EIV (see NMR spectrum 11b) is at 4 δ whereas methanol appears at ca 3.5 δ.



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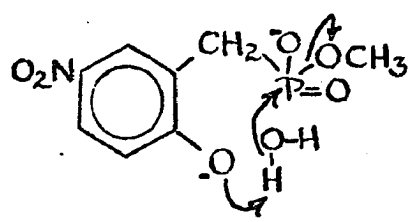
A water-mediated phenolate-catalyzed $S_N2(P)$ attack was considered:



[3] R.Rabinowitz, J. Amer. Chem. Soc., 82, 4564 (1960).

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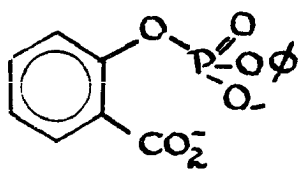
If this mechanism were operative, then MEIII should also undergo hydrolysis in an alkaline medium--it doesn't.

Kirby and co-workers (ref. 3c, Chap. I) hydrolyzed the aryl groups from the diphosphate esters 3 at rates 10^7-10^8 times faster than diphosphates without the benefit of a carboxyl group. The phenyl diphosphate ester 49 did hydrolyze some 10^3 times slower than the phenyl triphosphate ester 50, however. Thus, despite



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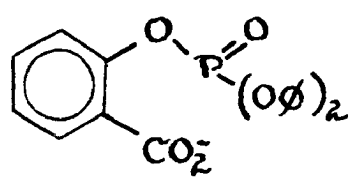

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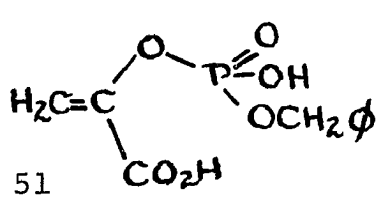
the unfavorable concentration of negative charges, nucleophilic catalysis may still occur. Kirby's suggested mechanism involves formation of a penta-coordinated intermediate; the products of the hydrolysis are consistent with the rules of pseudo-rotation (ref. 22, Chap. I). Schray and Benkovic (ref. 5b, Chap. I) suggested a similar mechanism for the hydrolysis of 51.



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Intramolecular attack at phosphorus by oxygen is not unreasonable in the present case. Figure 24 illustrates the proposed mechanism for hydrolysis under basic conditions. This mechanism is seen to be entirely analogous to that proposed for the buffer-catalyzed hydrolysis of EV (see Figure 19). In this case, water may act as the general acid. The positive deviation of the kinetic rate constants determined at pD's 8 to 10 from the calculated rate curve shown in Figure 20 may be a result of general acid catalysis by the ammonia and tris buffers. This could be easily determined by doing buffer concentration experiments as described in Section E, Chapter III.

The rate expression for this scheme, as derived in Appendix 1 is:

$$\text{Rate} = \frac{k_A[S]}{1 + \frac{[H^+]}{K_2} + \frac{[H^+]^2}{K_1 K_2}}$$

where $k_A = = \dfrac{k_{H_2O}\ k_5}{k_{-5} + k_{H_2O}}$

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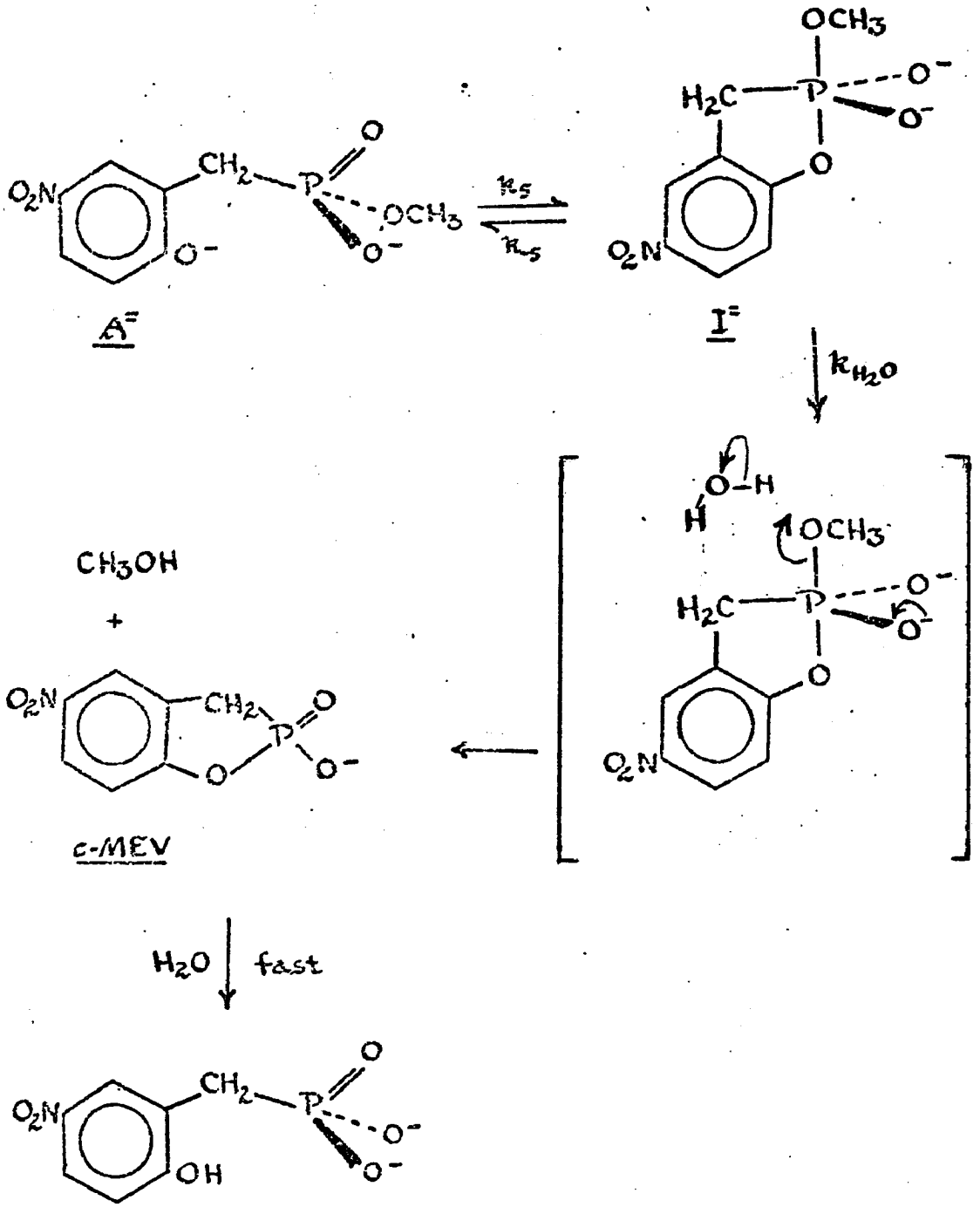

Figure 24

Proposed mechanism for the hydrolysis of MEV under basic conditions

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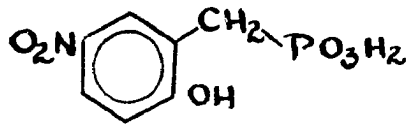
The cyclic phosphonate c-MEV would not be expected to be isolable under alkaline conditions due to the high ring strain and due to the presence of a good leaving group (i.e. p-nitrophenoxide) as in compound 24. In fact, addition of the phosphonic acid 52[4] to a partially hydro-



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lyzed sample of MEV in NaOD enhanced the NMR signal from one of the methylene doublets.[5] This confirms the identity of the product and shows that the postulated cyclic phosphonate does not accumulate.

d. Comparison of Mechanism for the Hydrolysis of EV and MEV

In most respects, the proposed mechanisms for the hydrolysis of EV and MEV are quite analogous. As would be

---

[4]Compound 52 was prepared by acid ahydrolysis of EV. The resultant material was dissolved in NaOD.

[5]As hydrolysis proceeds, the original methylene doublet (3.4 δ) is shifted upfield. It was this shifted doublet that was enhanced by addition of 52. The signals for both doublets were weak in NaOD due to deuterium exchange.

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expected from earlier studies[3], MEV would not exhibit the hydroxide $S_N2(P)$ attack noted in the hydrolysis of EV. For both EV and MEV, an intramolecular attack by phenolic oxygen (either protonated or unprotonated) at phosphorus is assumed. Penta-coordinated intermediates with structures consistent with the strain rule and the electronegativity preference rule have been indicated. Pseudo-rotation of the intermediates is unnecessary (see ref. 4, Chap. I). The mechanisms involving intramolecular nucleophilic attack at phosphorus were formulated in order to account for the accelerated rates of hydrolysis. EV was found to hydrolyze up to 10^2 times faster than EII or EIII. MEV underwent hydrolysis an estimated 10^6 times faster than MEII at pD 7.

By taking into account the difference in temperatures (ca 50°C) at which the rate constants for hydrolysis of EV and MEV were determined, a comparison reveals that EV hydrolyzes more readily than MEV. This is as expected, particularly at the pH's where the phenolic oxygen is ionized. Electrostatic repulsion would make the intramolecular attack of oxygen at phosphorus more difficult for MEV.

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e. Calculation of pD-Rate Profiles

The calculated and experimental pD-dependence of the rate of hydrolysis of Monoesters V and II at 94.3°C are shown in Figures 20 and 21, respectively.

The observed rate constant for hydrolysis of MEII is easily calculable:

$$\text{Rate} = \frac{k_1[S]}{1 + \frac{K_1}{[H^+]}}$$

$$\text{so } k_{obs} = \frac{k_1}{1 + \frac{K_1}{[H^+]}}$$

where

$$k_1 = 10^{-3} \text{ min.}^{-1}$$

$$K_1 = 10^{-2} \text{ M (estimated}^6\text{)}$$

For MEV, the observed rate constant is the sum of three terms:

$$k_{obs} = \frac{k_{H_2A}}{1 + \frac{K_1}{[H^+]} + \frac{K_1K_2}{[H^+]^2}} + \frac{k_{HA}}{1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}}$$

$$+ \frac{k_{A=}}{1 + \frac{[H^+]}{K_2} + \frac{[H^+]^2}{K_1K_2}}$$

---

[6]H.H. Jaffé, L.D. Freedman, and G.O. Doak, J. Amer. Chem. Soc., 75, 2209 (1953).

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This expression is derived in Appendix 1. The following values were used in calculating the pD-rate curve:

$K_1 = 10^{-2}$ M (estimated[6])

$K_2 = 10^{-8}$ M (measured and corrected, see below)

$k_{H_2A} = 2.6 \times 10^{-2}$ min.$^{-1}$

$k_{HA^-} = 2.5 \times 10^{-3}$ min.$^{-1}$

$k_{A^=} = 5 \times 10^{-4}$ min.$^{-1}$

A correction for K_2 (ionization of the phenol) in D_2O was applied:

$$\Delta pK = pK_{DA} - pK_{HA}$$

$$= 0.48 \text{ for p-nitrophenol}^{7}$$

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[7] A.O. McDougall and F.A. Long, J. Phys. Chem., 66, 429 (1962).

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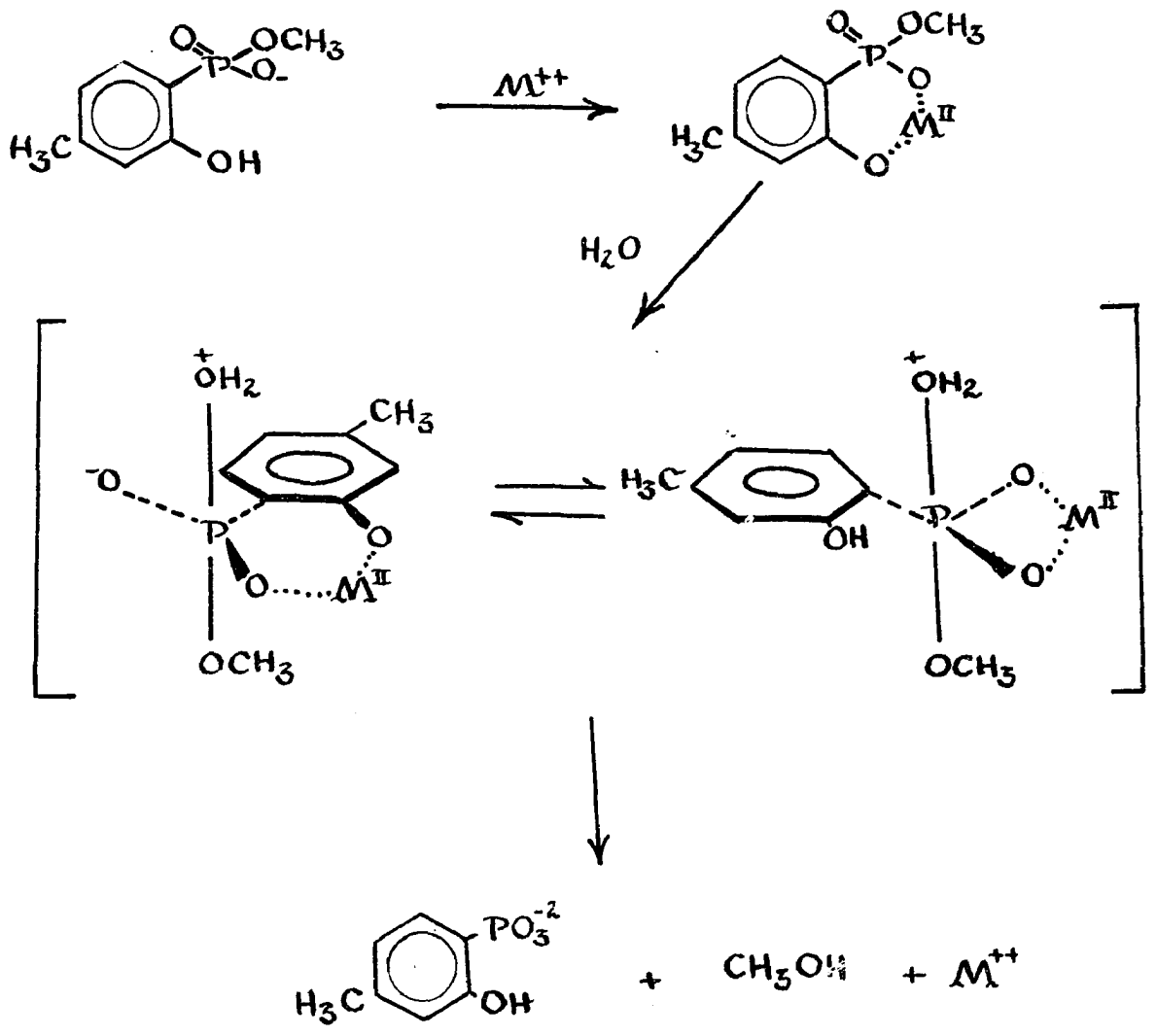
C. Metal Ion Catalysis in the Hydrolysis of Monoesters I and II-- A Preliminary Search

From enzymic and non-enzymic examples (see Section B-2, Chapter I), one may postulate that metal ions could be effective catalysts in the hydrolysis of the phosphonate monoesters by the following pathway (shown for MEI):



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The divalent metal ion could render the phosphorus more electrophilic by dispersing charge from the negatively charged oxygens.

Preliminary studies using nitrate salts of some divalent metal ions with Monoesters I and II were briefly pursued.

Experimental and Results

With a Hamilton syringe, 0.2 ml of 0.4 M D_2O solutions of MEI and MEII were transferred to NMR tubes. To these were added 0.2 ml of D_2O solutions of magnesium nitrate, cadmium nitrate, zinc nitrate, or nickel nitrate. The following combinations produced precipitates: cadmium nitrate and MEI, zinc nitrate and MEII, zinc nitrate and MEI (after heating). The NMR tubes were sealed and placed in a thermostated oil bath at 84.5°C.

Aftter one month of heating, neither MEI nor MEII with $Mg(NO_3)_2$ or $Cd(NO_3)_2$ showed signs of hydrolysis; likewise for MEI and II with $Ni(NO_3)_2$[8] or $Zn(NO_3)_2$ after

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[8]Paramagnetic Ni^{++} did not allow for resolution of the NMR spectra.

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one week. After these discouraging results, this study was abandoned.

Obviously, the search for a metal ion catalyst is far from complete. The NMR method is rather limited in that such biologically important metals as iron, manganese, and copper cannot be effectively studied due to their paramagnetism.[9]

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[9]F.A. Cotton and G. Wilkinson, "Advanced Inorganic Chemistry," Interscience Publishers, USA, 1962, pp. 505-509.

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APPENDICES

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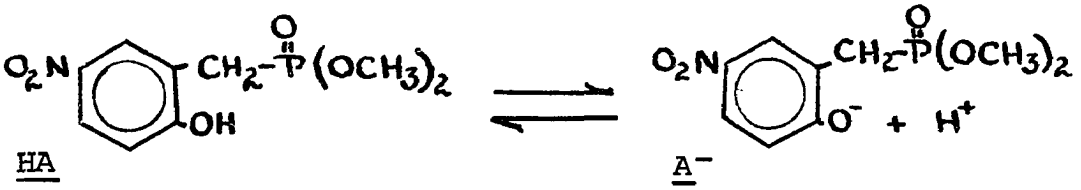
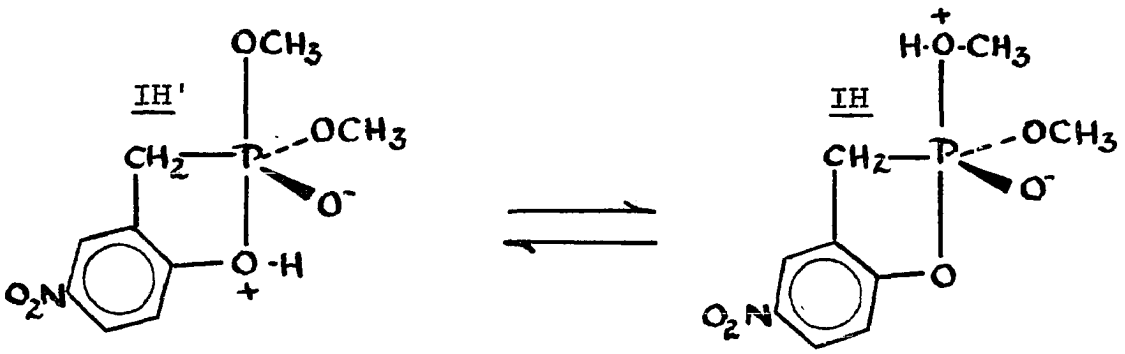
Appendix 1: DERIVATION OF RATE EXPRESSIONS

A. Hydrolysis of EV



HA $\rightleftharpoons$ A^- + H^+

$$K_1 = \frac{[A^-][H^+]}{[HA]} \qquad 1)$$



IH' $\rightleftharpoons$ IH

$$K_2 = \frac{[IH]}{[IH']} \qquad 2)$$

$$[S] = [HA] + [A^-] \qquad 3)$$

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i) In acid solution (refer to Figure 16)

$$\text{Rate} = k_2[IH]$$

From the steady-state assumption:

$$\frac{d[IH' + IH]}{dt} = k_1[HA] - k_{-1}[IH'] - k_2[IH] = 0$$

Solving for $[IH']$ using eq. 2

$$[IH'] = \frac{[IH]}{K_2}$$

Solving for HA using eq. 1 and eq. 3

$$[HA] = [S] - [A^-]$$

$$[A^-] = \frac{K_1[HA]}{[H^+]}$$

so

$$[HA]\left[1 + \frac{K_1}{[H^+]}\right] = [S]$$

$$[HA] = \frac{[S]}{1 + \frac{K_1}{[H^+]}} \qquad 4)$$

Substituting these into the steady-state equation:

$$\frac{d[IH' + IH]}{dt} = \frac{k_1[S]}{1 + \frac{K_1}{[H^+]}} - \frac{k_{-1}[IH]}{K_2} - k_2[IH] = 0$$

$$[IH] = \frac{k_1[S]}{\left[1 + \frac{K_1}{[H^+]}\right]\left[\frac{k_{-1}}{K_2} + k_2\right]}$$

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so

$$\text{Rate} = \frac{k_1 k_2 [S]}{\left[\frac{k_{-1}}{K_2} + k_2\right]\left[1 + \frac{K_1}{[H^+]}\right]}$$

Grouping the rate constants

$$k_o = \frac{k_1 k_2}{\frac{k_{-1}}{K_2} + k_2}$$

The expression for k_{obs} in acid solution becomes simply:

$$k_{obs} = \frac{k_o}{1 + \frac{K_1}{[H^+]}} \qquad 5)$$

ii) In neutral solution, buffer catalyzed rate (refer to Figure 19).

$$\text{Rate} = k_{HB}[HB][I^-]$$

Using the steady state assumption

$$\frac{d[I^-]}{dt} = k_3[A^-] - k_{-3}[I^-] - k_{HB}[HB][I^-] = 0$$

Solving for A^- using eq. 3 and 4

$$[A^-] = [S] - [HA]$$

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$$= [S] - \frac{[S]}{1 + \frac{K_1}{[H^+]}}$$

$$= [S]\left[1 + \frac{K_1}{[H^+]}\right] - [S]$$
$$\frac{}{1 + \frac{K_1}{[H^+]}}$$

$$[A^-] = \frac{[S]K_1}{[H^+] + K_1} \qquad 6)$$

Substituting these into the steady-state equation:

$$\frac{d[I^-]}{dt} = \frac{k_3 K_1 [S]}{[H^+] + K_1} - k_{-3}[I^-] - k_{HB}[I^-] = 0$$

$$[I^-] = \frac{k_3 K_1 [S]}{\left[k_{-3} + k_{HB}[HB]\right]\left[[H^+] + K_1\right]}$$

so

$$\text{Rate} = \frac{k_{HB} k_3 K_1 [S]}{\left[k_{-3} + k_{HB}[HB]\right]\left[[H^+] + K_1\right]}$$

And if $k_{-3} \gg k_{HB}[HB]$, then

$$\text{Rate} = \frac{k_{HB} k_3 K_1 [S][HB]}{k_{-3}\left[[H^+] + K_1\right]}$$

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Letting $k_{2_{HB}} = \frac{k_{HB}k_3}{k_{-3}}$

this becomes, summing for all buffers,

$$k_{obs} = \sum \frac{k_{2_{HB}}[HB]}{\frac{[H^+]}{K_1} + 1} \qquad 7)$$

The same expression is derived if one assumes that the mechanism shown on page 145 is rate-determining.

Including only the term for water as the general acid catalyst, expression 7 becomes

$$k_{obs} = \frac{k_{H_2O}}{\frac{[H^+]}{K_1} + 1} \qquad 8)$$

where $k_{H_2O} = k2_{H_2O}H_2O$

iii) In base, a second-order displacement on A^- occurs so

$$\text{Rate} = k_{OH}[OH^-][A^-]$$

Using eq. 7

$$\text{Rate} = \frac{k_{OH}K_1[OH^-][S]}{[H^+] + K_1}$$

so

$$k_{obs} = \frac{k_{OH}K_1[OH^-]}{[H^+] + K_1} \qquad 9)$$

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In strong base, this becomes

$$k_{obs} = k_{OH}[OH^-]$$

Combining expressions 5, 8, and 9 with some rearranging:

$$k_{obs} = \frac{k_o}{1 + \frac{K_1}{[H^+]}} + \frac{k_{H_2O}}{1 + \frac{[H^+]}{K_1}} + \frac{k_{OH}[OH^-]}{1 + \frac{[H^+]}{K_1}}$$

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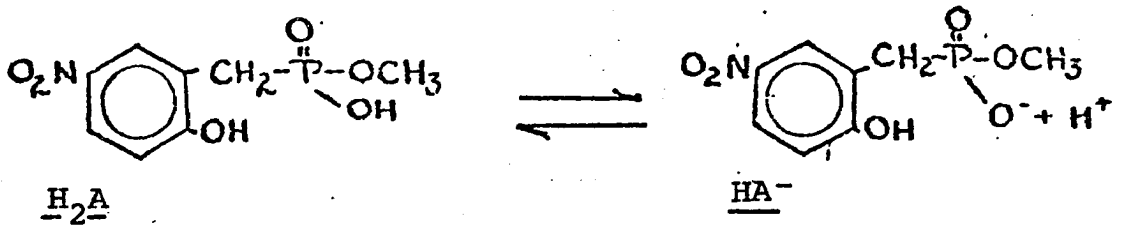
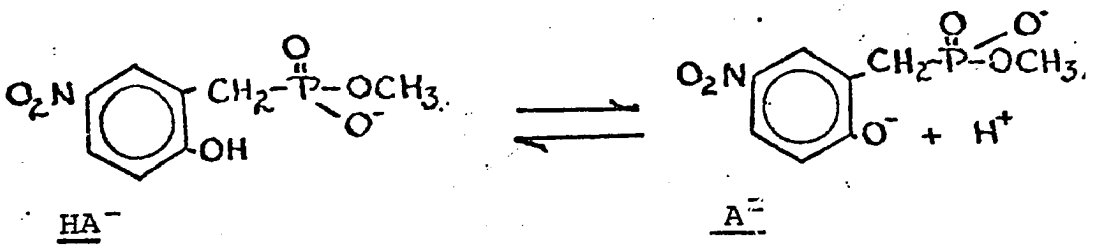
B. Hydrolysis of MEV



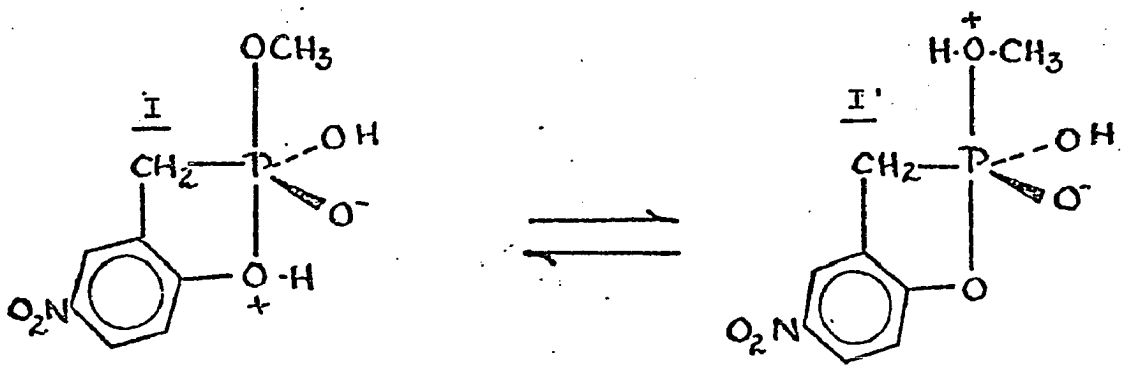
H_2A HA^-

$$K_1 = \frac{[H^+][HA^-]}{[H_2A]} \qquad 1)$$



HA^- $A^=$

$$K_2 = \frac{[H^+][A^=]}{[HA^-]} \qquad 2)$$



$$K_3 = \frac{[I']}{[I]} \qquad 3)$$

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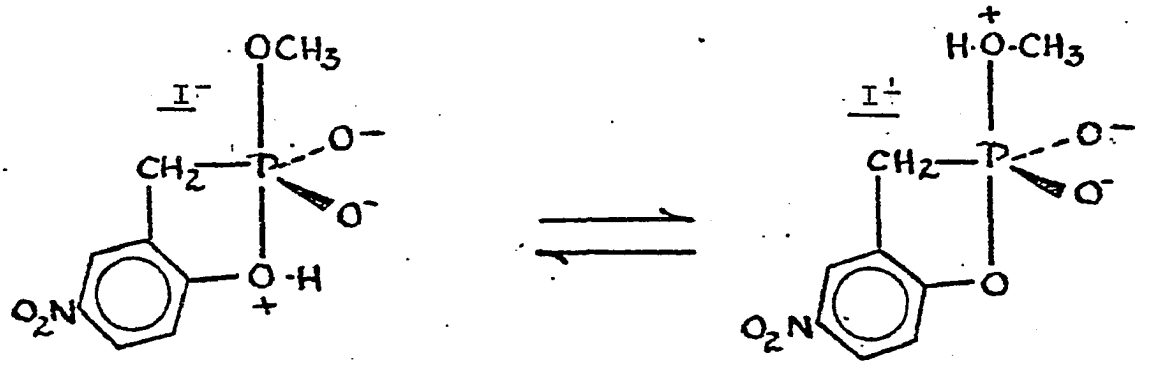

$$K_4 = \frac{[I^{\pm}]}{[I^-]} \qquad 4)$$

$$[S] = [H_2A] + [HA^-] + [A^=] \qquad 5)$$

i) In acid solution (refer to Figure 22)

Rate = $k_2[I']$

= $k_{H_2A}[H_2A]$ (as seen previously in the derivation of hydrolysis of EV in acid)

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Solving for $[H_2A]$

$$[H_2A] = [S] - [HA^-] - [A^=]$$

Using eq. 1

$$[HA^-] = \frac{K_1 [H_2A]}{[H^+]}$$

Using eq. 2 and the above

$$[A^=] = \frac{K_2 [HA^-]}{[H^+]} = \frac{K_1 K_2 [H_2A]}{[H^+]^2}$$

so

$$[H_2A]\left[1 + \frac{K_1}{[H^+]} + \frac{K_1K_2}{[H^+]^2}\right] = [S]$$

$$[H_2A] = \frac{[S]}{1 + \frac{K_1}{[H^+]} + \frac{K_1K_2}{[H^+]^2}} \qquad 6)$$

so

$$k_{obs} = \frac{k_{H_2A}}{1 + \frac{K_1}{[H^+]} + \frac{K_1K_2}{[H^+]^2}} \qquad 7)$$

where $k_{H_2A} = \frac{k_1 k_2}{\frac{k_{-1}}{K_3} + k_2}$

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ii) In mildly acidic solution (refer to Figure 23)

$$\text{Rate} = k_4 [I^{\cdot -}]$$

$$\frac{d[I^- + I^{\cdot -}]}{dt} = k_3[HA^-] - k_{-3}[I^-] - k_4[I^{\cdot -}]$$

Solving for $[HA^-]$

$$[HA^-] = [S] - [H_2A] - [A^=]$$

Using eq. 1

$$[H_2A] = \frac{[H^+][HA^-]}{K_1}$$

and from eq. 2

$$[A^=] = \frac{K_2[HA^-]}{[H^+]}$$

$$[HA^-]\left[1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}\right] = [S]$$

$$[HA^-] = \frac{[S]}{1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}}$$

From eq. 4

$$[I^-] = \frac{[I^{\cdot -}]}{K_4}$$

therefore

$$\frac{d[I^- + I^{\cdot -}]}{dt} = \frac{k_3[S]}{1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}} - [I^{\cdot -}]\left[\frac{k_{-3}}{K_4} + k_4\right]$$

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$$[I^-] = \frac{k_3[S]}{\left[1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}\right]\left[\frac{k_{-3}}{K_4} + k_4\right]}$$

and

$$k_{obs} = \frac{k_{HA^-}}{1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}} \qquad 8)$$

where $k_{HA^-} = \frac{k_3 k_4}{\frac{k_{-3}}{K_4} + k_4}$

iii) In basic solution (refer to Figure 24)

$$\text{Rate} = k_{H_2O}[I^=]$$

$$\frac{d[I^=]}{dt} = k_5[A^=] - k_{-5}[I^=] - k_{H_2O}[I^=] = 0$$

$$[A^=] = [S] - [H_2A] - [HA^-]$$

Using eq. 2

$$[HA^-] = \frac{[H^+][A^=]}{K_2}$$

Using eq. 1 and the above

$$[H_2A] = \frac{[H^+][HA^-]}{K_1} = \frac{[H^+]^2[A^=]}{K_1 K_2}$$

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so

$$[A^{=}]\left[1 + \frac{[H^+]}{K_2} + \frac{[H^+]^2}{K_1K_2}\right] = [S]$$

$$[A^{=}] = \frac{[S]}{1 + \frac{[H^+]}{K_2} + \frac{[H^+]^2}{K_1K_2}} \qquad 9)$$

Therefore

$$\frac{d[I^{=}]}{dt} = \frac{k_5[S]}{1 + \frac{[H^+]}{K_2} + \frac{[H^+]^2}{K_1K_2}} - k_{-5}[I^{=}] - k_{H_2O}[I^{=}]$$

$$[I^{=}] = \frac{k_5 S}{\left[k_{-5} + k_{H_2O}\right]\left[1 + \frac{[H^+]}{K_2} + \frac{[H^+]^2}{K_1K_2}\right]}$$

so

$$\text{Rate} = \frac{k_{H_2O}\, k_5[S]}{\left[k_{-5} + k_{H_2O}\right]\left[1 + \frac{[H^+]}{K_2} + \frac{[H^+]^2}{K_1K_2}\right]}$$

let $k_A^{=} = \frac{k_{H_2O}\, k_5}{k_{-5} + k_{H_2O}}$

so

$$k_{obs} = \frac{k_A^{=}}{1 + \frac{[H^+]}{K_2} + \frac{[H^+]^2}{K_1K_2}} \qquad 10)$$

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In basic solution, this expression reduces simply to

$$k_{obs} = k_{A=}$$

Combining equations 7,8, and 10

$$k_{obs} = \frac{k_{H_2A}}{1 + \frac{K_1}{[H^+]} + \frac{K_1K_2}{[H^+]^2}} + \frac{k_{HA^-}}{1 + \frac{[H^+]}{K_1} + \frac{K_2}{[H^+]}} + \frac{k_{A=}}{1 + \frac{[H^+]}{K_2} + \frac{[H^+]^2}{K_1K_2}}$$

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Appendix 2: ERROR ANALYSIS

Because of the inaccuracies inherent in NMR kinetics due to poor integrals, the crudest possible error analysis was performed. When plotting the percentage of $[Ester]/[Ester]_o$ vs. time for first-order kinetics, the ratio was allowed an error of $\pm$ 2%. After a run was completed, the best line, by eye, was drawn through the points and its slope, k_1, calculated. Using the error brackets, lines of steepest and shallowest possible slopes were drawn and their k_1's calculated. (see Figure 22). The difference between the slope of the best line and the slopes of the "worst" probable lines was usually on the order of $\pm$ 10%.

The same graphical method of error analysis was also used in the uv kinetics. It was assumed that A_∞ could be determined to $\pm$ 0.01 absorbance units and that each A_t could be read to $\pm$ 0.005 units. Therefore, each point $|A_\infty - A_t|$ had an assigned error of $\pm$ 0.015 absorbance units. As before, the difference in slopes was

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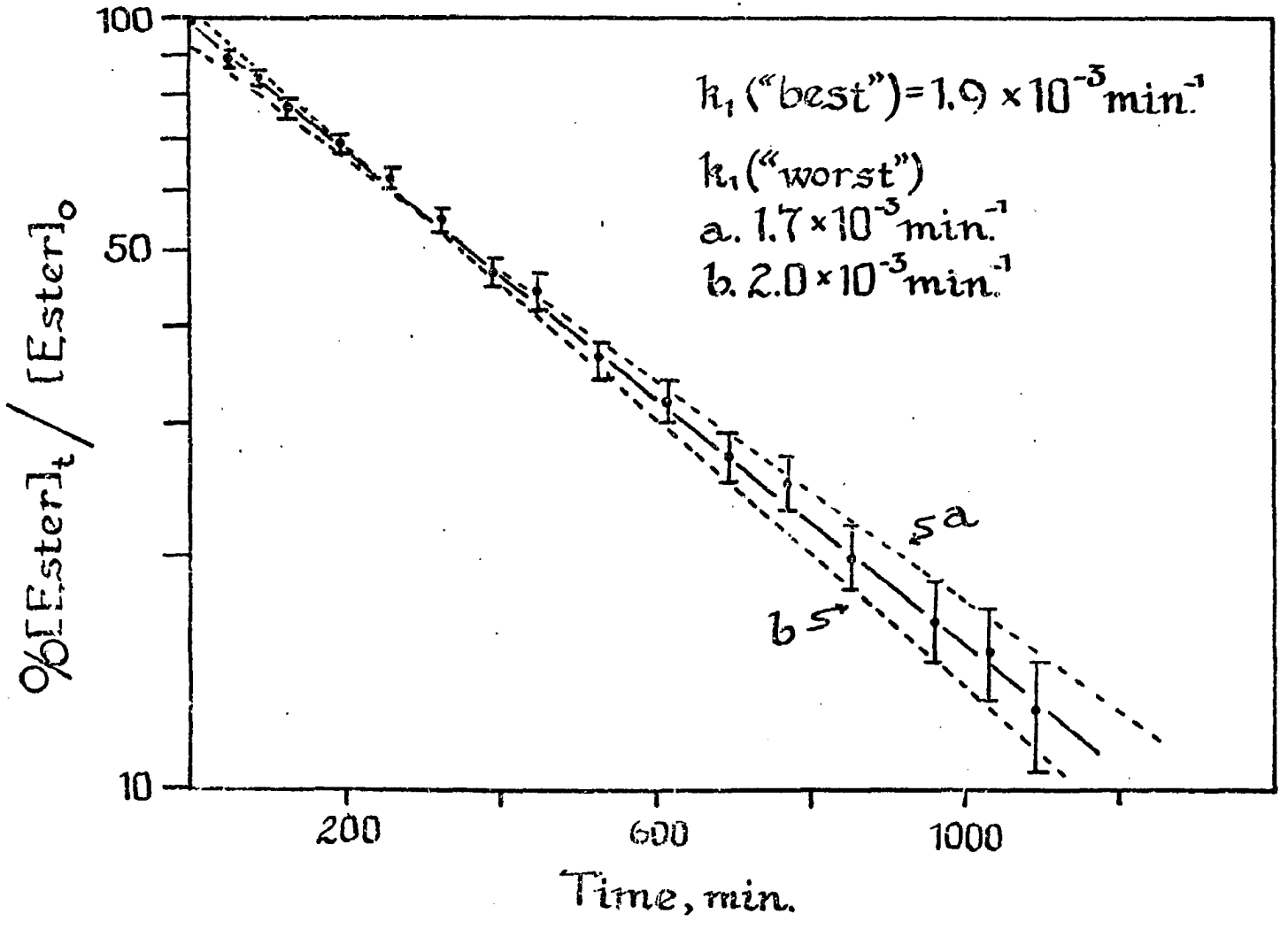

Figure 25

A typical first-order rate plot from NMR kinetics showing the method of error analysis used in this work

on the order of $\pm$ 10%.[1]

In the hydrolysis of EV at 45°C, an alternative, more sophisticated method was used to re-determine the rate constants--"the method of averages."[2] For all cases, the agreement between the "graphical" k_1 and the "method of averages" k_1 was good, differing by less than 10%.

In plotting second-order reactions where [A] = [B], $t_1/[\text{Ester}]$ rests in the value $1/[\text{Ester}]_t$ which equals $1/[\text{Ester}]_o$ (x) where (x) is the fraction of unreacted starting material, a quantity easily determined by NMR as described in Section C, Chapter III. $[\text{Ester}]_o$ is, of course, a known quantity. A best linear approximation method[3] may be used to find the error function:

$$\Delta u = \left[\frac{\partial u}{\partial x}\right]\Delta x$$

thus,

---

[1]For the rate constants determined by the uv aliquot method, and where less than one half-life was followed by NMR, a larger error of $\pm$ 15-20% is in order.

[2]F. Daniels, J.W. Williams, P. Bender, R.A. Alberty, and C.D. Cornwell, "Experimental Physical Chemistry," McGraw-Hill, New York, 1962, p.411.

[3]Ibid., p. 398.

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$$\frac{1}{c} = \frac{1}{c_o x} = u \qquad \text{where } c = [\text{Ester}] \text{ and } c_o = [\text{Ester}]_o$$

so,

$$\frac{\partial u}{\partial x} = -\frac{1}{c_o x^2}$$

and

$$|\Delta u| = \left|\frac{\Delta x}{c_o x^2}\right|$$

For most points, as with the first-order treatment, Δx (error in the determination of unreacted ester) was $\pm$ 2%. The brackets, $|\Delta u|$, were then placed around each point and the best line and worst lines were again drawn, slopes calculated, and the difference taken.

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Appendix 3: ON THE CARE AND FEEDING OF CHROME ALUM ($KCr(SO_4)_2 \cdot 12\ H_2O$) CRYSTALS

To prepare a crystal as seen in Figure 1, dissolve about one part chromic potassium sulfate ($KCr(SO_4)_2$) in two parts boiling water. As the supersaturated solution cools, small crystals will precipitate out. Select a nice one, place it in a beaker, and keep it covered with the chrome alum solution at room temperature. From day to day, freshen the solution and turn the crystal over to a different face. When the crystal has reached the desired size, place it in a saturated solution of alum (aluminum potassium sulfate, $KAl(SO_4)_2$). The clear alum coating will prevent the chrome alum crystal from dehydrating. Alum itself will not dessicate below 60-65°C.[4]

The crystal shown in Figure 1[5] is two years old and weighs 3 kg. The growth of this crystal was discontinued because a suitable large tank was not available. I would like to thank Dr. Jerry J. Smith for suggesting this project.

---

[4]Merck Index, 8th Ed., Merck and Co., Inc., Rahway, N.J., 1968, p. 46.

[5]Figure 1- photo credit: Dr. Gary W. Allen; crystal holder design: Prof. F.H. Westheimer

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