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MECHANISMS OF NUCLEOPHILIC SUBSTITUTION IN PHOSPHATE ESTERS

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CONTENTS

I. Introduction 317
II. Nucleophilic Substitution in Triesters of Phosphoric Acid and Related Compounds 319
A. Alkaline Hydrolysis of Triesters of Phosphoric Acid 319
B. Reaction with Nucleophiles Other than Hydroxide Ion 323
III. Hydrolysis of Diesters of Phosphoric Acid and Related Compounds 329
A. Acyclic Compounds 329
B. Five-Membered Cyclic Diesters of Phosphoric Acid 332
IV. Nucleophilic Substitution of Monosubstituted Derivatives of Phosphoric Acid 335
A. Solvolysis of the Monoanion 336
B. Solvolysis of the Neutral Species 341
C. Solvolysis in Strong Acid 343
D. Summary of Reactions of Monoesters 344
V. Oxidative Reactions Resulting in Phosphorus–Oxygen Cleavage 345
VI. Further Processes Resulting in Carbon–Oxygen Cleavage 345
VII. Catalytic Processes Related to Mechanisms of Enzymatic Hydrolysis of Phosphate Esters 346
A. Metal Ion Catalysis 346
B. Intramolecular Catalysis 347
C. Polyfunctional Catalysis 348
D. Enzymatic Catalysis 349
VIII. References 349

I. INTRODUCTION

The reactions of carboxylic acids and their derivatives have been subjected to intensive study since the early days of organic chemistry. The culmination of this study has been the recent elucidations of the mechanisms of these reactions, ably reviewed by Bender (24a). The chemistry of analogous derivatives of phosphoric acid was much slower to be exploited, and only with the realization that an understanding of the processes by which phosphate esters, anhydrides, amides, etc., are formed and solvolyzed is basic to an understanding of the chemistry of life, was intensive study of these systems undertaken.

The factors complicating these studies are legion. Classical synthetic procedures were few in number and frequently required great skill in isolation of even small amounts of pure products. Characterization of products was made difficult by the poor analytical results frequently obtained by standard combustion techniques, and by the fact that many compounds are isolated as metal salts which seldom melt and are often extensively hydrated. Further, in solution, a chemically pure substance may exist as an equilibrium mixture of several distinct species which differ *inter se* as to degree of protonation. Each of these species may then undergo reaction with nucleophiles, possibly by more than one mechanism. Thus considering the difficulty of synthesis, isolation and characterization, and the complicated solvolytic behavior of these compounds, it is not surprising that early progress was slow.

Modern synthetic methods for derivatives of phosphoric acid owe much to studies of reaction mechanisms, as do sophisticated synthetic techniques in many other fields. Since our interest is principally in the area of reaction mechanism, rather than in synthesis, we shall, in general, not comment extensively on synthesis, except when this sheds light on the mechanism. For leading information to synthetic approaches to biologically important molecules, the reader is referred to the recent review by Khorana (159).

Earlier workers who have partially reviewed progress in understanding mechanisms of reaction of phosphate esters have stressed the multiplicity of reactions these compounds undergo. A system of nomenclature similar to that in general use for carbon compounds has been proposed to simplify discussion of the possibilities (22), but this system proves to be inadequate to contain all the reaction paths currently known. Although the classification system for nucleophilic substitutions as originally proposed by Ingold and his collaborators is a perfectly general one (see ref. 148 for a brief historical account), the term SN2, in particular, has been loosely used to the extent that many readers infer from it mechanistic details which are true only of SN2 reactions

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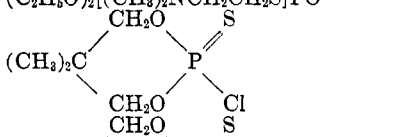
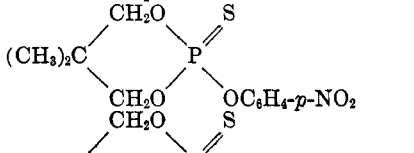
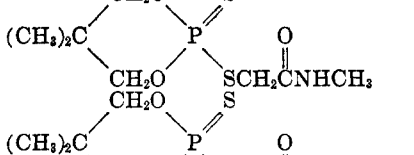
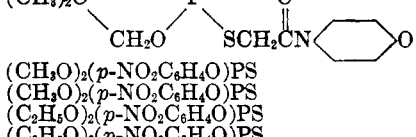
TABLE I

SECOND-ORDER RATE CONSTANTS FOR THE REACTION OF TRIESTERS OF PHOSPHORIC ACID WITH AQUEOUS BASE

Compound	Medium	Temp., °C.	k_2 (mole/l.)$^{-1}$ sec.$^{-1}$	E, kcal./mole	log PZ, (mole/l.)$^{-1}$ sec.$^{-1}$	Ref.
$(CH_3O)_3PO$	H_2O	35	3.36×10^{-4}	16.2	8.1	23, 143
$(CH_3O)_3PO$	75% dioxane–25% H_2O	35	2.46×10^{-4}			232
$(C_2H_5O)_3PO$	H_2O	37.5	3.73×10^{-5}	15.0	6.16	157
$(C_2H_5O)_3PO$	50% dioxane–50% H_2O	35	1.75×10^{-5}	14.9	5.8	222
$(C_2H_5O)_2(C_6H_5O)PO$	50% ethanol–50% H_2O	37.5	2.47×10^{-4}	15.0	6.98	158
$(C_6H_5O)_3PO$	75% ethanol–25% H_2O	37.5	2.67×10^{-4}	16.1	13.57	157
$(C_6H_5O)_3PO$	60% dioxane–40% H_2O	35	2.32×10^{-2}	10.2	4.9	23
$(C_6H_5O)_3PO$	75% dioxane–25% H_2O	35	2.40×10^{-2}			23
$(C_2H_5O)_2[(CH_3)_3N^+CH_2CH_2O]PO$	H_2O	25	4.3×10^{-2}			221
$(C_2H_5O)_2[m\text{-}(CH_3)_3N^+C_6H_4O]PO$	50% ethanol–50% H_2O	37.5	9.1×10^{-2}	15.8	10.12	157
$(CH_3O)_2(p\text{-}NO_2C_6H_4O)PO$	H_2O	37	8.8×10^{-2}	12.20 ± 0.20	7.55	117
$(C_2H_5O)_2(p\text{-}NO_2C_6H_4O)PO$	50% ethanol–50% H_2O	37.5	1.58×10^{-2}	12.4	6.86	157
$(C_2H_5O)_2(p\text{-}NO_2C_6H_4O)PO$	H_2O	37	2.67×10^{-2}	13.52 ± 0.21	7.95	117
$(n\text{-}C_3H_7O)_2(p\text{-}NO_2C_6H_4O)PO$	H_2O	37	1.67×10^{-2}	13.03 ± 0.36	7.43	117
$(i\text{-}C_3H_7O)_2(p\text{-}NO_2C_6H_4O)PO$	H_2O	37	2.5×10^{-3}	14.51 ± 0.21	7.64	117
$(n\text{-}C_4H_9O)_2(p\text{-}NO_2C_6H_4O)PO$	H_2O	37	1.8×10^{-2}	13.19 ± 0.38	7.55	117
$(i\text{-}C_4H_9O)_2(p\text{-}NO_2C_6H_4O)PO$	H_2O	37	1.46×10^{-2}	13.25 ± 0.19	7.52	117
$(sec\text{-}C_4H_9O)_2(p\text{-}NO_2C_6H_4O)PO$	H_2O	37	1.22×10^{-3}	14.34 ± 0.30	7.20	117
$(CH_3O)(p\text{-}NO_2C_6H_4O)_2PO$	50% acetone–50% H_2O	37	1.29	13.4	9.57	158
$(C_2H_5O)(p\text{-}NO_2C_6H_4O)_2PO$	50% acetone–50% H_2O	37	1.37	14.3	10.2	158
$(p\text{-}NO_2C_6H_4O)_3PO$	50% acetone–50% H_2O	25	3.34×10	4.1	4.56	157
$(C_2H_5S)_3PO$	50% dioxane–50% H_2O	25	1.23×10^{-2}	14.1	8.4	222
$(C_2H_5O)_2(C_2H_5SCH_2CH_2S)PO$	H_2O	25	1.4×10^{-2}			114
$(C_2H_5O)_2(C_2H_5SCH_2CH_2O)PS$	50% ethanol–50% H_2O	25	1.5×10^{-4}			114
$(C_2H_5O)_2[(CH_3)_3N^+CH_2CH_2S]PO$	H_2O	25	7×10^{-1}			221
$(C_2H_5O)_2[(CH_3)_3N^+CH_2CH_2O]PO$	H_2O	25	4.3×10^{-2}			221
$(C_2H_5O)_2[(CH_3)_2NCH_2CH_2S]PO$	25% ethanol–75% H_2O	37	9.3×10^{-3}			113
	50% dioxane–50% H_2O	?	2×10^{-3}			102
	50% dioxane–50% H_2O	?	4×10^{-4}			102
	50% dioxane–50% H_2O	?	3.6×10^{-1}			102
	50% dioxane–50% H_2O	?	8.3×10^{-2}			102
$(CH_3O)_2(p\text{-}NO_2C_6H_4O)PS$	H_2O	15	1.53×10^{-3}	15.4	8.93	154
$(CH_3O)_2(p\text{-}NO_2C_6H_4O)PS$	50% acetone–50% H_2O	25	1.4×10^{-3}	16.7	9.48	158
$(C_2H_5O)_2(p\text{-}NO_2C_6H_4O)PS$	H_2O	15	3.6×10^{-4}	16.6	9.17	154
$(C_2H_5O)_2(p\text{-}NO_2C_6H_4O)PS$	50% acetone–50% H_2O	25	2.1×10^{-4}	19.2	10.18	158
$(i\text{-}C_3H_7O)_2(p\text{-}NO_2C_6H_4O)PS$	H_2O	25	5.5×10^{-5}			225
$(CH_3O)(p\text{-}NO_2C_6H_4O)_2PS$	50% acetone–50% H_2O	25	2.2×10^{-2}	13.9	8.55	158
$(C_2H_5O)(p\text{-}NO_2C_6H_4O)_2PS$	50% acetone–50% H_2O	25	1.6×10^{-2}	12.1	6.95	158
$(p\text{-}NO_2C_6H_4O)_3PS$	50% acetone–50% H_2O	25	2.1×10^{-1}	5.7	3.43	158
$(C_2H_5O)_2(p\text{-}NO_2C_6H_4S)PO$	H_2O	37	2.5			8
$(C_2H_5O)(C_2H_5S)(p\text{-}NO_2C_6H_4O)PO$	H_2O	37	1.33×10^2			8

at a saturated carbon atom. On this account the present authors prefer to avoid that terminology.

It may be argued that reactions of phosphate esters in which a bond between carbon and oxygen is broken are merely special cases of substitution or elimination at carbon in which the leaving group happens to be the anion of a phosphoric acid. The interest of this review is in defining the ways by which phosphoric esters react and the factors influencing their reactivity. Hence the reactions of these compounds at carbon are of interest within its context and will be included. While the reactions in which the bond between phosphorus and oxygen is broken probably predominate in the biological transformations of phosphate derivatives, biological sequences are known in which the carbon–oxygen bond of a phosphate ester is broken (193). Hence both of these types of reaction are important biochemically.

This review of the reactions of phosphoric esters and other derivatives will consider in turn: the reactions of the fully substituted derivatives (such as the triesters and triamides of phosphoric acid); those of the disubstituted derivatives which possess one acidic hydroxyl group; those of the monosubstituted derivatives, which possess two ionizable hydroxyl functions; and, finally reactions of phosphoric acid itself which fall

TABLE II

SECOND-ORDER RATE CONSTANTS FOR THE REACTION OF SOME PHOSPHONATE ESTERS WITH AQUEOUS BASE

Compound	Solvent	Temp., °C.	k, (mole/l.)$^{-1}$ sec.$^{-1}$	E, kcal./mole	log PZ, (mole/l.)$^{-1}$ sec.$^{-1}$	Ref.
$(CH_3O)_2(CH_3)PO$	H_2O	100	2.35×10^{-1}[a]	13.3[a]	7.17[a]	144
$(C_2H_5O)_2(CH_3)PO$	H_2O	100	1.80×10^{-2}[a]	11.6[a]	5.06[a]	144
$(i\text{-}C_3H_7O)_2(CH_3)PO$	H_2O	100	4.82×10^{-4}[a]	16.1[a]	5.3[a]	144
$[(CH_3)_3CCH_2O]_2(CH_3)PO$	H_2O	100	2.25×10^{-4}[a]	9.6[a]	1.87[a]	144
$(C_6H_5O)_2(CH_3)PO$	H_2O	100	3.81[a]	12.85[a]	8.11[a]	144
$(C_6H_5CH_2O)_2(CH_3)PO$	H_2O	100	5.9×10^{-2}[a]	9.18[a]	4.15[a]	143
$(CH_3O)_2(C_2H_5)PO$	H_2O	100	1.43×10^{-1}[a]	22.2[a]	5.5[a]	143
$(C_2H_5O)_2(C_2H_5)PO$	H_2O	100	9.40×10^{-3}[a]	13.9[a]	11.3[a]	144
$(i\text{-}C_3H_7O)_2(C_2H_5)PO$	H_2O	100	8.67×10^{-5}[a]	16.2	5.42[a]	144
$(n\text{-}C_3H_7O)_2(n\text{-}C_3H_7)PO$	H_2O	100	4.60×10^{-4}[a]	14.4[a]	6.64[a]	144
$(i\text{-}C_3H_7O)_2(n\text{-}C_3H_7)PO$	H_2O	100	5.0×10^{-6}[a]	22.9[a]	5.30[a]	144
$(C_2H_5O)_2(n\text{-}C_4H_9)PO$	H_2O	100	2.05×10^{-3}[a]	15.35[a]	6.31[a]	144
$(i\text{-}C_3H_7O)_2(n\text{-}C_4H_9)PO$	H_2O	100	1.51×10^{-5}[a]	22.7[a]	8.49[a]	144
$(i\text{-}C_3H_7O)_2(t\text{-}C_4H_9)PO$	H_2O	125	4.16×10^{-6}			144
$(C_2H_5O)_2(C_6H_5)PO$	H_2O	100	3.75×10^{-2}[a]	12.6[a]	5.94[a]	144
$(CH_3O)(C_2H_5)_2PO$	H_2O	100	7.08×10^{-2}[a]	14.3[a]	7.22[a]	143
$(p\text{-}NO_2C_6H_4O)_2(C_2H_5)PO$	20% ethanol–80% H_2O	25	6.17	8.7	7.17	155
$(p\text{-}NO_2C_6H_4O)(C_2H_5O)(C_6H_5)PS$	20% ethanol–80% H_2O	25	1.52×10^{-1}	17.5	12.03	155
$(p\text{-}NO_2C_6H_4O)(C_2H_5O)(CH_3)PO$	H_2O	25	4.0×10^{-2}	12.0	9.17	144
$(C_2H_5O)[(CH_3)_3N^+C_2H_4S](CH_3)PO$	H_2O	25	2×10^{-1}			221
$(C_2H_5O)[(CH_3)_3N^+C_2H_4O](CH_3)PO$	H_2O	25	4×10^{-2}			221
$(i\text{-}C_3H_7O)_2(CH_3)PO$	H_2O	25	6.31×10^{-7}[a]	14.9[a]	4.74[a]	144
$(i\text{-}C_3H_7S)_2(CH_3)PO$	H_2O	25	4×10^{-2}	11.4	5.5	145

[a] Calculated from the data in the reference cited.

within the category of nucleophilic substitutions. The attempt will also be made to correlate the reactions of formally similar species, for example, those of $(RO)_3PO$ with a molecule of water, where $(RO)_3$ may represent any combination of alkoxy and hydroxyl groups, in terms of mechanisms of reaction common to members of such a group.

II. Nucleophilic Substitution in Triesters of Phosphoric Acid and Related Compounds

A. Alkaline Hydrolysis of Triesters of Phosphoric Acid

It has been known qualitatively for many years that triesters of phosphoric acid are hydrolyzed in basic solution rather readily to the corresponding diesters, but that subsequent stages of hydrolysis are relatively slow (165). It is surprising, therefore, that this reaction has been studied very little in a quantitative fashion. The available data are assembled in Table I. The behavior of trimethyl phosphate and of triphenyl phosphate, recently examined in detail by Barnard, Bunton, Llewellyn, Vernon, and Welch (23), may probably be taken as characteristic of the reactions of the triesters of primary alcohols, phenols, and thiols with hydroxide ion.

The hydrolysis of trimethyl phosphate in aqueous base is first order in hydroxide ion and first order in the ester. Isotopic tracer experiments show that the phosphorus–oxygen bond is broken exclusively (30); furthermore, with the limit of experimental error of the isotopic analysis no isotopic exchange occurs prior to hydrolysis between the phosphoryl oxygen and the oxygen atoms of the solvent (23). A small depression of rate is observed on changing the solvent from water to 75% dioxane–25% water. The few available data suggest that changes of neutral salt concentration do not noticeably affect the rate of saponification of the triesters. The hydrolysis of triphenyl phosphate in 75% dioxane–25% water was found also to be first order in both hydroxide ion and the ester.

The kinetic order of these hydrolyses together with the position of bond fission establishes that hydroxide ion attacks the phosphorus atom in the rate-controlling step of the sequence. Formation of an intermediate addition product of hydroxide ion and the ester, which is in equilibrium with the reactants and decomposes to give the products, as formulated in Eq. 1, is excluded

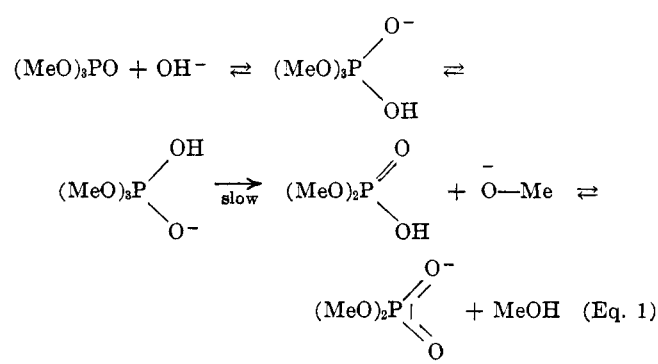


TABLE III, GROUP 1

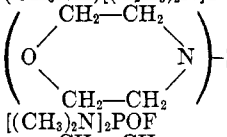
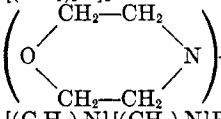
SECOND-ORDER RATE CONSTANTS FOR THE REACTION OF SOME PHOSPHORYL, PHOSPHONYL, AND PHOSPHINYL HALIDES AND PSEUDO-HALIDES WITH AQUEOUS BASE[a]

Compound	k, (mole/l.)$^{-1}$ sec.$^{-1}$	E, kcal./mole	log PZ, (mole/l.)$^{-1}$ sec.$^{-1}$	Ref.
$(CH_3O)_2POF$	1.82×10	11.6	9.78[b]	6
$(C_2H_5O)_2POF$	1.8			146
$(i\text{-}C_3H_7O)_2POF$	1			161
$(CH_3O)(CH_3)POF$	1.06×10^2	10.5	7.80[b]	170
$(C_2H_5O)(CH_3)POF$	6.07×10	11.2	9.58[b]	170
$(BrCH_2CH_2O)(CH_3)POF$	1.62×10^2	11.3	10.5[b]	170
$[(CH_3)_3N^+CH_2CH_2O](CH_3)POF$	9.35×10^2	9.0	9.59[b]	170
$(n\text{-}C_3H_7O)(CH_3)POF$	5.4×10	10.4	9.36[b]	170
$[(CH_3)_3N^+CH_2CH_2CH_2O](CH_3)POF$	3.05×10^2	11.6	11.00[b]	170
$(i\text{-}C_3H_7O)(CH_3)POF$	2.58×10	9.1	8.08[b]	170
$[(CH_3)_3N^+CH(CH_3)CH_2O](CH_3)POF$	3.81×10^2	10.1	9.99[b]	170
$[(CH_3)_3CCH_2CH_2O](CH_3)POF$	4.93×10	12.0	10.49[b]	170
$(CH_3O)(CH_3CH_2)POF$	4.91×10	10.1	9.09[b]	6
$(i\text{-}C_3H_7O)(C_2H_5)POF$	9.35	10.7	9.54[b]	170
$(i\text{-}C_3H_7O)(i\text{-}C_3H_7)POF$	2.03	9.2	6.98[b]	170
$(C_2H_5)_2POF$	7.26×10^2	8.3	8.94[b]	6
$(C_2H_5S)(CH_3)POF$	1.95×10^4	6.2	8.83[b]	173
$[(CH_3)_2N](C_2H_5O)POCN$	7.49	10.1	8.28[b]	173

[a] In water at 25°. [b] Calculated from the data in the reference cited.

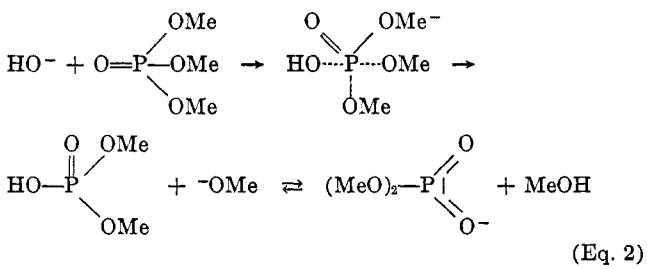
TABLE III, GROUP 2

SECOND-ORDER RATE CONSTANTS FOR THE REACTION OF SOME PHOSPHORYL HALIDES WITH AQUEOUS BASE

Compound	Solvent	Temp., °C.	k, (mole/l.)$^{-1}$ sec.$^{-1}$	E, kcal./mole	log PZ, (mole/l.)$^{-1}$ sec.$^{-1}$	Ref.
$(CH_2{=}CHCH_2NH)_2POF$	H_2O	16	1.6×10^2			136a
$(i\text{-}C_3H_7NH)_2POF$	H_2O	25.09	8.1×10^{-1}	11.2	8.12[a]	136a
$(CH_3NH)[(CH_3)_2N]POF$	H_2O	25.08	2.9×10^{-1}	11.2	7.67[a]	136a
$(C_2H_5NH)[(CH_3)_2N]POF$	H_2O	25	2×10^{-1}	11.4	7.65[a]	136a
$(n\text{-}C_4H_9NH)[(CH_3)_2N]POF$	H_2O	25	1.9×10^{-1}	11.4	7.63[a]	136a
$(i\text{-}C_3H_7NH)[(CH_3)_2N]POF$	H_2O	25	1.4×10^{-1}	11.9	7.87[a]	136a
$(CH_3NH)[(C_2H_5)_2N]POF$	H_2O	25	2.6×10^{-2}			136a
 $[(CH_3)_2N]POF$	H_2O	25	1.5×10^{-4}			136a
$[(CH_3)_2N]_2POF$	H_2O	28.9	7.8×10^{-5}	14.7	6.52[a]	138
 $[(C_2H_5)_2N]POF$	H_2O	25	1.6×10^{-5}			136a
$[(C_2H_5)_2N][(CH_3)_2N]POF$	H_2O	25	4.79×10^{-6}	16.6	6.85[a]	136a
$[(C_2H_5)_2N][(C_2H_5)_2N]POF$	H_2O	25.2	4.1×10^{-7}	17.1	6.14[a]	136a
$[(CH_3)_2N](CH_3)POF$	H_2O	25	1.04	11.9	8.74[a]	173
$(p\text{-}ClC_6H_4O)(CH_3NH)_2PO$	50% ethanol–50% H_2O	25	1.13×10^{-1}	16.7	$\Delta S^* = -19$ e.u.	143
$(p\text{-}ClC_6H_4O)(C_2H_5NH)_2PO$	50% ethanol–50% H_2O	25	6.0×10^{-2}	15.2	$\Delta S^* = -13.7$ e.u.	143

[a] Calculated from the data in the reference cited.

by the failure of the phosphoryl group to exchange oxygen with the solvent prior to hydrolysis. Although pentaalkoxyphosphoranes, $(RO)_5P$, have been characterized (194, 196, 197), the reaction cannot, then, proceed by an addition–elimination sequence analogous to that believed to represent the course of hydrolysis of carboxylic acid esters (24a), but must consist either of a one-step process in which the leaving group is being expelled at the same time the substituting group is entering (Eq. 2) or of a two-step process in which the intermediate decomposes so very rapidly that it cannot equilibrate with the solvent. The latter sequence is, therefore, indistinguishable by present experimental criteria from a one-step process.



(Eq. 2)

Recorded rate constants for basic hydrolysis of trisubstituted derivatives of phosphoric acid other than simple esters are listed in Tables II and III, groups 1 and 2. When the leaving group is the anion of a sufficiently strong acid, the phosphoryl derivative as-

TABLE III, GROUP 3

PSEUDO-UNIMOLECULAR RATE CONSTANTS FOR THE SOLVOLYSIS OF SOME PHOSPHATE ESTERS, PHOSPHONATE ESTERS, AND SOME PHOSPHORYL, PHOSPHONYL, AND PHOSPHINYL HALIDES IN NEUTRAL OXYGEN SOLVENTS WITHOUT ADDED BASE

Compound	Solvent	Temp., °C.	k, sec.$^{-1}$	E, kcal./mole	log PZ, sec.$^{-1}$	Ref.
$(CH_3O)_2POCl$	5% H_2O–95% acetone	0	2.9×10^{-5}	10.6	3.9	146
$(CH_3O)(CH_3)POCl$	5% H_2O–95% acetone	0	2.9×10^{-3}	8.3	4.1	146
$(C_2H_5O)(CH_3)POCl$	5% H_2O–95% acetone	0	1.5×10^{-3}	8.5	4.0	146
$(i\text{-}C_3H_7O)(CH_3)POCl$	5% H_2O–95% acetone	0	6.8×10^{-4}	8.7	3.6	146
$(C_6H_5O)(CH_3)POCl$	5% H_2O–95% acetone	0	2.2×10^{-3}	8.1	3.8	146
$(CH_3O)(C_2H_5)POCl$	5% H_2O–95% acetone	0	1.6×10^{-3}	8.4	3.9	146
$(C_2H_5O)(C_2H_5)POCl$	5% H_2O–95% acetone	0	4.9×10^{-4}	8.9	3.8	146
$(i\text{-}C_3H_7O)(C_2H_5)POCl$	5% H_2O–95% acetone	20	4.2×10^{-4}			146
$(C_2H_5O)_2POCl$	5% H_2O–95% acetone	0	9.9×10^{-4}	8.60	3.88	146
$(C_2H_5)_2POCl$	5% H_2O–95% acetone	0	2.5×10^{-2}	7.3	4.1	146
$(CH_3O)_2POCl$	100% C_2H_5OH	0	5.6×10^{-5}	12.0	5.4	96
$(C_2H_5O)_2POCl$	100% C_2H_5OH	0	2.3×10^{-5}	13.4	6.1	96
$(i\text{-}C_3H_7O)_2POCl$	100% C_2H_5OH	0	9.7×10^{-6}	12.8	5.2	96
$(C_6H_5CH_2O)_2POCl$	100% C_2H_5OH	0	3.16×10^{-5}	14.6	7.2	96
$(C_6H_5O)_2POCl$	100% C_2H_5OH	0	5.82×10^{-5}	11.9	5.3	96
$(CH_3)_2POCl$	100% C_2H_5OH	−8.5	6.0×10^{-3}			96
$(C_6H_5)_2POCl$	100% C_2H_5OH	−0.2	1.3×10^{-3}	11.4	6.2	96
$(i\text{-}C_3H_7O)(CH_3)POCl$	H_2O	25	1.5×10^{-2}			171
$(CH_3)_2POF$	H_2O	25	4×10^{-4}			127
$(i\text{-}C_3H_7O)_2POF$	H_2O	25	1.7×10^{-6}			127
$(i\text{-}C_3H_7O)_2POCl$	H_2O	25	8.1×10^{-3}			127
$(i\text{-}C_3H_7O)_2POF$	D_2O	25	1.59×10^{-6}			127
$(C_2H_5O)(p\text{-}NO_2C_6H_4O)_2PO$	H_2O	25	3.4×10^{-6}	14.3	4.95	158
$(p\text{-}NO_2C_6H_4O)_3PO$	H_2O	25	1×10^{-8}	4.1	0.06	158
$(C_6H_5O)_3PO$	40% H_2O–60% dioxane	100	3×10^{-9}[a]			23
$(CH_3O)_3PO$[b]	H_2O	80	3.36×10^{-4}			143
$(CH_3O)_2(C_2H_5)PO$[b]	H_2O	100	2.5×10^{-5}			143
$(C_6H_5CH_2O)_2(CH_3)PO$[b]	25% acetone–75% H_2O	100	1.07×10^{-3}			143
$(C_2H_5S)_3PO$	60% dioxane–40% H_2O	82	4.17×10^{-8}			222
$(C_2H_5O)_3PO$	60% dioxane–40% H_2O	82	8.8×10^{-8}			222
$(C_2H_5O)_2(p\text{-}NO_2C_6H_4O)PS$	H_2O	37	1.2×10^{-8}			136b
$(C_2H_5O)_2(p\text{-}NO_2C_6H_4S)PO$	H_2O	37	7.8×10^{-6}			136b
$(C_2H_5O)_2(p\text{-}NO_2C_6H_4O)PO$	H_2O	37	6.5×10^{-7}			136b
$(C_2H_5O)_2(C_6H_5O)PO$	H_2O	37	2.7×10^{-9}			136b

[a] k_2 for H_2O in (mole/l.)$^{-1}$ sec.$^{-1}$. [b] These reactions probably give carbon–oxygen bond fission (see text), but are included to allow comparison of rates with those of attack of water at phosphorus.

sumes anhydride character, and the rate of attack of neutral nucleophiles becomes significant. Rates of these solvolyses are given in Table III, groups 3 and 4. Probably all of the reactions of Tables II and III constitute examples of direct nucleophilic substitution at phosphorus (99,147), with the exception of certain of the compounds of group 2 of Table III, which are discussed separately below. The rate constants quoted in Table II and in groups 1 and 2 of Table III refer to bimolecular attack of hydroxide ion; those of Table III, groups 3 and 4, are pseudo-unimolecular rate constants and must be divided by the concentration of the neutral solvent to be directly comparable to the bimolecular rates. The mechanism is probably similar to that of the alkaline hydrolysis of the simple esters, and these reactions may thus be considered as a group. The effect of substituents on the reaction rate is obviously very large, and both steric and electronic factors must be considered; rationalization of the observed rates requires a semi-

TABLE III, GROUP 4

PSEUDO-UNIMOLECULAR RATE CONSTANTS FOR THE NEUTRAL SOLVOLYSIS OF SOME PYROPHOSPHATE ESTERS[a]

Compound	k, sec.$^{-1}$	Source
$(CH_3O)_2PO\text{–}O\text{–}PO(OCH_3)_2$	4×10^{-4}	[b]
$(CH_3O)_2PO\text{–}O\text{–}PO(OC_2H_5)_2$	1.1×10^{-4}	[b]
$(CH_3O)_2PO\text{–}O\text{–}PO(O\text{-}n\text{-}C_3H_7)_2$	9.3×10^{-5}	[b]
$(CH_3O)_2PO\text{–}O\text{–}PO(O\text{-}i\text{-}C_3H_7)_2$	2×10^{-5}	[b]
$(C_2H_5O)_2PO\text{–}O\text{–}PO(OC_2H_5)_2$	2.6×10^{-5}	[b]
$(C_2H_5O)_2PO\text{–}O\text{–}PO(O\text{-}n\text{-}C_3H_7)_2$	1.7×10^{-5}	[b]
$(C_2H_5O)_2PO\text{–}O\text{–}PO(O\text{-}i\text{-}C_3H_7)_2$	4.7×10^{-6}	[b]
$(C_2H_5O)_2PO\text{–}O\text{–}PO(O\text{-}n\text{-}C_4H_9)_2$	1.6×10^{-5}	[b]
$(n\text{-}C_3H_7O)_2PO\text{–}O\text{–}PO(O\text{-}n\text{-}C_3H_7)_2$	1.1×10^{-5}	[b]
$(n\text{-}C_3H_7O)_2PO\text{–}O\text{–}PO(O\text{-}i\text{-}C_3H_7)_2$	3.3×10^{-6}	[b]
$(n\text{-}C_4H_9O)_2PO\text{–}O\text{–}PO(O\text{-}i\text{-}C_3H_7)_2$	3.3×10^{-6}	[b]
$(i\text{-}C_3H_7O)_2PO\text{–}O\text{–}PO(O\text{-}i\text{-}C_3H_7)_2$	$\sim 1.5 \times 10^{-6}$	[b]
$[(CH_3)_2N]_2PO\text{–}O\text{–}PO[N(CH_3)_2]_2$[c]	$\sim 2 \times 10^{-9}$	[d]
$[(CH_3)_2N]_2PO\text{–}O\text{–}PO(NHCH_3)_2$[c]	$\sim 3 \times 10^{-4}$	[d]

[a] In water at 25°. [b] Calculated by Heath (136b) from the data of Toy (227). [c] pH 8. [d] From data of Spencer (213).

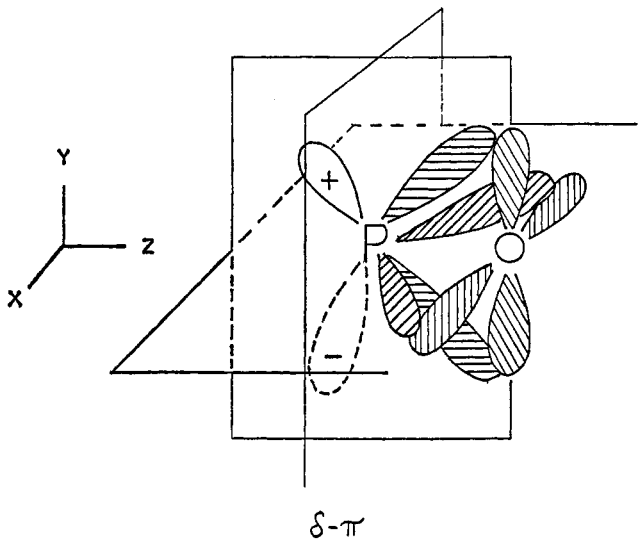
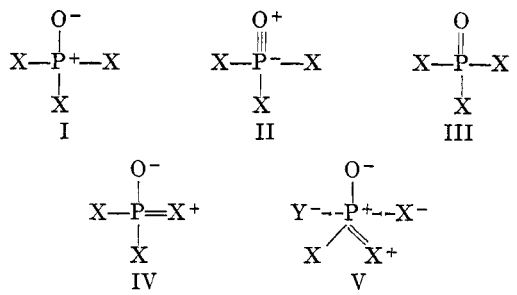


Fig. 1.—Orbital overlap in the phosphoryl P–O bond.

quantitative assessment of several opposing effects. It is valuable in understanding the electronic factors to consider the simple electronic picture given by Lucken and Whitehead (179) of the bonding of the four groups attached to phosphorus in a phosphoryl derivative with three substituents.

Assuming that the molecule, phosphoryl trichloride, for instance, is held together by a framework of σ-bonds to the phosphorus atom in a sp^3 state of tetrahedral hybridization, consider the extreme, coordinate form of the P–O bond analogous to the N–O bond of amine oxides (I). The vacant 3d-orbitals of the phosphorus atom can now form multiple bonds to oxygen in order to remove the negative charge. They can also overlap with the unshared electrons in orbitals of the chlorine atoms, and both of these effects do occur.



The phosphoryl chloride molecule has C_{3v} symmetry. Let the z-axis lie along the P–O bond and the two occupied p-orbitals of the oxygen atom in the x–y plane. Consideration of the transformation properties of these two orbitals and of the five d-orbitals of phosphorus shows that the p_x- and p_y-orbitals can interact to form a multiple bond with the pair d_{xy}, d_{yz}. The overlap is illustrated in Fig. 1, and the calculations of Craig, Maccoll, Nyholm, Orgel, and Sutton (74, 75) assign to the overlap integral a sufficiently large value for efficient bond formation. Thus, since both pairs of orbitals are equally capable of overlap, the P–O bond has the characteristics of a triple bond, rather than those of a double bond. Charge transfer to the phosphorus atom is probably far from complete, and the oxygen atom retains a net negative charge. In resonance language, the P–O bond is best represented as a hybrid of the canonical forms I and II, and the form III has little, if any contribution.

While of the remaining d-orbitals the d_z-orbital does not have the correct symmetry for $d\pi$–$p\pi$ double bonding, the $d_{x^2-y^2}$- and d_{xy}-orbitals are correctly oriented to overlap with the unshared electron pair orbitals of chlorine. Although all three chlorine orbitals overlap equally with the degenerate pair of d-orbitals, the overlap will be less than that of the phosphorus and oxygen orbitals because the chlorine atoms lie below the x–y plane containing the phosphorus atom, and the P–Cl bond is longer than the P–O bond. The $POCl_3$ molecule is, therefore, a hybrid principally of the forms I, II, and IV.

While the three substituents of fully substituted phosphoryl derivatives can conjugate weakly with the phosphorus atom and hence among themselves, there is no direct conjugation between them and the P–O bond. The value of the overlap integral of the phosphorus d-orbitals with the oxygen p-orbitals is very sensitive to the effective charge on the phosphorus atom, however, and in this way inductive substituents can exert a conjugative effect indirectly on such properties as depend on the electron density of the oxygen atom.

Let us assume that nucleophilic substitution at phosphorus in the trisubstituted derivatives in a one-step process, as the available evidence indicates. One important contributing form of the transition state for this process is V, from which it is clear that increasing polarity of the P–O bond should lower the energy of activation for the substitution by increasing the electrostatic interaction of the nucleophilic and electrophilic centers. However, if the transition state is more polar than the reactants, the more polar the reactants are, the more polar the transition state is required to be; but the more polar the transition state is, the greater is the ordering of the solvent around it. Hence there is observed a tendency, at least among groups of compounds in which the leaving group remains constant, for a compound which shows a favorable energy of activation (that is, the substituent X forms weak $d\pi$–$p\pi$ bonds with P) to display an unfavorable entropy of activation.

The origin of the entropy advantage of the compounds whose energy advantage is least (X is able to π bond well with P) can be visualized on the basis of the same transition state. Since these molecules are best able to distribute the developing charge over a larger number of atoms in the sense of formula V, the necessary amount of solvent ordering is less. If

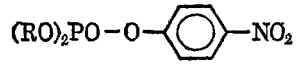
the substituent cannot participate in such structures, the amount of solvation required is greater.

Aksnes (6) has pointed out the above trends in the activation parameters for nucleophilic displacement in a group of selected phosphoryl compounds and has substantiated his interpretation by a comparison with the corresponding thermodynamic functions for the equilibrium involving hydrogen bond formation between phenol and some of the same phosphoryl compounds (4, 5).

Qualitatively, the electronic effects of substituents may be estimated on the basis of their ability to π bond with the d-orbitals of phosphorus. In amides, for instance, the nitrogen atom can readily reduce the positive charge of phosphorus, reducing its ability to attract the nucleophile. In addition, the bond-breaking process is hindered due to the difficulty of separating an amide ion. The unreactivity of amides to basic hydrolysis (with the exception of amides of imidazole) is thereby accounted for. Similarly, although fluorine is more electronegative than chlorine, it is able to π bond much more readily. Thus, the remainder of the molecule being the same, a phosphoryl fluoride is attacked by nucleophiles more slowly than is the corresponding chloride.

More subtle effects can be accommodated by this picture. When sulfur is substituted for oxygen in the $P^+–O^-$ position, the rate of hydrolysis is decreased, while substitution of S for O in the P–O–C position increases the rate of hydrolysis. Since the atom bonded to the lone pair always is relatively negative, the atom of greater electronegativity, oxygen, will produce the greater amount of positive character on phosphorus. In the ester link the oxygen atom can conjugate effectively with phosphorus, reducing its electron demand, while sulfur, which does not so readily form multiple bonds with phosphorus, cannot. In addition, it should be noted that this primary effect is enhanced by the fact that the mercaptide anion is a better leaving group than is the alkoxide ion. This interpretation is strengthened by the fact that phosphates possess larger dipole moments than the corresponding phosphorothioates (157).

Although it is presently impossible to disentangle completely the steric and inductive effects of alkyl groups, it is nevertheless not difficult to assess their over-all effect. Thus, in a series of compounds of the same structural type, say a series of trialkyl phosphates, $(RO)_3PO$, of dialkyl alkyl phosphonates, $(RO)_2RPO$ (112), or of dialkyl *p*-nitrophenyl phosphates



progressive increase in the size of the alkyl group, whether attached to oxygen or to phosphorus, lowers the frequency factor, resulting in a decrease in reaction rate. This difference is most pronounced when an α-hydrogen is replaced by an alkyl group, but negligible among series of homologous primary or homologous secondary groups (see Table I and II). Dostrovsky and Halmann noted a pronounced effect on the reaction of various dialkyl phosphorochloridates with amines (98), and Keay has found that the rates of reaction of several secondary amines with ethyl methylphosphonochloridate, $(C_2H_5O)CH_3POCl$, are similarly influenced by steric factors (150).

The very large acceleration of the rate of attack of basic and neutral oxygen nucleophiles at phosphorus which results from the inclusion of two of the ester functions in a five-membered ring (166) has been shown to be associated with thermodynamic strain of the ring which is relieved upon ring opening (73, 149). In order for the rate to be affected by the factor of 10^7 to 10^8 which is observed, essentially all of the strain must be relieved in the transition state for the hydrolysis reaction. Since the transition state for nucleophilic attack at phosphorus in the cyclic compounds must be closer in geometry to the ground state than is that of acyclic compounds, the suggestion is strong that the geometry of the normal nucleophilic attack at phosphorus involves rehybridization of phosphorus so that the substituents are located similarly to those of the ground state of the five-membered ring compound. The exact source of the stereoelectronic strain has not yet been identified. Nevertheless, certain conclusions can be drawn from the information at hand. The argument advanced by Haake and Westheimer (124) (see section IIIB) for the geometry of the transition state for the attack of water on the conjugate acid of ethylene phosphate is probably equally valid in the case of the triesters. If, in addition, one draws from the data of Green and Hudson (120, 121), of Michalski (184), and of Aaron, Uyeda, Frack, and Miller (1) the conclusion that inversion accompanies nucleophilic attack at phosphorus, the trigonal bipyramid with entering and leaving groups occupying adjacent basal positions is the geometrical disposition of the involved groups that uniquely accommodates all the experimental data. If retention of configuration occurs, then the geometry may be that of a square pyramid.

Hudson and Green have recently reviewed the stereochemistry of displacement reactions at phosphorus atoms (142). If the requirement be released that the species represented be a transition state, rather than an intermediate of finite lifetime, then the water molecule may enter by an apical position and depart by a basal, or *vice versa*, without violating the law of microscopic reversibility. No experimental data from which a choice can be made have yet been adduced.

B. REACTION WITH NUCLEOPHILES OTHER THAN HYDROXIDE ION

When an alkoxide ion is the nucleophile attacking a

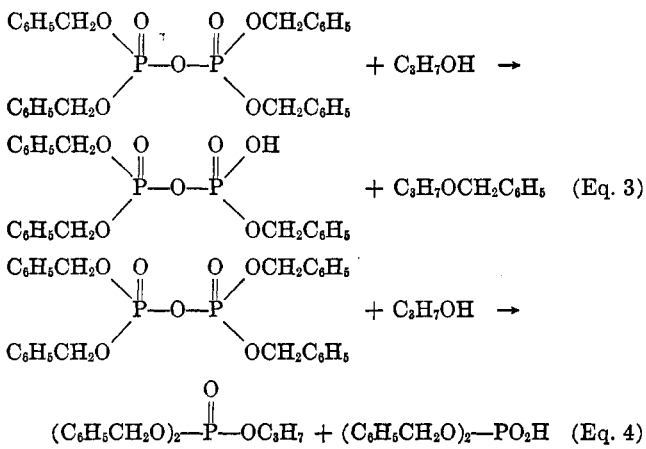
TABLE IV

KINETIC DATA FOR REACTIONS OF TETRABENZYL PYROPHOSPHATE AT 50° (100)

Reaction	Rate constant	Ethanol	Propanol		k_H/k_D
			C_3H_7OH	C_3H_7OD	
Solvolysis, total	10^5k_1, sec.$^{-1}$	6.58	4.84	4.12	1.18
Solvolysis, C–O cleavage only	10^5k_1, sec.$^{-1}$		4		
Lutidine-catalyzed	10^5k_2, sec.$^{-1}$ (mole/l.)$^{-1}$	18.9	11.3	3.33	3.39
Collidine-catalyzed P–O cleavage	10^5k_2 sec.$^{-1}$ (mole/l.)$^{-1}$		20.8	6.38	3.24

triester of phosphoric acid, transesterification results, with the establishment of equilibrium among the various possible products. While this reaction has been employed for synthetic purposes (202), the mechanism has not been studied extensively. It may be presumed to resemble the reaction with hydroxide ion.

Although trialkyl phosphates are rather unreactive in alcoholic solution in the absence of base, the solvolysis of tetrasubstituted pyrophosphates in alcohols proceeds readily at moderate temperatures. Dudek and Westheimer (100) undertook a study of the solvolysis of tetrabenzyl pyrophosphate in propanol in order to elucidate the mechanism of cleavage of the pyrophosphate bond and supplement the general picture of phosphate ester reactivity. Although tetrabenzyl pyrophosphate is of low toxicity, in contrast to tetraalkyl pyrophosphates of lower molecular weight, its chemistry is complicated by the fact that the benzyl groups are easily removed by C–O cleavage, and the following reactions were both observed.



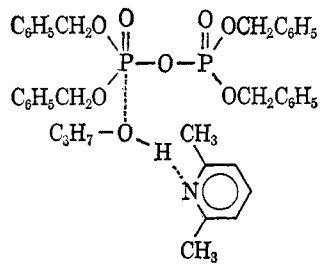
The rate of production of acid by the sum of the above reactions is first order in pyrophosphate. The rate is essentially constant with variation in the concentration of 2,6-N,N-tetramethylaniline or of tribenzylamine present, but increases linearly with increasing concentrations of lutidine and collidine. Reactions carried out in the absence of a proton acceptor were autocatalytic. With propoxide ion the rate was very fast.

Product analysis revealed that the ratio of the rate constant for Eq. 3 to that of Eq. 4 is about 3:1 for the uncatalyzed reaction. Thus, in the presence of tribenzylamine, 75% of tribenzylpyrophosphoric acid was produced and 25% of dibenzylphosphoric acid. With 0.05 *M* lutidine the rate is only slightly increased, and the same ratio of products was observed as with tribenzylamine. In the presence of 0.9 *M* lutidine, 55% of dibenzylphosphoric acid was produced, while with propoxide ion this was the only acidic product.

Rate constants for these reactions are given in Table IV.

The carbon–oxygen cleavage appears not be catalyzed by lutidine. If the solvolyses of various benzyl derivatives proceed by similar mechanisms, then the tribenzyl pyrophosphate ion is a remarkably good leaving group, since the solvolysis of tetrabenzyl pyrophosphate is much more rapid than that of benzyl chloride, and about one-fifth as great as that of the tosylate.

The mechanism for the amine-catalyzed reactions suggested as being best, in accord with the findings of a primary kinetic isotope effect and of first-order rate dependence upon amine concentration, is direct attack by propanol upon the phosphorus–oxygen bond, the reaction rate being enhanced by the simultaneous attack of the base on the proton of the alcohol. If this mechanism is correct, the activated complex would have the structure VI. The mechanism of the uncatalyzed

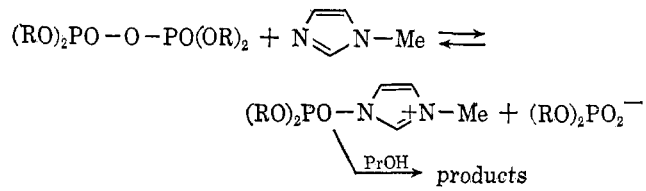


VI

carbon–oxygen cleavage is probably unimolecular solvolysis, but this point was not established.

There is suggestion that heterocyclic amines, in particular, imidazole, possess unique nucleophilicity toward phosphoryl phosphorus (79, 223, 233, 239, 241). Thus, the hydrolysis of diisopropyl phosphorofluoridate is catalyzed by imidazole, pyridine, histidine, and a number of their derivatives in the order of their relative basicities (233), as well as by such ionic nucleophiles as acetate, bicarbonate, and phosphate (161). 2-Methylpyridine was about as reactive as pyridine itself, though, and since failure to observe steric hindrance in such a system has come to be regarded as diagnostic of general base, rather than nucleophilic, catalysis (48, 68), delineation of the exact mechanisms

of these reactions must await the results of further experiments. Although tetrabenzyl pyrophosphate is solvolyzed in propanol in the presence of lutidine by general base catalysis, in the presence of N-methylimidazole catalysis occurs by way of nucleophilic attack on phosphorus (28).

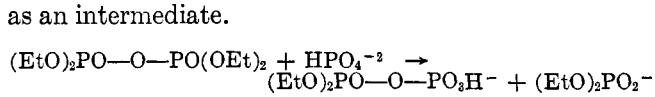


The presence of the intermediate, dibenzylphosphoryl-N-methylimidazolium ion, is demonstrated by the sharp diminution of the rate of solvolysis in the presence of added dibenzyl phosphate ion. Catalysis by imidazole is complicated by additional acid–base equilibria, but the process has been shown to be well described by a reaction sequence involving dibenzylphosphorylimidazole as intermediate (28, 29).

The hydrolysis of methyl ethylene phosphate has recently been shown by Covitz and Westheimer (68) to be catalyzed by heterocyclic bases with a solvent deuterium isotope effect, k_{H_2O}–k_{D_2O}, of about two. 2,6-Lutidine is a poorer catalyst than would have been expected from its base strength by a factor of ten. Since the general base catalysis of the mutarotation of glucose and the general acid catalysis of the inversion of menthone were shown to be subject to steric hindrance to a similar degree, these data suggest that the hydrolysis of methyl ethylene phosphate is subject to general base, rather than to nucleophilic, catalysis.

Much evidence has accumulated that the imidazole moiety of histidine is vital to the functioning of ribonuclease and possibly of all phosphorolytic enzymes (207, 242a). Although the synthetic studies of Cramer (79) and the reactions of tetrabenzyl pyrophosphate with N-methylimidazole and with imidazole (28) clearly show that imidazole moieties can serve as nucleophiles at phosphorus, the fact that, in the hydrolysis of methyl ethylene phosphate, imidazole serves as a general base catalyst suggests that the role histidine plays in catalysis by ribonuclease is also that of a general base–general acid catalyst (242a).

The hydrolysis of tetraethyl pyrophosphate is strongly accelerated by orthophosphate dianion (35). This seems most reasonably attributed to nucleophilic catalysis with formation of diethyl pyrophosphate ion as an intermediate.



The intermediate is a powerful phosphorylating agent for alcohols, producing monoesters, and for orthophosphate, producing inorganic polyphosphates. Sulfite ion had a similar catalytic effect, as did pyridine. 2,6-Lutidine, a stronger base than pyridine, failed to catalyze the hydrolysis, presumably due to steric hindrance of nucleophilic attack.

The rate of hydrolysis of isopropyl methylphosphonofluoridate is greatly increased by hydroperoxide ion, two molecules of which are consumed per mole of the phosphorus halide hydrolyzed. One mole of oxygen gas is evolved (172). Diethyl *p*-nitrophenyl phosphate reacts similarly with hydroperoxide ion (106). Among other nucleophiles, catechol monoanion (108), hydroxamate ions (217, 218), oximes and oximate anions (118, 119), hypohalite ions (105), and nitrite ion (84) have been reported to effect catalysis of similar phosphoryl halides. Dimethylformamide catalyzes the reaction of phosphorochloridates with alcohols, amines, and acids in anhydrous media. Nucleophilic catalysis by phosphorylation of the carbonyl oxygen was demonstrated (80, 200). A number of similar reactions have been discussed by Larsson (174). Nucleophilic catalysis of hydrolysis is, thus, a well-established phenomenon.

Pyridine has long been known to catalyze the reactions of alcohols with phosphoryl chloride, and argument of polemic dimensions has occurred in the literature over whether a phosphorylated pyridinium intermediate accounts for this catalysis. Cryoscopic measurements on carefully purified materials revealed no detectable compound formation (247), and it seems likely that the precipitate observed by other workers (122) is, in fact, due to hydrolysis by adventitious moisture. Since an equilibrium quantity of the proposed intermediate too small to be detected by this means could still account for the observed catalysis, the mechanism remains undefined.

Unless the phosphoryl compound acting as substrate has a leaving group which is the anion of a reasonably strong acid available for displacement, most nucleophiles in which the attacking atom is something other than a negative oxygen tend to form a bond with carbon, rather than phosphorus. Some recent studies have attempted to study reactions of selected phosphate esters with various nucleophiles in order to be able to separate the reactions at phosphorus from those at carbon, and to study the rates of each quantitatively. From such studies it should be possible to construct a scale of nucleophilicity toward phosphoryl phosphorus similar to that established by Swain and Scott (216) for nucleophilic attack at saturated carbon.

Miller (186) has studied the rates of reaction in *t*-butyl alcohol–dioxane of several anions of oxygen and sulfur with O,O-diphenyl phosphorochloridothioate, in which attack of the nucleophile at the aromatic carbon is very unlikely. Of the nucleophiles listed in Table V, all except *t*-butoxide anion and carboxylate anions reacted with simple second-order kinetics in the region

TABLE V

REACTION OF O,O-DIPHENYL PHOSPHOROCHLORIDOTHIOATE WITH ANIONIC NUCLEOPHILES AT 58°[a]

Conjugate acid of anion	pK_a	$k \times 10^2$, l. mole^{-1} sec.$^{-1}$	$-\log k$
t-Butyl alcohol	*ca.* 19	121[b]	−0.11
1-Pentanethiol	10.66	35.7[b]	0.448
Phenol	9.94	26.7 ± 0.4	0.573
2-Hydroxypyridine	8.66	14.5 ± 0.1	0.840
p-Hydroxyacetophenone	7.75	9.20 ± 0.05	1.035
p-Cyanophenol	7.23	7.75 ± 0.15	1.111
Thiophenol	6.52	4.48 ± 0.15	1.35
2-Hydroxypyrazine		3.11 ± 0.005	1.507
2-Pyridinethiol	5.13	0.517 ± 0.009	2.29
Trimethylacetic acid	5.05	0.283 ± 0.010	2.56
Acetic acid	4.76	0.343 ± 0.011	2.47

[a] Data from ref. 186 and references cited therein. [b] Extrapolated from runs at lower temperatures.

of concentration 0.1–0.3 *M*, as would be expected from Eq. 5. Subsequent reaction of the product of Eq. 5

$$A^- + (C_6H_5O)_2P(=S)Cl \rightarrow (C_6H_5O)_2P(=S)A + Cl^- \quad \text{(Eq. 5)}$$

complicates the behavior of the other three anions, and for this reason their behavior was examined separately in greater detail (185).

The data of Table V give the rates of reaction at 58° of O,O-diphenyl phosphorochloridothioate with anionic nucleophiles. These data are plotted in Fig. 2 as a function of the acidity of the conjugate acid of the anion. As Miller notes, "The most striking aspect of this plot is the excellent correlation...between reactivity and basicity, and the complete and unique lack of distinction between oxygen and sulfur anions."

Dimethyl sulfoxide has been reported to react with a phosphoryl chloride by attack of its oxygen atom on phosphorus (201).

The "α effect" (104) is, however, of great importance in determining the nucleophilicity of a reagent toward phosphoryl phosphorus, even though polarizability seems to contribute little, if anything. Such nucleophiles as hydroxylamine, oximate anions, hydroperoxide ion, and hypochlorite ion, which show the common structural feature of an electronegative atom which has unshared electrons α to the attacking atom, all exhibit reactivity much greater than would be predicted from their basicities (6, 12, 105, 106, 108, 118, 119, 172, 186, 217, 218). The origin of this effect has

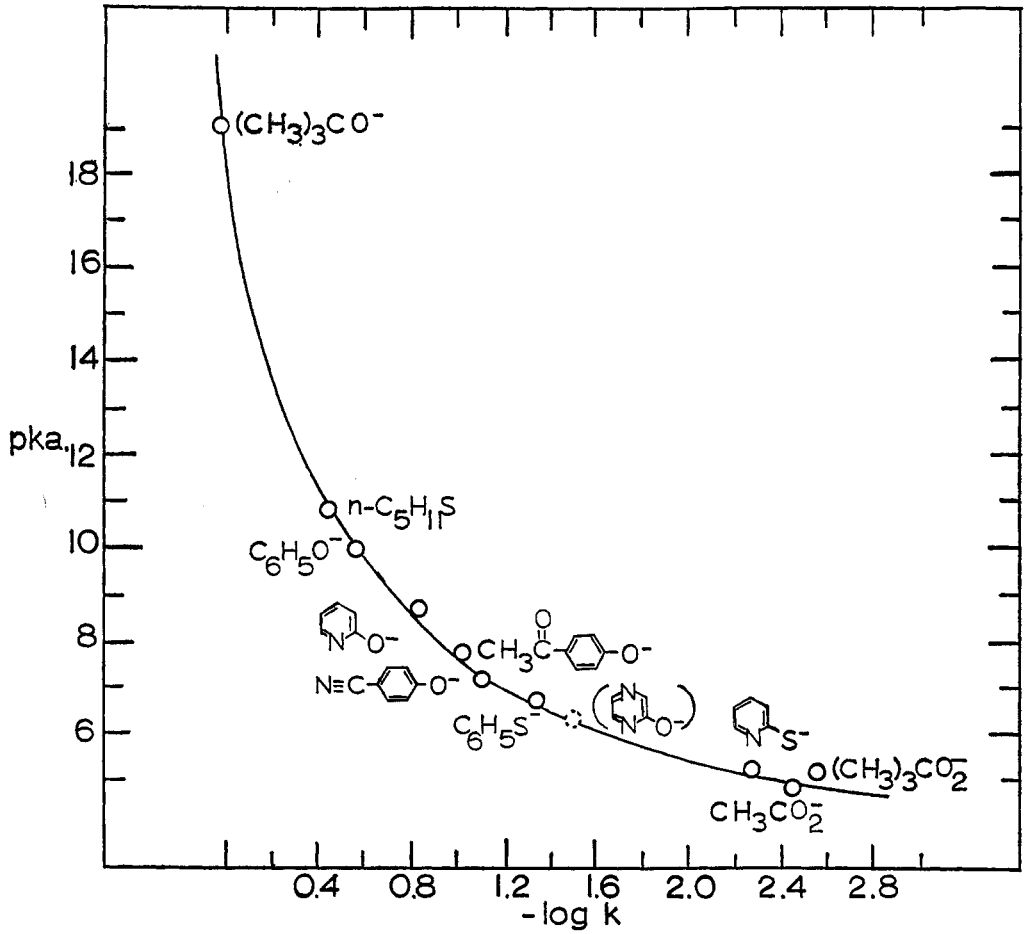


Fig. 2.—Reactions of anions with O,O-diphenyl phosphorochloridate at 58° (186).

TABLE VI

RATE CONSTANTS FOR THE REACTION OF SOME ANIONIC NUCLEOPHILES WITH SOME PHOSPHATE ESTERS (143)

Nucleophilic reagent	$P(OMe)_3$[a] k_1, min.$^{-1}$	$P(OMe)_3$[a] log k_2/k_{H_2O}	$Et{\cdot}PO(OMe)_2$[a] k_1, min.$^{-1}$	$Et{\cdot}PO(OMe)_2$[a] log k_2/k_{H_2O}	$MePO(O{\cdot}CH_2Ph)_2$[b] k_1, min.$^{-1}$	$MePO(O{\cdot}CH_2Ph)_2$[b] log k_2/k_{H_2O}
Water	2.02×10^{-2}	0	1.5×10^{-3}	0	6.4×10^{-2}	0
Chloride	0.49×10^{-2}	1.83	0.3×10^{-3}	1.74	8.3×10^{-2}	2.46
Bromide	2.40×10^{-2}	2.52	1.8×10^{-3}	2.52	11.9×10^{-2}	2.61
Thiocyanate	8.71×10^{-2}	3.07	3.04×10^{-3}	3.75	72.4×10^{-2}	3.38
Iodide	4.65×10^{-2}	2.81	7.02×10^{-3}	3.10	4.7×10^{-2}	3.41
Thiosulfate	4.51	4.79	0.21	4.58	3.47	4.07
Hydroxide	1.67	6.09	4.70	7.40	59.0	5.31
Hydroxide (estd.)[c]		2.75		2.60		2.60

[a] With 0.2 N anion in water at 80°. [b] With 0.2 N anion in 25% acetone + 75% water at 100°. [c] Estimated from the equation $\log k_2 = \alpha E_r + \log k_2^0$, where α is a constant characteristic of the substrate, E_r is the oxidation–reduction potential of the anion, and k_2^0 is the rate of a reference reaction, here that of the substrate with water. The k_1 is the apparent first-order rate constant for the reaction with an anion of concentration $[X^-]$; $k_2 = k_1/[X^-]$.

been suggested to lie in a greater electron availability for bond formation (104), but the alternative explanation of bifunctional catalysis has not been excluded (186). Neither of these explanations appears compelling, and the striking reactivity of fluoride ion toward the phosphoryl group (91, 97) remains unexplained.

Hudson and Harper (143) utilized the known proportionality between the oxidation–reduction potential of an ion and its reactivity in bimolecular substitution reactions at saturated carbon (103, 104) as a criterion of mechanism in the reaction of several phosphate esters with anions. The reactivity of chloride, bromide, iodide, thiocyanate, and thiosulfate ions could be readily correlated with their oxidation-reduction potentials (Table VI). Hydroxide ion is about a 1000 times as reactive as would be predicted on the basis of its oxidation–reduction potential. Since hydroxide ion is known from O^{18}-tracer studies to attack several triesters at phosphorus, the inference seems justified that the other ions are carrying out SN2 displacement at carbon. Although product studies were not carried out in this investigation, products of C–O cleavage by anionic nucleophiles have frequently been obtained, and the reaction has found wide synthetic application (46, 64, 66, 82, 90, 176, 248).

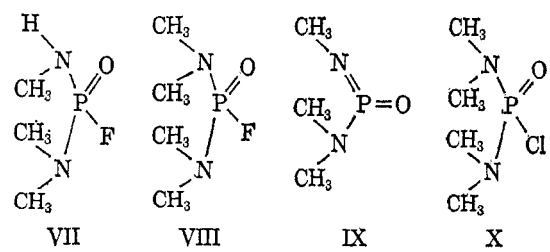
N,N-Diethyl dithiocarbamate dealkylates phosphate esters (212), as does thiophenoxide ion (187). Thiourea is also alkylated by phosphate esters, but the alkyl group becomes attached to nitrogen rather than to sulfur (192).

Ammonia at room temperature attacks trialkyl phosphates principally at the carbon but displaces phenoxide ion from triaryl phosphates to form phosphoramides (162). Amines, with the possible exception of imidazole, react with neutral esters of phosphoric acid to effect SN2 reaction at the carbon, alkylating the amine (15, 27, 51, 60). This reaction has been of considerable value as a step in the preparation of various esters of phosphoric acid; the phosphorylating agent, dibenzyl phosphorochloridate, can be used to phosphorylate an alcohol, and then one benzyl group can be removed cleanly by reaction of the triester with a suitable amine. Though the negative charge on the anion prevents further attack by amines, the second protective benzyl group may be removed by catalytic hydrogenation.

Organometallic reagents have long been known to react with trialkyl and triaryl phosphates at phosphorus (see ref. 116 for leading references). Gilman and Gaj (116) have recently shown that the steric requirements of the ester and of the carbon–metallic compounds are important in determining the course of the reaction. Sterically hindered Grignard reagents react preferentially with trialkyl phosphates at the carbon, with alkylation of the carbanionoid carbon, while unhindered Grignard reagents react with formation of a carbon–phosphorus bond. Triphenylsilyllithium was alkylated by several phosphate esters (115).

Saunders and co-workers have found that, whereas phenylmagnesium bromide reacts with diisopropyl phosphorochloridate to form mainly triphenylphosphine oxide, its reaction with diisopropyl phosphorofluoridate can be controlled to yield diisopropyl phenylphosphonate (205).

From the data of Table III, group 2, it is clear that substituted phosphoramides in which one nitrogen atom still bears a hydrogen substituent are much more reactive with hydroxide ion than are similar compounds in which all hydrogens on nitrogen have been replaced by alkyl groups. Thus, at 25° the reaction of VII with hydroxide ion is more than 10^3 times as fast as that of VIII (134, 136a). Examination of the kinetic

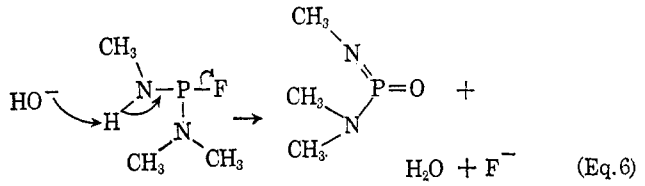


VII VIII IX X

parameters for the two series reveals that the activation energy for the group of compounds in which the hydrogen is present is nearly constant (*ca.* 11 kcal. per mole),

while that for the series in which the nitrogen is fully substituted is 3 to 5 kcal. per mole larger, becoming greater with increasing size of the alkyl substituents. The frequency factors in the fully substituted series are, in addition, slightly lower.

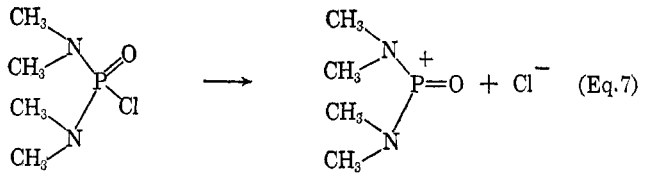
This striking difference does not seem to be explicable in terms of simple steric effects, but instead suggests that the series which undergoes ready reaction has available to it a reaction pathway of lower energy than that of the substituted series. Westheimer (240) suggested that the base in this case removes a proton from the nitrogen, rather than attacking phosphorus, aiding the departure of the anion of the leaving group (Eq. 6), a variety of general base catalysis. The resultant species IX, the nitrogen analog of a monomeric metaphosphate ester, then would be expected to undergo rapid hydrolysis to the observed product.



(Eq. 6)

VII IX

Hall (125) has observed that the rate of hydrolysis of X was not significantly increased by the addition of hydroxide ion, and suggested that the rate-determining step of the reaction is ionization in the sense of Eq. 7.



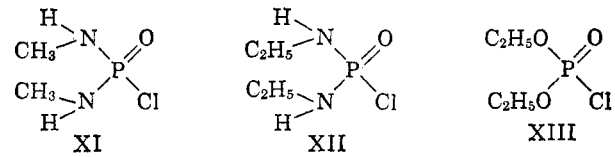
(Eq. 7)

X

Crunden and Hudson found that the rate of hydrolysis of X is increased by nitrite ion and by acetoxime, both of which presumably serve as nucleophilic catalysts (84), and Samuel and Westheimer showed that the rate of reaction of X is increased by addition of azide ion and that the phosphoryl azide could be isolated in good yield (204). At high hydroxide ion concentrations a slight increase in the rate of hydrolysis was observed as well. Thus, the process probably involves bimolecular attack of the nucleophile at phosphorus. The circumstance that hydroxide ion and water exhibit nearly the same nucleophilicity toward X served to obscure the molecularity of the hydrolysis. The hydrolysis of X is catalyzed by pyridine, but not by 2,4- or 2,6-dimethylpyridine (241). The base-catalyzed hydrolysis, therefore, presumably occurs by way of nucleophilic attack by the heterocyclic base on the phosphorus atom.

Hall and Lueck (126) have recently reported that mercuric perchlorate greatly increases the rate of the neutral hydrolysis of X and offer this fact in support of an ionization mechanism in the sense of Eq. 7, assisted by coordination of the halide ion with mercuric ion. Since no criterion of molecularity has been offered, and since many examples of bimolecular nucleophilic attack at phosphorus are known to be catalyzed by coordination of a metal ion which acts as a general acid, this observation is a pertinent fact, but it alone does not establish the reaction mechanism.

Although X and XI are solvolyzed at nearly the same rate in slightly acidic solution, producing only chloride ion, in basic solution the rate of reaction of XI is much the faster (204). Similarly, Crunden and



Hudson (83) have found that the hydrolysis of N,N-diethyl phosphorochloramidate (XII) is catalyzed by 2,6-lutidine, while that of diethyl phosphorochloridate (XIII), is not. Since both compounds should have similar steric factors operative as well as qualitatively similar (although quantitatively different) electronic factors, the different response to catalysis was ascribed to change in mechanism from nucleophilic attack at phosphorus in (XIII) to base-catalyzed proton elimination in XII.

Waters and de Worms (236a), Kilpatrick and Kilpatrick (161), and Halmann (127) have shown that the hydrolysis of diisopropyl phosphorofluoridate is subject to general acid catalysis, while Dostrovsky and Halmann (96) have shown that the solvolysis of diisopropyl phosphorochloridate in a wide range of solvents is not acid-catalyzed. Apparently under these conditions the P–F bond is broken, although in formic acid the chloride is dealkylated as well (96).

Selim and Thanh (209) determined the rate of acid-catalyzed hydrolysis at 58.8° as a function of pH of a series of N,N-dialkylamides of dimethylphosphoric acid. The reaction rate in each case was first order in hydronium ion and first order in the amide. Presumably the reaction product was in every case the dialkylammonium salt of dimethylphosphoric acid. Representative pseudo-first-order rate constants at pH 2.35 are quoted in Table VII. Similarly, Heath and

TABLE VII

RATES OF ACID-CATALYZED HYDROLYSIS OF SOME PHOSPHORAMIDIC ESTERS (209)

$(CH_3O)_2PONR_2$ R_2	Rate of hydrolysis,[a] $k_1 \times 10^5$, sec.$^{-1}$
$(CH_3)_2$–	130
–$(CH_2)_5$–	43.7
–$(CH_2)_2O(CH_2)_2$–	15.9
$(C_2H_5)_2$–	4.21

[a] At pH 2.35 at 58.8°.

Casapieri (138) found triamides of phosphoric acid to be very stable in base but readily hydrolyzed in acidic solution. Diphenyl N,N-dimethyl phosphoramidate affords diphenyl phosphorochloridate with gaseous hydrogen chloride (211).

One may conclude that if a sufficiently basic group is attached to phosphorus—here, a fluorine atom or an amino group—it may be protonated, leading to faster attack of the nucleophile at phosphorus, and hence acidic catalysis of the hydrolysis (7).

In neutral or acidic aqueous solution trimethyl phosphate undergoes reaction with water at a rate which is not appreciably increased by the addition of perchloric acid up to 3 *M* (23). Studies carried out in O^{18}-enriched water showed that the tracer appears in the methanol, rather than in the dimethyl phosphate produced; hence the reaction is an SN2 displacement at carbon, the leaving group probably being the dimethyl phosphate anion.

Since many nucleophiles may compete in the C–O cleavage with water, the rate of production of dimethyl phosphate from trimethyl phosphate in neutral or acidic solutions is found to be increased when buffers or acids are used, the anions of which possess good nucleophilicity toward carbon. Domange and Masse (95) have studied the rate of hydrolysis of trimethyl phosphate in several buffer mixtures varying in hydrogen ion concentration from 1 *N* $HClO_4$ to pH 11. Complex behavior was observed, the most likely explanation of which seems to be that, in the regions of low pH, water and the buffer anions competitively attack the ester at carbon, while at higher pH attack of hydroxide ion at phosphorus supersedes other reactions.

Hudson and Keay (144) studied the hydrolysis in 1 *N* benzenesulfonic acid of a series of dialkyl alkylphosphonates, $(RO)_2R'PO$. The data suggest that, whereas the methyl esters are hydrolyzed by bimolecular attack of water, the higher esters undergo unimolecular fission to give carbonium ions and alkyl alkylphosphonate ions. The observation (151) that (−)-2-octyl ethyl methylphosphonate, $(C_6H_{13}CH_3C^*HO)(C_5H_5O)CH_3PO$, is racemized at the same rate that acid is produced under the same experimental conditions used for the other phosphonates substantiates this conclusion. In neither study was it shown that the rate of the solvolysis is dependent upon the concentration of added acid; indeed, it is probable that the reactive species is the neutral ester, not its conjugate acid.

In contrast to the behavior of other reported triesters of phosphoric acid, the rate of hydrolysis of tri-*t*-butyl phosphate is not increased by the addition of hydroxide ion, and pronounced depression of the rate occurs when the solvent is made less polar (72, 188). These observations suggest that the rate-determining step is ionization of the ester to the *t*-butyl carbonium ion and the di-*t*-butyl phosphate ion. This reaction appears to be strongly catalyzed by the acid produced in the reaction (188). Qualitatively similar behavior has been found for triisopropyl phosphate (70). Similarly, the propanolysis of tetrabenzyl pyrophosphate in the presence of added base was found by Dudek and Westheimer (100) to be autocatalytic. However, since this reaction shows a positive salt effect, it is possible that the observed catalysis is due simply to the ions produced and is not truly an acid catalysis (29). In general, in the studies reported so far, the attempt has been made to minimize the occurrence of such acid-catalyzed processes, since they complicate the kinetic behavior of these systems.

The rate of the reaction of neutral triethyl phosphate with water is greatly reduced by the change of solvent from pure water to 50% dioxane–water (222), and in the mixed solvent acid catalysis was observed. The position of bond fission under acid catalysis in the mixed solvent was not reported, so that it is unknown whether substitution at phosphorus or at carbon was being observed. The rate of hydrolysis of triphenyl phosphate in 75% dioxane–water at 100° first increases in rough proportion to the stoichiometric acidity up to 0.5 *M* perchloric acid, then falls off (232).

A convincing explanation of this behavior has not been offered since the ester was shown not to be significantly protonated in this solvent in the presence of 1 *M* perchloric acid. Full publication of the experimental facts of the matter has not been made.

Under neutral or acidic conditions, alcohols attack triesters of phosphoric acid with primary alcohols chiefly at carbon, rather than at phosphorus (153, 189, 226). Although trimethyl phosphate may thus be used as a methylating reagent in a manner similar to the use of dimethyl sulfate, it appears to be less reactive than the sulfate ester and serves no unique synthetic purpose. The mechanisms of these reactions have not been studied.

III. Hydrolysis of Diesters of Phosphoric Acid and Related Compounds

A. Acyclic compounds

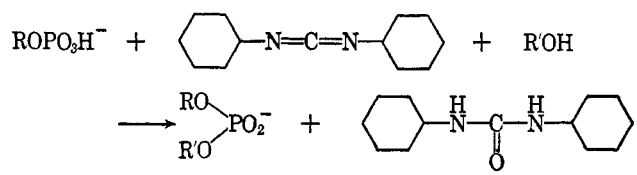
With the exception of esters in which the phosphorus is included in a five-membered ring, or esters of 2-hydroxyalkyl groups in which conversion to a five-membered ring diester can occur by intramolecular transesterification, diesters of phosphoric acid are as a group the least reactive of the series of phosphates. The unusually reactive types of diesters will be discussed at length later.

As yet, of the group of normal diesters, only dimethyl phosphate and dibenzyl phosphate have been studied in detail. Kumamoto, Cox, and Westheimer (166) found the hydrolysis of the monoanion of dimethyl

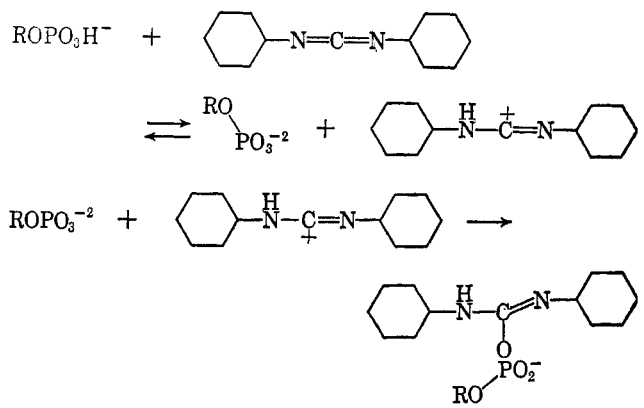
phosphate to be extremely slow. In strongly alkaline solution the reaction was first order in hydroxide ion and first order in the ester, but the absolute rate constant was somewhat dependent upon the composition of the solution. The ionic strength principle ordinarily does not apply to such highly concentrated solutions. However, only about 10% of this reaction results in cleavage of the phosphorus–oxygen bond, the remainder involving nucleophilic attack at carbon (123, 124). The second-order rate constant for attack at phosphorus is $k_2 = 5 \times 10^{-7}$ (mole/l.)$^{-1}$ sec.$^{-1}$ at 125° in water.

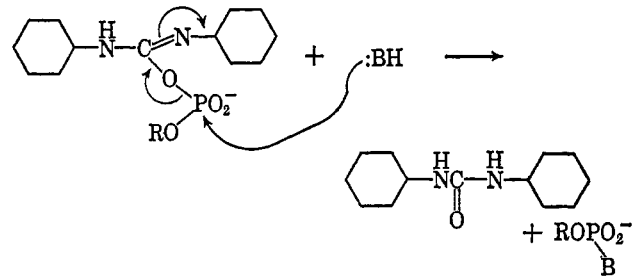
When a good leaving group is present in a disubstituted phosphate, more rapid attack at phosphorus is possible. Thus, the dihalo acids and their salts, $HOPOX_2$, which presumably are intermediates in the hydrolysis of the phosphoryl halides, are not known as isolable substances (231). The compound EtOP(O)(OH)F has been prepared by cautious hydrolysis of the corresponding chlorofluorophosphate (210). It is hydrolyzed only slowly.

The monoanion of di-*p*-nitrophenyl phosphate reacts slowly with hydroxide ion; $k_2 = 6 \times 10^{-6}$ (mole/l.)$^{-1}$ sec.$^{-1}$ at 25° in 20% ethanol–80% water, $\Delta E^* = 18.3$ kcal./mole, log $PZ = 8.2$ (155). Many modern phosphorylation techniques involve "activating" phosphoric acid or one of its monoesters so as to incorporate a good leaving group in a disubstituted derivative (159). A wide variety of reagents is available for this purpose, of which the following equation is typical.

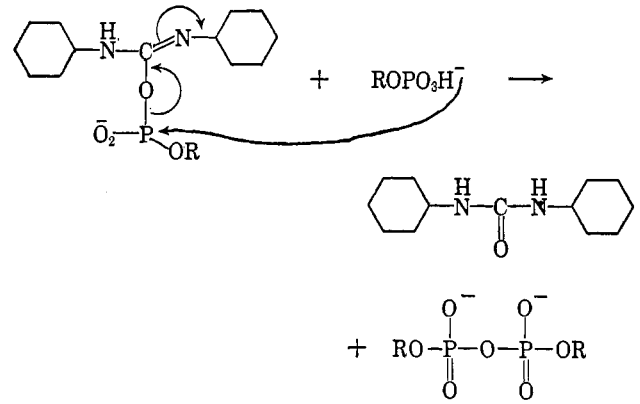


Although the mechanism of this kind of reaction has not been established, the following interpretation seems to accommodate most of the known facts.

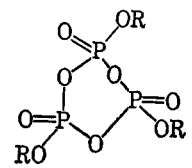




A variant of this pathway has been suggested by Weimann and Khorana (237, 238).

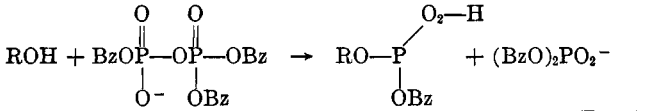


In two similar steps the dialkyl pyrophosphate is transformed into a trialkyl trimetaphosphate



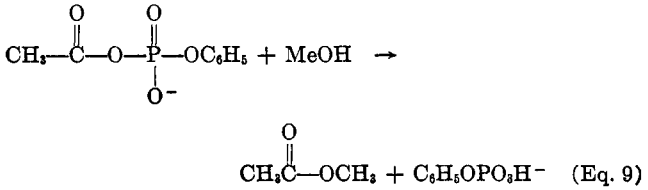
which is believed to be the actual phosphorylating agent. Nucleophilic attack at phosphorus in the trimetaphosphate results in formation of diesters, etc.

Similarly, Cramer and Wittmann (81) reported that tribenzyl pyrophosphate phosphorylates alcohols, amines, and acids in the manner of Eq. 8 rather than



(Eq. 8)

by attack at the fully substituted phosphorus atom. Di Sabato and Jencks (91) have shown, though, that nucleophiles attack the monoanion of an acyl phenyl phosphate at carbon, expelling the phenyl phosphate anion as in Eq. 9.



(Eq. 9)

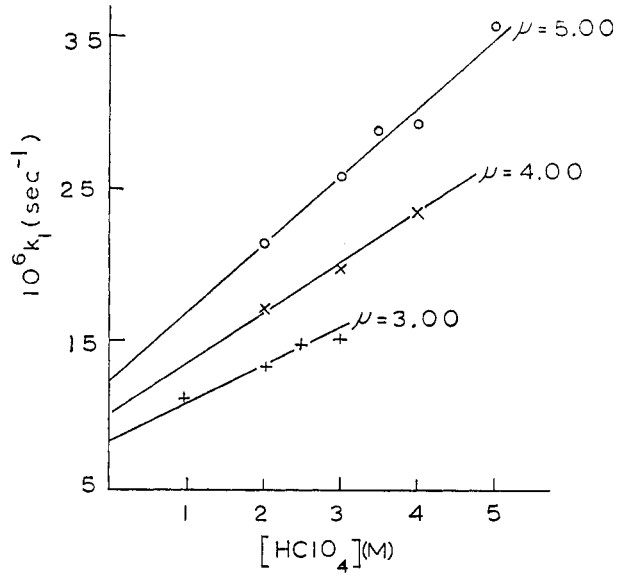


Fig. 3.—Hydrolysis of dimethyl phosphate in perchloric acid at constant ionic strengths at 100° (46).

A study of the hydrolysis of dimethyl phosphate in solutions of varying acidity has been made by Bunton and co-workers (46). In solutions that are not more acidic than pH 0 the only species present in large concentration which reacts at a significant rate is the undissociated acid, $(MeO)_2PO_2H$. The value of the dissociation constant was estimated by measurement at several lower temperatures and graphical extrapolation to 100°, the temperature at which the kinetic measurements were made. Using pK = 1.60, so obtained, to evaluate C_NC_P, the fraction of the dimethyl phosphate present in neutral form, the specific rate constant given by $k_N = k_{obsd}C_PC_N$, where k_{obsd} is the observed rate constant at any pH, was shown to be sensibly constant over the range from pH 0.72 to 4.17, a variation in C_NC_P of about 350-fold. At 100° the mean value of k_N = 4.2 × 10^{-6} sec.$^{-1}$. The reaction shows a positive salt effect, the magnitude of which can be evaluated from the intercepts of a series of plots of observed rate constants *vs.* stoichiometric acidity, each plot at constant ionic strength (Fig. 3). The data can be represented by the equation $k_{N'} = k_Ne^{0.182\mu} \times 10^{-6}$ sec.$^{-1}$, where k_N = 4.92. Although the value of k_N obtained by this method differs somewhat from that obtained by the first method described above, considering the complexities of the system the two values may be said to be in rather good agreement.

In solutions 1 to 5 M in perchloric acid, but of constant ionic strength, the rate of hydrolysis of dimethyl phosphate increases linearly with acid concentration (Fig. 3) and can be represented by the equation $k_{obsd} = k_{N'} + k_{A'}[H^+]$, where $k_{N'}$ and $k_{A'}$ are the rate constants for reaction at zero ionic strength of the neutral species and of its conjugate acid, respectively. The reaction of the conjugate acid exhibits a positive salt

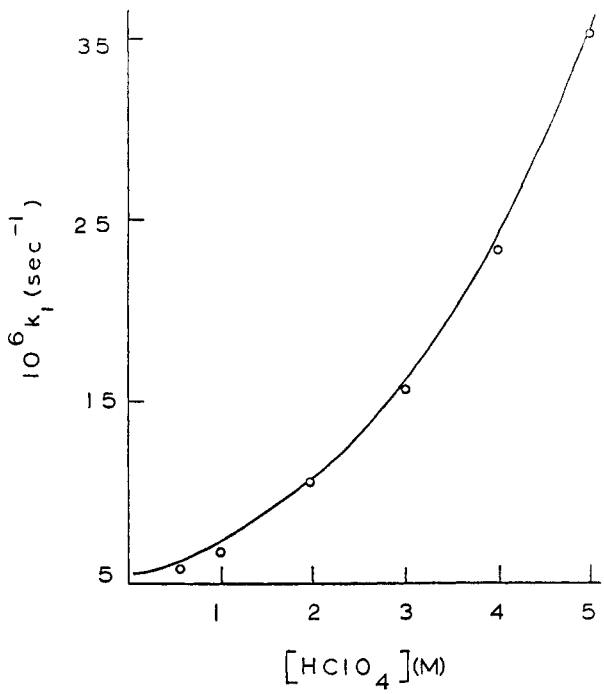


Fig. 4.—Hydrolysis of dimethyl hydrogen phosphate in perchloric acid at 100°; the curve corresponds to Eq. 10 (46).

effect, which, as in the case of the neutral species can be fitted by the second empirical term of the Debye–Hückel equation (178).

The dependence of the rate of hydrolysis of dimethyl phosphate on hydrogen ion concentration and ionic strength over the range from pH 4 to 5 M $HClO_4$ is, then, satisfactorily represented by Eq. 10 (46). In

$$k_{obsd} = (4.92e^{0.182\mu} + 1.02[H^+]e^{0.306\mu})C_N/C_P \times 10^{-6} \text{ sec.}^{-1} \quad \text{(Eq. 10)}$$

Fig. 4, the curve is a plot of this equation, while the experimental values are the plotted points.

The position of bond fission involved in the hydrolysis of the neutral species and of the conjugate acid has been investigated by Bunton and co-workers (46) and by Haake and Westheimer (124). Bunton and co-workers analyzed the methanol for isotopic composition after hydrolysis in water enriched in O^{18}, while Haake and Westheimer analyzed the monomethyl phosphate produced. Considering the diversity of methods and the complexity of the system, the results obtained by the two sets of workers are in reasonably good agreement. The hydrolysis of the neutral species proceeds with 20–30% P–O cleavage. However, the value of the percentage of P–O cleavage in the reaction of the conjugate acid depends critically on small differences in large numbers, and only considerable additional careful work can resolve the question as to whether the amount is of the order of 11% (46), or near 0% (124).

A careful kinetic investigation of the hydrolysis of dibenzyl phosphate by Kumamoto and Westheimer (167) showed that the rate of hydrolysis may, as in the

case of dimethyl phosphate, be represented by an equation such as Eq. 11 where [E] is the concentration of the

$$v = k_{obsd}[E] = k_1[H^+][E] + k_2[E] + k_3[E^-] \quad \text{(Eq. 11)}$$

ester and at ionic strength = 1 and 100°, $k_1 = 4.53 \times 10^{-4}$ sec.$^{-1}$, $k_2 = 3.16 \times 10^{-5}$ sec.$^{-1}$, and $k_3 = 4.16 \times 10^{-8}$ sec.$^{-1}$. The monoanion of the monoester was found to be 23 times as reactive as that of the diester; the reactivity per benzyl group is, therefore, some 46 times as great. Since this ratio is that of total reactivity, and since the reaction of the monoanion of the monoester probably proceeds with exclusive phosphorus–oxygen cleavage, while that of the diester probably proceeds primarily with carbon–oxygen fission, the ratio of reactivities at phosphorus is probably much higher than this uncorrected result.

In summary, then, each of the molecular species in which dimethyl phosphate exists in aqueous solution, differing only in degree of protonation, undergoes hydrolysis by at least two mechanisms, one involving attack at phosphorus, the other at carbon. Since unimolecular separation of a methyl carbonium ion or of an oxyphosphonium cation seems unlikely, all of these processes may be presumed to involve water as a nucleophile in a bimolecular, rate-determining step. Application of his empirical criterion of mechanism of acid-catalyzed reactions by Bunnett (42) to the C–O cleavage reaction of the conjugate acid species gives a result in agreement with the one based on intuition. Bunnett's criterion fails, however, in the case of monomethyl phosphate in acidic solution, where the intuitive argument may also be considered reliable. It must, therefore, be applied with caution to situations in which no independent check is available, as the acid-catalyzed reactions of α-D-glucose-1 dihydrogen phosphate (44).

Mechanisms similar to each of these may be expected to occur generally, and, in addition, if the alkyl group is able to form a stable carbonium ion, additional mechanisms which result in carbon–oxygen cleavage by unimolecular processes may occur. Furthermore, the possibility that solvolysis of certain disubstituted phosphoric esters proceeds *via* a monomeric ester of metaphosphoric acid must be kept in mind.

Monoalkyl esters of phosphoramidic acid react readily with nucleophiles, apparently by attack of the nucleophile upon the zwitterion of the starting material, $ROP(NH_3^+)O_2^-$ (65). This reaction has been widely applied synthetically.

B. FIVE-MEMBERED CYCLIC DIESTERS OF PHOSPHORIC ACID

In sharp contrast to the behavior of usual diesters of phosphoric acid, the reactivity of diesters in which a hydroxyl group is present on a carbon adjacent to the esterified hydroxyl group is high. Thus, for instance, while methyl 2-methoxyethyl phosphate is nearly inert

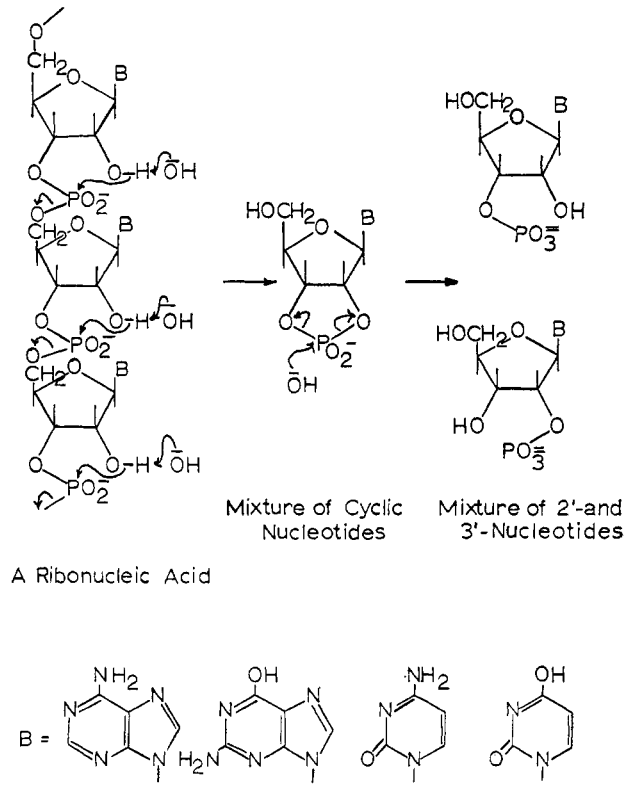


Fig. 5.—Hydroxide ion-catalyzed hydrolysis of a ribonucleic acid.

to mild, basic hydrolysis, methyl 2-hydroxyethyl phosphate is rapidly hydrolyzed under the same conditions to 2-hydroxyethyl phosphate (18). Proceeding on the assumption that this structural feature makes available a reaction pathway of low activation energy which is not possessed by ordinary diesters, and reasoning partly from the demonstration by Chargaff (57) that the establishment of equilibrium between 1- and 2-glycerophosphates in acid is intramolecular, Brown and Todd (40) interpreted these reactions as involving five-membered ring, cyclic esters of phosphoric acid as intermediates, and reviewed reactions from their work and from the work of others which could be interpreted to support this hypothesis. The formation of a cyclic diester could thus account for the rapid depolymerization in base of ribonucleic acid as pictured in Fig. 5. Desoxyribonucleic acid, from which the hydroxyl groups requisite for cyclic ester formation are absent, is relatively stable in alkaline solution.

Experimental evidence to support this hypothesis was soon forthcoming, principally in the form of the isolation of the presumed intermediate cyclic mononucleotides (181–183). Todd and co-workers were able to synthesize these esters in sufficient quantity to characterize them thoroughly and to show that they were hydrolyzed by base rapidly enough to fit the proposed scheme (37).

Lipkin, Talbert, and Cohn (177) carried out the

TABLE VIII

RATES OF HYDROLYSIS OF ESTERS OF SUBSTITUTED 2-HYDROXYETHYL PHOSPHATES AT 60° AND IONIC STRENGTH = 1 (33)

Compound	Cation of salt	$10^6 k_2$, sec.$^{-1}$ (mole/l.)$^{-1}$
Benzyl *cis*-2-hydroxycyclohexyl phosphate	$C_6H_{11}NH_3^+$	15
Benzyl *trans*-2-hydroxycyclohexyl phosphate	$C_6H_{11}NH_3^+$	2.6
Benzyl glycerol-1-cyclohexyl phosphate	$C_6H_{11}NH_3^+$	35
Methyl *cis*-2-hydroxycyclohexyl phosphate	$C_6H_{11}NH_3^+$	25
1-Glyceryl *cis*-2-hydroxycyclohexyl phosphate	Ba^{+2}	115
1-Glyceryl *trans*-2-hydroxycyclohexyl phosphate	Ba^{+2}	89

alkaline hydrolysis of ribonucleic acid to nucleotides in H_2O^{18}, and then obtained inorganic phosphate from the nucleotides; this inorganic phosphate was analyzed for isotopic oxygen. Only one atom of oxygen is introduced from the solvent into the inorganic phosphate during this sequence of reactions. This showed that the intermediate could not possibly be a triester of the type first suggested by Fonó (110) and by Brown and Todd (40), but could well be a cyclic diester. It also suggested that in the opening of the five-membered ring the P–O bond is broken almost exclusively.

In order to understand more clearly the several processes involved in ribonucleic acid hydrolysis, Westheimer and co-workers (68, 69, 123, 124, 149, 167, 235) have examined the mechanism of hydrolysis of the simplest cyclic ester, ethylene phosphate, while Brown and co-workers have studied the properties of model systems for the internal transesterification which constitutes the first step of the hydrolytic sequence (33, 34, 41).

In all cases reported by the Cambridge group (33, 34, 41) the rate-determining step of the hydrolysis of a β-hydroxy diester in base was found to be ring closure. In contrast, the corresponding step in ribonucleic acid depolymerization is faster than the subsequent ring opening of the cyclic diester. The rates summarized in Table VIII were reported. Since a marked barium ion catalysis was observed, rates of hydrolysis of esters with different cations are not directly comparable, but some generalizations may be drawn. The conformation of the six-membered ring with the two hydroxyls *cis*, that is, one axial, one equatorial, is more favorable in the ring closure than that of the *trans*, presumably with both equatorial, but is slightly less favorable than the case in which free rotation is allowed. Ring closure in the nucleic acid series appears to be much faster, likely due to the more favorable geometry of the *cis* hydroxyl groups on the five-membered ring of the ribose residue. Glycerol appears to be a better leaving group than methanol, which is better than benzyl alcohol. The studies of Barker, Montague, Moss, and Parsons (21) and of Brown and Usher (41) suggest that the reactivity decreases sharply with increase of bulk of the leaving group.

The question of whether the lability to hydrolysis of the cyclic nucleotides results wholly or principally from the presence of the five-membered ring, or whether other structural factors of these complex molecules are important in this regard has been the subject of several independent investigations. Kumamoto, Cox, and Westheimer (166) reported the synthesis of ethylene phosphate and determined its rate of alkaline hydrolysis to be about 10^7 times that of its acyclic analog, dimethyl phosphate; $k_2 = 4.7 \times 10^{-4}$ (mole/l.)$^{-1}$ sec.$^{-1}$ at 25° in water. Other groups have obtained this cyclic ester by various routes and agree as to its high reactivity (152, 160, 175, 228–230).

The study of the kinetics of the hydrolysis of ethylene phosphate, and of the oxygen exchange with the solvent which accompanies the acid-catalyzed hydrolysis, has yielded insight into the nature of the transition state for nucleophilic substitution at phosphorus. The reaction of ethylene phosphate anion with hydroxide ion is first order in each of the reactants; the appropriate kinetic parameters are given in Table IX. Hydrolysis

TABLE IX

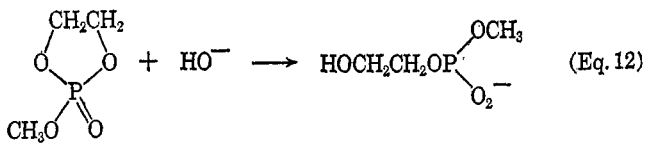
HYDROLYSIS OF ETHYLENE PHOSPHATE (69, 166)

KOH, mole/l.	$HClO_4$, mole/l.	Potassium ethylene phosphate, mole/l.	$BaCl_2$, mole/l.	Temp., °C.	μ	$10^4 k_2$, sec.$^{-1}$ (mole/l.)$^{-1}$
0.14		0.032		25	0.67[a]	4.74
0.13		0.032	0.083	25	0.67[a]	27.5
0.14		0.043		25	0.18[a]	3.65
	0.0049	0.0078	[c]	30	0.200[b]	0.052
	0.0100	0.0078	[c]	30	0.200[b]	0.233
	0.0101	0.0062		30	0.016[b]	0.335
	0.0146	0.0078	[c]	30	0.200[b]	0.49
	0.100	0.0078	[c]	30	0.200[b]	21.40
	0.150	0.0078	[c]	30	0.200[b]	31.60

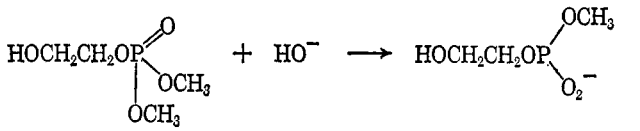
[a] Maintained with potassium *p*-toluenesulfonate. [b] Maintained with sodium perchlorate. [c] Barium ethylene phosphate used.

under basic conditions in water enriched in O^{18}, followed by isolation of the products and determination of their isotopic composition, revealed that the reaction proceeds with exclusive P–O fission. The limit of detection of C–O fission by the method employed was estimated at 1 part in 300. In contrast, dimethyl phosphate was found to be hydrolyzed in base, a process also first order in the ester monoanion and in hydroxide ion, with about 90% C–O fission. Thus, ethylene phosphate reacts with hydroxide ion *at phosphorus* about 10^8 times as fast as does dimethyl phosphate.

That this enormous reactivity is associated with thermodynamic strain of the five-membered ring was shown by Cox, Wall, and Westheimer (73), who compared the heats of hydrolysis of methyl ethylene phosphate and dimethyl hydroxyethyl phosphate (Eq. 12 and 13). The opening of the five-membered ring was found to be accompanied by the release of about 5.5 kcal./mole in excess of the heat of hydrolysis of a simple triester (149). Wall (235) found a high reactivity for the cyclic triester *vis-à-vis* its acyclic analog, comparable to that already found for the diester compared with its acyclic analog.



(Eq. 12)



(Eq. 13)

The same reactivity at phosphorus characterizes the acid-catalyzed hydrolysis of ethylene phosphate (Table IX). In contrast to the kinetic behavior of dimethyl phosphate in acidic solution, the only significant term in the rate equation for the acid hydrolysis of ethylene phosphate involves the conjugate acid of ethylenephosphoric acid. The rate of the reaction is given, then, by the equation

$$v = k[\mathrm{E}][\mathrm{H}^+]$$

where E is ethylenephosphoric acid. By studying the variation of rate with acidity in the region in which the apparent order in protons is changing from 2 to 1, Cox and Westheimer were able to assign to ethylenephosphoric acid the dissociation constant, $K_A = 0.1$ at ionic strength = 0.20 and 30°. Using this value of K_A, a constant value for k was found for the acidity range 0.15 to 0.005 M $HClO_4$, which encompasses a 700-fold variation in reaction rate.

This relatively simple kinetic behavior made possible a more precise analysis of exchange of oxygen with the solvent than has proved possible with methyl and dimethyl phosphate, since a minimum of correction of the observed data is necessary to account for side reactions. Haake and Westheimer found that after hydrolysis of ethylene phosphate in acid solution in a solvent enriched in O^{18}, the phosphate group of the hydroxyethyl phosphate obtained contained more than one atom of oxygen derived from the solvent per phosphate residue (124). When the hydrolysis was interrupted before completion of the reaction and the unreacted ethylene phosphate was isolated and analyzed for O^{18}, there was found to be excess O^{18} present in the phosphate group. The phosphoryl group had thus undergone acid-catalyzed exchange of oxygen with the water of the solvent prior to hydrolysis, the ratio of the rate constant for hydrolysis to that for exchange being about 5. The analysis of exchange into dimethyl phosphate in acid solution was much less precise, but it is apparent that although exchange of oxygen with the solvent *via* either of the mechanisms available in acid solution (that of the neutral species or that of the conjugate acid) is considerably slower than hydrolysis, exchange of the phosphoryl oxygen with the solvent occurs in both cyclic and acyclic diesters in acidic solution. Thus, the inclusion of the phosphoryl group in a five-membered ring has effected the acceleration of *both* the hydrolysis and the exchange without hydrolysis, and by a factor that is approximately the same for both processes, some 10^8.

Prior to the discovery that the exchange process was subject to the same large acceleration as the hydrolysis it had been argued that relief of the strain by opening the ring resulted in the enhanced rate of hydrolysis observed. It now appears, however, that the strain must be relieved in the transition state for both the hydrolysis and the exchange, although the final product of the hydrolysis, hydroxyethyl phosphate, no longer contains the strain, while in the "final product" of the exchange reaction, recovered ethylene phosphate, the strain is still present. This result therefore considerably limits the number of possible geometries of the transition state of the reaction, provided that the two processes are of the same mechanistic type. Proceeding on this last assumption, one may then consider the possible activated complexes for hydrolysis and exchange.

The geometries of three such complexes are illustrated in formulas XIV, XV, and XVI of Fig. 6. Each of these formulas represents one ethylenephosphoric acid, one water, and one extra proton, in accord with the kinetic equation. The resultant positive charge is divided between the oxygen atoms marked +.

A choice among these may be made on the basis of the following considerations. Since the five-membered ring is responsible for an increase in rate of about 10^8 for both the hydrolysis and the exchange reactions, it may be presumed to have the same geometrical place-

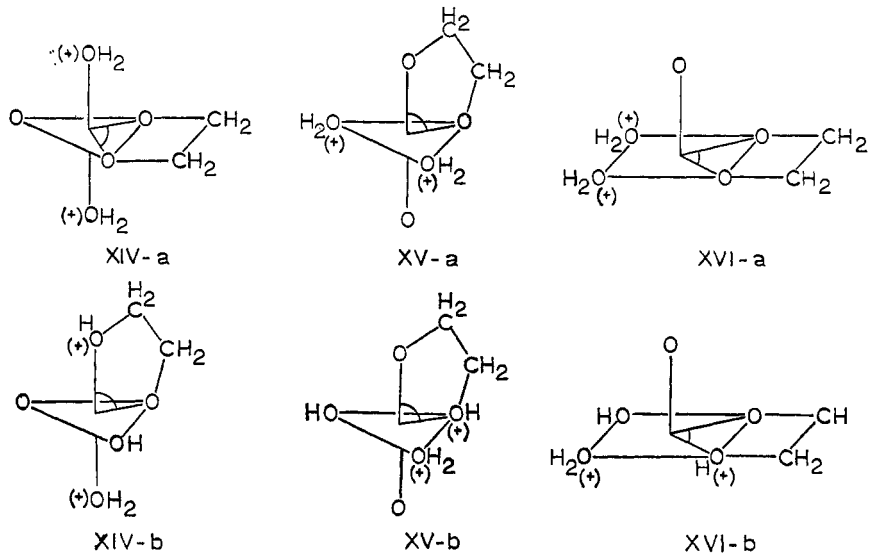


Fig. 6.—Possible geometric arrangements of the activated complexes for the acid-catalyzed hydrolysis and exchange of ethylene hydrogen phosphate (124).

ment in the transition state for both of these reactions.

If the transition state is assumed to have the geometry of a trigonal bipyramid, the geometry most often associated with pentacoordinated phosphorus, as in gaseous PF_5 and PCl_5 (31), then the attacking and the departing groups may *a priori* occupy both basal or both axial positions, but not one of each, without violation of the principle of microscopic reversibility. The square pyramid may not be arbitrarily ruled out, though, as recent evidence suggests this structure for pentaphenylphosphorane, $(C_6H_5)_5P$ (242b). XIVa represents the transition state for oxygen exchange when both groups are axial, and XIVb represents that for hydrolysis when both groups are axial. In XIVa the O-P-O bond angle in the ring is 120°, while in XIVb it is 90°. Hence, these transition states have quite different ring geometries and must be rejected.

XVa and XVb, however, satisfy the requirement and therefore represent a possible geometry for the transition state.

XVIa and XVIb, geometrical models based on a square pyramid, also satisfy the requirement of similar ring geometry. Reaction *via* XVI leads to retention of configuration about phosphorus, however, while reaction *via* XV leads to inversion. Since all the reported instances of nucleophilic attack at phosphorus lead to inversion of configuration (1, 120, 121, 184), the transition states XV are to be preferred, although the stereochemistry of the particular reaction has not been determined.

Confirmation that the presence of the five-membered phospho diester ring in a molecule labilizes nucleophilic attack at phosphorus, even when the ring remains intact in the reaction product, has recently come from other sources (38, 68, 196).

The five-membered ring sulfates show a similar acceleration of rate of nucleophilic attack at sulfur (149). Possible explanations of the basis of these stereoelectronic effects have been considered in detail (86, 149).

Wall (235) has recently reviewed the numerous reported examples of hydrolysis of six- and seven-membered ring esters. Trimethylene phosphate, the parent six-membered diester, reacts at very nearly the same rate as an acyclic ester (58, 160), and tetramethylene phosphate reacts slightly more slowly (160, 235). Isotopic labeling experiments to determine the position of bond cleavage in these hydrolyses have not yet been reported.

IV. Nucleophilic Substitution of Monosubstituted Derivatives of Phosphoric Acid

In pioneering investigations of the variation of rate of hydrolysis of monoesters of phosphoric acid with acidity of the medium, Bailly (16, 17) and Desjobert (87, 88) learned that the rate of hydrolysis of ethyl phosphate passes through a maximum at about pH 4 to 5. In more alkaline solution the rate steadily decreases, while in more acidic media a minimum rate is reached at about 1 *M* acid, after which the rate again increases with increasing acidity. A characteric plot of such behavior is shown in Fig. 7. Since from the approximately known values of the ionization constants of ethylphosphoric acid it could be calculated that the maximum rate in the region of pH 4 corresponds to the maximum concentration in solution of the monoanion, it was suggested that the monoanion has available to it a special mechanism for nucleophilic substitution which supersedes all other mechanisms in the pH range over which the monoanion is present in significant concentration. Extrapolating from the rates of reaction of the monoanion of dimethyl phosphate with hydroxide ion measured at 125 and 115° (166) and comparing with the

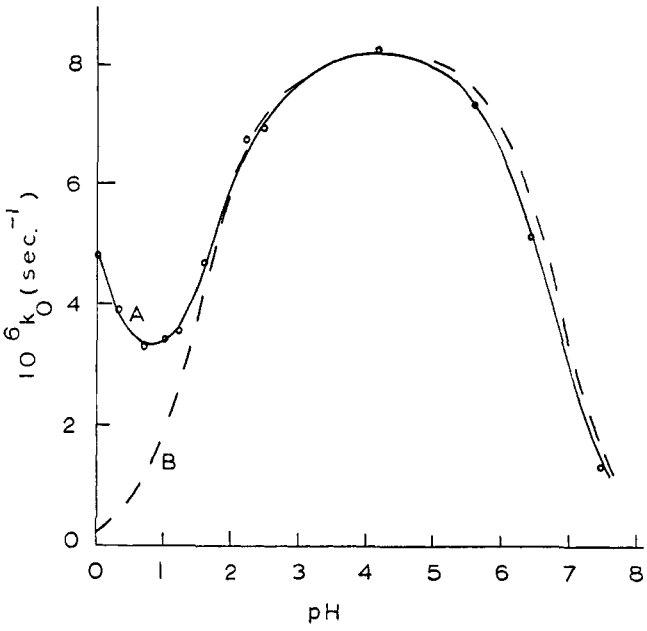


Fig. 7.—Hydrolysis of monometyhl phosphate at 100.1° (43) A, exptl.; B, calcd. from Eq. 14.

rate of reaction measured at 100° of the monoanion of methyl phosphate (43) and taking into account the position of bond cleavage (124), it can be calculated that the first-order rate constant for the hydrolysis of the monoanion of monomethyl phosphate is about 10^2 times as great as the second-order rate constant for the reaction of dimethyl phosphate monoanion with hydroxide ion at phosphorus. If the hydrolysis of monomethyl phosphate is presumed to be first order in water, as well as in the monoanion, a point not yet demonstrated by experiment, this rate factor should be divided by about 55 to be directly comparable. In any event, when it is taken into account that the nucleophilicity of hydroxide ion relative to water would be expected to be high, the rate factor is so large as to suggest that a different mechanism operates in the monoester from that in the diester.

This interpretation was confirmed by the study of 1-methoxypropyl-2 phosphate by Butcher and Westheimer (47), who showed in addition that the phosphorus–oxygen bond, rather than the carbon–oxygen bond, is cleaved in the process. In a careful study of methyl phosphate Bunton, Llewellyn, Oldham, and Vernon (43) estimated the dissociation constants of methylphosphoric acid and thus were able to calculate the concentration of each molecular species at a given pH. Assignment to the monoanion of an absolute rate of reaction permitted prediction of the rate of hydrolysis over the range of hydrogen ion concentration from pH 1 to strongly basic solution by means of the equation

$$\text{rate} = k_1[\text{ROPO}_3\text{H}^-] \qquad \text{(Eq. 14)}$$

in which k_1, the specific first-order rate constant for reaction of the monoanion, has the value 8.23×10^{-6} sec.$^{-1}$ at 100.1°. This relationship failed in strongly acidic solution due to the incursion of acid catalysis of the hydrolysis. Here two additional species were found to undergo hydrolysis: the neutral acid (that is, undissociated methylphosphoric acid) and its conjugate acid. The complete rate expression, therefore, involves three terms which refer to the rates of reaction of the monoanion, the neutral species, and the conjugate acid of the neutral species; since these three species are in equilibrium with each other and with added mineral acid, the total rate at any acidity can be predicted, provided the individual rates and dissociation constants have been evaluated.

A. SOLVOLYSIS OF THE MONOANION

Rate constants which have been reported for solvolysis of the monoanion of monosubstituted phosphoric acids are listed in Table X. In many cases these specific rate constants were calculated from the rate data and from the equilibrium constants for dissociation of the acid. In some cases these are rate constants for the maximum rate observed when the rate was studied as a function of pH. In a number of these derivatives, the reactivity of the neutral species is much greater than is that of simple alkyl esters, so that its reaction becomes predominant at higher pH. The result of this is that the maximum in the pH–rate profile is obscured, and a plateau rather than a maximum occurs. Compounds in Table X which show this behavior are indicated by an asterisk.

That the bond between phosphorus and oxygen is broken exclusively in the reaction of the monoanion has been established in a sufficient number of cases to indicate the generality of the result. Thus, hydrolysis at pH 4, the rate maximum, of optically active 1-methoxypropyl-2 phosphate yielded optically pure alcohol of retained configuration (47). Similarly, when water enriched in O^{18} is used as solvent, the alcohol produced has been found in a large number of cases (to which no exceptions have been reported) to be of normal isotopic composition, while the inorganic phosphate produced is found to contain one oxygen atom derived from the solvent per phosphate ion, within experimental error, as predicted by uncomplicated cleavage of the phosphorus–oxygen bond. It should be stressed that this result obtains only in the region of the pH maximum.

The polarity and polarizability of the leaving group influence the rate of the reaction but to a rather small extent; substitution of a more acidic leaving group usually increases the rate slightly but 2,4-dinitrophenyl phosphate is reported to be very much less stable than would be predicted from the comparative rates of hydrolysis of phenyl and *p*-nitrophenyl phosphates (14). As in the case of the triesters, reduction of the

TABLE X

RATE CONSTANTS FOR THE HYDROLYSIS OF THE MONOANIONS OF MONOSUBSTITUTED PHOSPHATES, $RXPO_3H^-$[b]

RX[a]	k, sec.$^{-1}$	Temp., °C.	ΔE^*, kcal. mole^{-1}	ΔS^*, e.u. (or A, sec.$^{-1}$)	P–X cleavage demonstrated	Ref.
HO–	4.03×10^{-6}	100.1			Yes	45
MeO–	8.23×10^{-6}	100.1	30.6	(6.47×10^{12})	Yes	43
EtO–	6.09×10^{-6}	100			No	88
$MeOCH_2CHO(Me)$–	7.1×10^{-6}	78			Yes	47
Glycerol-1–	1.35×10^{-5}	100	29.9	(4.28×10^{12})	Yes	220
Glycerol-2–	2.77×10^{-5}	100	30.3	(1.52×10^{13})	Yes	220
$H_3N^+CH_2CH_2O$–	3.07×10^{-5}	100	29.6	(6.71×10^{12})	Yes	220
$C_6H_5CH_2O$–*	9.6×10^{-7}	75.6			No	167
α-D-Glucose-1–*	1.45×10^{-6}	82	30.0	(5.04×10^{12})	Yes	44
$CH_2{=}C(CO_2H)O$–	2×10^{-4}	80			No	236b
C_6H_5O–	2.6×10^{-4}	100	28.3	+0.9	Yes	52, 215, 232
p-MeC_6H_4O–	2.2×10^{-4}	100			Yes	232
p-$NO_2C_6H_4O$–	5.5×10^{-4}	100	29.7	(2.46×10^{13})	Yes	232
p-$NO_2C_6H_4O$–	1.1×10^{-3}	100			No	54
p-$NO_2C_6H_4O$–	1.0×10^{-4}	80	26.0		No	141
m-$HO_2CC_6H_4O$–	4.47×10^{-5}	80	27.3	−1.4	No	52
p-$HO_2CC_6H_4O$–	8.52×10^{-5}	80	27.7	+0.9	No	52
α-Naphthyl-O–	1.24×10^{-5}	80	30.0	+4.1	No	52
β-Naphthyl-O–	4.08×10^{-5}	80.6	28.9	+5.5	No	52
$Me_3N^+CH_2CH_2O$–	1.8×10^{-5}	100	24.3	−16	No	11
BuS–	1.31×10^{-3}	37	23.8	+3.1	Yes	94, 139, 140
$H_2NCH_2CH_2S$–	$\sim 5 \times 10^{-4}$	37			Yes	2, 94
HS–	1.67×10^{-4}	52.8			Yes	93
H_2N–*	7.36×10^{-6}	20.0	23.6		Yes	54, see also 132
C_6H_5NH–*	9.6×10^{-5}	20			Yes	53
p-ClC_6H_4NH–*	1.06×10^{-4}	20	19.8	−6.3	Yes	53
p-$MeOC_6H_4NH$–*	1.02×10^{-4}	20			Yes	53
EtOCONH	2.12×10^{-4}	37	27.8	+14.4	Yes	128
C_6H_5CONH–	1.84×10^{-4}	37	25.8	+7.5	Yes	129
$(C_6H_5O)_2PONH$–	5.9×10^{-6}	37	30.0	+13.2	Yes	129
HO_2CCH_2NH–	9.8×10^{-4}	25				245
$C_6H_5SO_2NH$–	5.5×10^{-5}	37	26.1	+0.56	Yes	130
H_2NCOO–	2.05×10^{-4}	37	22.8	−3.98	20% P–O 80% C–O	131, see also 9
CH_3COO–	2.1×10^{-4}	39	22.5	−3.6	Yes	92
C_6H_5COO–	1.0×10^{-4}	39			No	92
p-CH_3O-C_6H_4COO–	8.0×10^{-5}	39			No	92
p-$CH_3C_6H_4COO$–	9.5×10^{-5}	39			No	92
p-ClC_6H_4COO–	9.33×10^{-5}	39			No	92
p-$NO_2C_6H_4COO$–	1.36×10^{-4}	39			No	92
m-$NO_2C_6H_4COO$–	1.37×10^{-4}	39			No	92
3,5-Di-$NO_2C_6H_4COO$–	2.83×10^{-4}	39			No	92
FCH_2COO–	$\sim 7.7 \times 10^{-5}$	24			No	180
CH_3CH_2S–	8.43×10^{-4}	34.8			P–S	2
$HOCH_2CH_2S$–	1.0×10^{-3}	34.8			P–S	2
$HOCH_2CH_2CH_2S$–	7.94×10^{-4}	34.8			P–S	2
$H_3N^+CH_2CH_2S$–	8.17×10^{-4}	34.8			P–S	2
$H_3N^+CH_2CH_2CH_2S$–	6.93×10^{-4}	34.8			P–S	2
$CH_3N^+H_2CH_2CH_2S$–	4.75×10^{-4}	34.8			P–S	2
$(CH_3)_2N^+HCH_2CH_2S$–	2.95×10^{-4}	34.8			P–S	2
$(CH_3)_3N^+CH_2CH_2S$–	3.57×10^{-4}	34.8			P–S	2
$(CH_3CH_2)_2N^+HCH_2CH_2S$–	2.40×10^{-4}	34.8			P–S	2
$(H_2N)_2C^+NHCH_2CH_2S$–	6.42×10^{-4}	34.8			P–S	2
$(H_2N)_2C^+NHCH_2CH_2CH_2S$–	7.40×10^{-4}	34.8			P–S	2
$HO_2CCH_2CH_2S$–	1.06×10^{-3}	34.8			P–S	2

[a] Compounds marked with an asterisk do not show a pH–rate maximum in the region of pH 4–5 due to the rapid reaction of the neutral species (see text). [b] Cherbuliez and co-workers have reported many rates at pH 4.5. For leading references see (59).

possibility of $d\pi$–$p\pi$ overlap in the ground state between phosphorus and an adjacent first row element, as by substituting sulfur for oxygen, may result in an even larger rate increase. The dependence of the rate of hydrolysis of n-butylthiol phosphate on acidity is qualitatively very similar to that of methyl phosphate, yet its maximum rate is several powers of ten times as fast (94a). It should be noted in this connection that monothiophosphoric acid is isolable as its salts (246), but its monoanion is rapidly hydrolyzed to phosphoric acid (93). Silicone grease has been reported to catalyze its hydrolysis in strong base (3). Similarly, monofluorophosphate is the only monohalophosphate stable enough to have been isolated (231). Presumably $d\pi$–$p\pi$ overlap stabilizes this molecule relative to monochlorophosphate.

In general, the kinetic results reported have been determined at only one ionic strength for each com-

pound. For methyl phosphate the rate of hydrolysis of the monoanion was found to be insensitive to changes of ionic strength (43), nor was the rate sensibly affected by change of solvent to deuterium oxide, insofar as the water pH scale is applicable to that solvent.

Although, as Kumamoto and Westheimer have pointed out, reaction between the uncharged acid and the hydroxide ion would show the same kinetic behavior as a reaction between the monoanion and water, such a mechanism may be discarded on several grounds. In particular it cannot account for the fact that the hydrolysis of the monoanion is much faster for monobenzyl than for dibenzyl phosphate (167); further, Bunton, *et al.*, have calculated (43) that the specific rate constant for such a reaction of methylphosphoric acid would have to be *ca.* 10^7 l. mole^{-1} sec.$^{-1}$ at 100°. This value, compared with that measured for the reaction under the same conditions between trimethyl phosphate and hydroxide ion, 3.3×10^2 l. mole^{-1} sec.$^{-1}$, clearly rules out such a possibility.

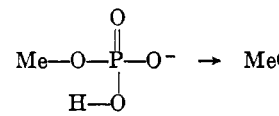
Since no experimental criterion of the kinetic order in water of the solvolysis of the monoanion has been adduced, mechanisms which must be considered for this process include direct nucleophilic attack of water on the monoanion as well as mechanisms which require the unimolecular dissociation of the monoanion into an intermediate which then is captured by the nucleophile.

Although Koshland and Herr have claimed that their observation of varying ratios of nucleophilicity of methanol to water for several esters rules out the unimolecular mechanism, inspection of the experimental conditions employed by them reveals that the experiments were carried out at a variety of acidities; therefore, different mechanisms were in operation. Actually, the few results obtained in the region of the pH–rate maximum suggest that an intermediate common to all these cases was formed and captured (139, 140, 164).

Thus, bimolecular attack of water on the monoanion with breaking of the old phosphorus–oxygen bond concerted with making of the new phosphorus–oxygen bond, and accompanied by simultaneous migration of a proton, is a possible path for the reaction. The addition of water to the phosphoryl group followed by the elimination of the elements of the alcohol from the addition compound is, however, ruled out by the results of using O^{18} as a tracer in the solvent. If the addition–elimination path were operative, it would be expected that the substrate would become enriched with O^{18} prior to hydrolysis, since the addition compound should be able to eliminate the elements of water to reform starting material as well as those of alcohol to form products. The inorganic phosphate produced should then contain more than one oxygen atom from the solvent. No such prior enrichment has been found to occur (43, 215).

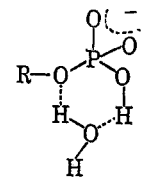
Westheimer and co-workers (47, 167) and Bunton and co-workers (22) have suggested that a unimolecular decomposition of the monoanion to an alcohol and the hypothetical intermediate, monomeric metaphosphate ion, occurs in the rate-determining step. This intermediate has been proposed in several instances in connection with phosphorylations; it would be expected to be rapidly hydrated to afford orthophosphate, polymerized to polymeric metaphosphates, particularly trimetaphosphate, and to react with nucleophiles other than water which are present in the solution. Such products do occur.

This process may be formulated as simple heterolysis of the monoanion (Eq. 15). A more attractive formu-



(Eq. 15)

lation of this process was offered by Westheimer and co-workers (47, 167). The intermediate XVII, in which a water molecule is hydrogen bonded to the monoanion, was supposed to constitute the actual reactive species. This intermediate can decompose readily because the alcohol is formed, rather than the high



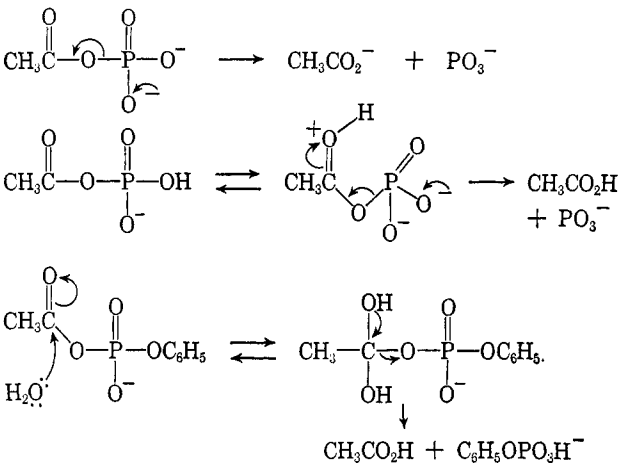
XVII

energy anion RO^-. The dianion $ROPO_3^{-2}$ does not react readily with hydroxide ion because of the electrostatic repulsion of the mutual negative charges of the ions; the dianion could enter reaction with water by way of an ion similar to XVII only by involving the endothermic ionization of water to produce hydroxide ion. These considerations explain why the rate is faster at pH 4 than in alkaline solution. Furthermore, in more acidic solution, where $ROPO_3H_2$ is present in high concentration, hydrolysis by way of an intermediate analogous to XVII would lead to the alcohol, ROH, and monomeric metaphosphoric acid, HPO_3. But presumably the presence of the negative charge in the monoanion greatly facilitates the cleavage of the phosphorus–oxygen bond, since the bonding pair of electrons accompanies the oxygen atom. The greater reactivity of the monoanion is thus explained, provided it be allowed that at moderate acidities proton transfer to the alcoholic oxygen is an obligatory part of the process.

Finally, phosphoric acid itself undergoes exchange of oxygen with water by a mechanism which appears from its kinetic characteristics to be analogous to the hydrolysis reactions identified above (45). At 100°

the specific rate constant for the exchange of the monoanion with water is 4.03×10^{-6} sec.$^{-1}$, as compared to 8.23×10^{-6} sec.$^{-1}$ for the hydrolysis of the monoanion of methyl phosphate.

Of the possible derivatives of phosphoric acid other than esters, the acyl phosphates, the amides, and the polyphosphates seem to have been accorded the most study. The elegant experiments of Di Sabato and Jencks (92) on the hydrolysis of acetyl phosphate provide considerable support for the hypothesis that both the dianion and the monoanion fragment to monomeric metaphosphate in the rate-determining step of the reaction, in contrast to the monoanion of acetyl phenyl phosphate which undergoes nucleophilic attack by water at the acyl group. The proposed pathways are given in the following equations. Evidence supporting



the above formulations is summarized as follows:

(a) Phosphorus–oxygen bond cleavage is the result of hydrolysis of AcP^{-2}, $CH_3COOPO_3^{-2}$, and of AcP^-, $CH_3COOPO_3H^-$ (26, 191), while solvolysis of $AcPhP^-$, $CH_3COOPOO^-OC_6H_5$, occurs with carbon–oxygen bond cleavage (91).

(b) Values of ΔS^* for hydrolysis are 3.8 e.u. for AcP^{-2}, -3.6 e.u. for AcP^-, and -28.8 e.u. for $AcPhP^-$. These are values consistent with fragmentation and with bimolecular reactions, respectively.

(c) The volumes of activation for the neutral hydrolysis of AcP^{-2}, AcP^-, and $AcPhP^-$ are -1.0 ± 1, -0.6 ± 1, and -19 ± 2 cm.^{3}/mole, respectively. A large negative ΔV^* is characteristic of a bimolecular reaction.

(d) The rates of solvolysis AcP^{-2} and of AcP^- are insensitive to changes in ionic strength, while that of $AcPhP^-$ is considerably decreased in concentrated salt solutions.

(e) Similarly, addition of 30–50% acetonitrile has little effect of the rate of hydrolysis of AcP^{-2} and AcP^-, but considerably decreases that of $AcPhP^-$.

(f) The postulated mechanism for solvolysis of monoanions of phosphate monoesters (22, 47), phosphoramidates (53, 128, 129), acyl phosphates (92), thiophosphates (93, 94a), and phosphoric acid (45) requires proton transfer either during the rate-determining step or in a prior equilibrium so as to allow fragmentation to the monometaphosphate ion (which may be stabilized by resonance) and a neutral molecule of alcohol, amine, thiol, water, etc. These reactions show rather small effects of substituents in the leaving groups, since, presumably, substituents which favor proton transfer make breaking the bond to phosphorus more difficult, and *vice versa*.

If the leaving group were a sufficiently stable anion, or if the bond between it and phosphorus is weak, it might be expected to depart as the anion, without requiring proton transfer. In this case the dianion of the ester would react by the elimination reaction. This pathway seems to be followed by the acyl phosphate dianions. Since proton transfer is not required, the rate of this process should be increased by electron-withdrawing substituents which stabilize the anion. Such an electronic effect is indeed observed in the hydrolyses of substituted benzyl phosphate dianions, which follow a Hammett σ–ρ relationship with $\rho = 1.2$. Presumably, the rapid decomposition of fluorophosphate dianion in base (89) and the hydrolysis of the thiophosphate dianion (93) occur by similar processes.

(g) The rates of neutral hydrolysis of AcP^{-2} and of AcP^- are not significantly changed in D_2O solution, while that of $AcPhP^-$ is decreased 2.5-fold. Although no solvent isotope effect was found for the hydrolysis of the monoanion of methyl phosphate (43), conclusion as to the generality of these results must await the outcome of further experiment, since Koshland and Herr (163) report that the monoanion of *n*-butylthiol phosphate is hydrolyzed over twice as fast in water as in D_2O. Halmann, Lapidot, and Samuel report that, whereas there is no solvent isotope effect in the hydrolysis of the monoanion of urethane phosphate, $C_2H_5OCONHPO(OH)_2$ (128), of diphenyl N-dihydroxyphosphinylphosphoramidate, $(C_6H_5O)_2PONHPO(OH)_2$ (129), or of N-benzenesulfonylphosphoramidic acid, $C_6H_5SO_2NHPO(OH)_2$ (130), the monoanion of N-benzoylphosphoramidic acid is solvolyzed about 20% faster in D_2O than in water (129). Jencks has presented reasoning that a small kinetic isotope effect is not necessarily inconsistent with a concerted proton transfer (92), but these results remain probably the most difficult to rationalize on the basis of the proposed mechanism.

(h) The low reactivity at phosphorus of dialkyl phosphate monoanions and the much smaller reactivity of $(C_6H_5COO)_2PO_2^-$ and $C_6H_5COOPO_2^-OC_6H_5$ than of $C_6H_5COOPO_3^{-2}$ clearly show that, except for the leaving group, the phosphate must be unsubstituted in order to undergo the metaphosphate elimination. Possibly part of the "driving force" of the fragmentation is formation of the resonance-stabilized PO_3^- ion.

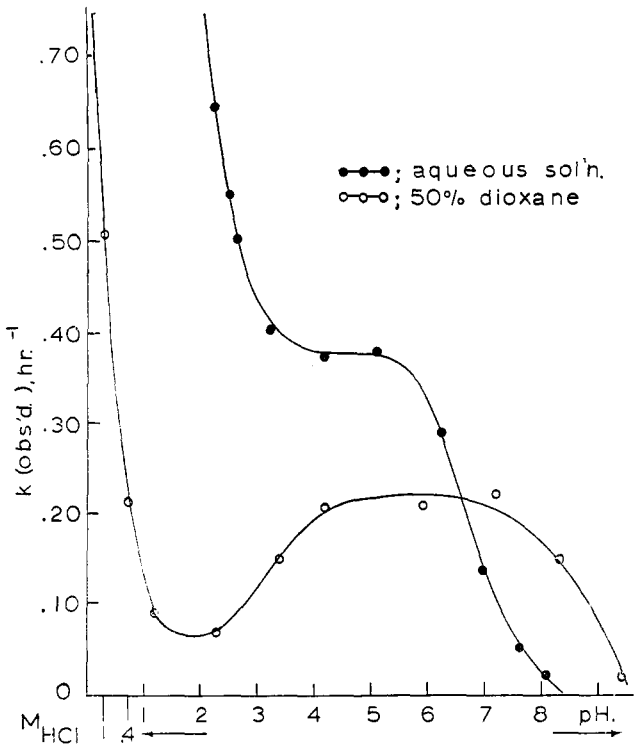


Fig. 8.—The observed hydrolysis rate as plotted against pH for N-(*p*-chlorophenyl)amidophosphoric acid at 20° (53).

(i) The formation of condensed phosphates accompanies solvolysis of the monoanions in solutions of low water activity (92) or in the presence of the orthophosphate ion (35, 94b, 224). While several paths to phosphoric anhydrides under these conditions can be imagined, their formation is at least consistent with a monomeric metaphosphate intermediate. In this connection the following observations are of interest.

Swoboda (219) found that change of solvent in the hydrolysis of the monoanion of glycerol-2 phosphate from 9% dioxane (mole fraction of H_2O = 1.00) to 90% dioxane (mole fraction of H_2O = 0.35) had no detectable effect on the rate. Brown and Hamer (35) solvolyzed phenyl hydrogen phosphate in concentrated aqueous solution and detected trimetaphosphate in the reaction mixture. The ester was stable to heating in pure dioxane, or in dioxane containing 1 or 2 moles of water per mole of ester. Thus, the aqueous solvent plays a role which equimolar quantities of water molecules cannot. This result could be rationalized on the basis of proton transfer in an equilibrium step or in a concerted manner during the rate-determining step.

Thus, many lines of evidence converge on fragmentation to monomeric metaphosphate as the best explanation of the "special mechanism" by which the phosphate monoanions (and dianions substituted with a good leaving group) react. While none of them is *per se* conclusive, together they form strong support for the original hypothesis of Butcher and Westheimer (47).

Extensions of this idea may be used to explain satisfactorily the catalysis of certain phosphate hydrolyses by metallic ions and by hydrolytic enzymes. Additional evidence for metaphosphates as intermediates is presented in connection with the hydrolysis of phosphoramidic halides.

The reactions of acetyl phosphate at neutral pH with hydroxylamine, aniline, morpholine, N-methylimidazole, glycine, and glycylglycine give C–O bond breaking, and involve a reaction between the amine as free base and the acetyl phosphate monoanion. These acyl transfer reactions are also subject to general base catalysis (91).

In water the aromatic monophosphoramides show a variation of rate of hydrolysis with pH rather similar to that of benzyl phosphate, glucose-1 phosphate and *t*-butyl phosphate. In the region of pH 4 to 5 a plateau, rather than a maximum appears, while the hydrolysis becomes much more rapid in more acidic media (53, 54). In water–dioxane mixtures, however, the rate of hydrolysis of the neutral species is sufficiently slower than that of the monoanion that a well-defined maximum appears, causing the pH–rate profile to resemble that of a typical aromatic phosphate ester (Fig. 8). Chanley and Feageson attribute the large diminution in rate of the neutral species on change of solvent to 50% dioxane–water to the hydrolysis of the neutral species *via* its zwitterionic form, $ArN^+H_2PO_3H^-$. Since in the medium of lower dielectric constant the ionic form would be less favored than the uncharged form, if the charged form were the reactive species, the rate would be decreased. It should be pointed out that the hydrolysis of the neutral species of monoesters shows a large positive ionic strength effect, which may well be associated with a similar cause.

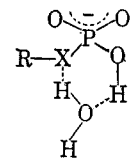
If the zwitterionic form of the monoanion, $ArN^+H_2PO_3^{-2}$, were the reactive form of this species, the rate of reaction of the monoanion should be similarly depressed by the change from water to 50% dioxane, whereas the observed depression of rate is relatively small compared to that of the neutral species. Thus, the monoanion probably does not react primarily through its zwitterionic form. If the reaction of the monoanion involved direct attack of water on the phosphorus atom, then the reaction of the amidophosphates would be expected to be much slower than that of the aromatic phosphate esters, since it has been shown that attack of hydroxide ion on triamidophosphates is very slow, while trialkyl phosphates are readily hydrolyzed by hydroxide ion (138). Actually, the hydrolysis of the monoanion of N-phenylphosphoramidic acid is about 10^3 times as fast as that of the monoanion of phenyl phosphate at 20°.

The entropy of activation of the hydrolysis of the monoanion of phosphoramidic acid is −1.6 e.u., calculated on the basis of a unimolecular reaction.

These several criteria which have been used to support the postulated metaphosphate intermediate in the

solvolysis of phosphate monoester monoanions would suggest that the same mechanism might be operative in the solvolysis of the monoanions of phosphoramidic acids as well.

The cyclic mechanism of Westheimer (47) accommodates all these facts, in that the intermediate XVIIa, where X is NH, is better able to hydrogen bond the requisite water molecule than that in which X is O.



XVIIa

If reaction occurs by this path, both intermediates would be presumed to decompose to the same reactive intermediate, monomeric metaphosphate ion. The insensitivity of the rate of reaction to the electronic nature of substituents in the aromatic nucleus is at least consistent with the proposed cyclic mechanism.

Recent studies of the hydrolysis of phosphoramidic acid and its derivatives and some of the earlier studies of acyl phosphates have produced results which show that direct attack on the monoanionic species by some nucleophiles occurs. By analogy with these displacement reactions, the hydrolysis may be also thought to be a direct nucleophilic displacement at phosphorus.

The evidence upon which this interpretation is based is summarized here. Several tertiary amines, including triethylenediamine, pyridine, 4-methylpyridine, and probably trimethylamine, catalyze P–O fission of the monoanion of acetyl phosphate (91, 191, 198). Fluoride ion analogously reacts with the monoanion to form fluorophosphoric acid, which can be detected in the reaction medium.

The monoanion of phosphoramidic acid similarly reacts with fluoride ion (132). Furthermore, the rate of solvolysis of phosphoramidate monoanion (but not the rates of the dianion, neutral species, or conjugate acid) in water or in water–alcohol mixtures is increased by the addition of pyridine, 3- and 4-methylpyridine, and the anions of nicotinic and isonicotinic acids. No catalysis of solvolysis was observed, however, on addition of 2-methylpyridine, 2,6-dimethylpyridine, aniline, the anion of picolinic acid, or the dianion of phosphoric acid (54, 199). This finding strongly supports interpretation of the observed base catalysis as a nucleophilic attack of the base on phosphorus, forming a reactive phosphoramidonium species as intermediate. The strong steric hindrance is in agreement with that found by Butler and Gold (48) in the pyridine-catalyzed solvolysis of acetic anhydride. Covitz and Westheimer have recently shown that general acid and general base catalysis is subject to a much smaller degree of steric hindrance (68).

One further criterion argues strongly against the intermediacy of metaphosphate in the solvolysis of the monoanionic species of both monoesters and monoamides. Such an intermediate, if produced in the presence of an array of nucleophiles each of which could react to form a stable product, should produce exactly the same ratio of products regardless of the source of the intermediate. Thus, the monoanions of phenyl phosphate and of phosphoramidic acid should, when solvolyzed in the same mixture of methanol and water at the same temperature and pH produce the same ratio of methyl phosphate to phosphoric acid, representing competition by methanol and water as nucleophiles for the intermediate metaphosphate. In fact, in 50% methanol–water mixtures, phosphoramidic acid produces about twice as much methyl phosphate as does *p*-nitrophenyl phosphate (54).

The alcoholysis of N-benzoylphosphoramidic acid was found by Zioudrou to be catalyzed by tertiary bases (250). A maximum in the reaction rate was observed at equimolar concentrations of acid and base. Substitution in the aromatic ring had negligible effect upon the reaction rate. The reaction thus appears to belong to the group which occur *via* the "special mechanism" of the monoanion.

Havinga, de Jongh, and Dorst discovered that the hydrolysis of *m*-nitrophenyl phosphate is subject to strong photochemical acceleration (135). An interpretation of this phenomenon has been given by Zimmerman and Somasekhara (249), but further investigation is required to show whether this reaction is mechanistically related to the other monoester solvolyses.

In summary, it seems to be established that all monoanionic species which display high reactivities toward hydrolysis cannot be hydrolyzed by a reaction pathway which requires formation of metaphosphate, but a large number of these cases are satisfactorily interpreted at present by this hypothesis.

B. SOLVOLYSIS OF THE NEUTRAL SPECIES

Although the neutral species, $ROPO_3H_2$, is the bulk component in solution at moderate acidities (about 1 *M* acid) in the case of simple alkyl esters, of which methyl phosphate may be taken as typical, it reacts so slowly that its reaction never is predominant (see Table XI). The reactions of the monoanion and the conjugate acid are kinetically more important at all points of the pH range. However, a plot (43) of the experimentally observed first-order rate constants against stoichiometric acidity for a series of equal ionic strength, $\mu = (NaClO_4 + HClO_4)$, yields a straight line at any given ionic strength (Fig. 9). The slope and intercept of this line are a measure, respectively, of the rate constant for the reaction of the conjugate acid and that for reaction of the neutral species. Since for a series of such plots at different total ionic strengths the slopes

TABLE XI

RATE CONSTANTS FOR THE HYDROLYSIS OF THE NEUTRAL SPECIES OF MONODERIVATIVES OF PHOSPHORIC ACID, $RXPO_3H_2$

$v = k_N e^{\beta\mu}$

RX–	k, sec.$^{-1}$	β	Temp., °C.	ΔE^*, kcal./mole	ΔS^*, e.u. (or A, sec.$^{-1}$)	Position of cleavage predominant	Ref.
MeO–	5.0×10^{-7}	0.423	100.1			C–O	43
α-D-Glucose-1–	3.03×10^{-2}		100.1	31.0	(4.30×10^{16})	C–O	44
C_6H_5O–	2.7×10^{-5}		100			P–O	22
HO–	1.28×10^{-6}		100.1			P–O	45
HS–	2.43×10^{-5}	Positive salt effect	52.8			P–S	93
BuS–	2.25×10^{-4}	Negative salt effect	37.1			P–S	94a
$H_2NCH_2CH_2S$–	$\sim 10^{-4}$		37			P–S	94a
$C_6H_5CH_2O$–	$\sim 10^{-6}$		75.6			C–O	167
H_2N–	6.6×10^{-6}		10.1			P–N	54
p-ClC_6H_4NH–	6.4×10^{-5}		0			P–N	53
C_6H_5CONH–	8.65×10^{-5}	Negative salt effect	37	26.4	+10	P–N	129
H_2NCOO–	1.9×10^{-4}		37	21	−4.1		131, see also 9

are constant, the acid-catalyzed reaction clearly is little affected by changes in ionic strength. The intercepts of such a family of lines increase with increasing μ; hence, the reaction of the neutral species is subject to a large, positive salt effect.

It has been suggested (178) that for reactions of uncharged reagents in not very dilute solution the dependence of rate constant on ionic strength has the form

$$k_N = k_N^0 e^{\beta\mu} \qquad \text{(Eq. 16)}$$

where k_N and k_N^0 are the specific rate constants at ionic strength μ and zero, respectively, and β is a constant. Since a straight line is obtained by plotting the values of log k_N calculated from the intercepts of the plot against μ, the hydrolysis of the neutral species obeys this equation. From the slope and intercept of the line, β and k_N^0 are found to be 0.423 and 0.50×10^{-6} sec.$^{-1}$, respectively. Hence, the first-order rate constant for the hydrolysis of the neutral species at any ionic strength is

$$k_N' = (C_N/C_P)(0.50 \times 10^{-6})e^{0.423\mu} \text{ sec.}^{-1} \qquad \text{(Eq. 17)}$$

where C_N/C_P is the fraction of monomethyl phosphate present as the neutral species (43).

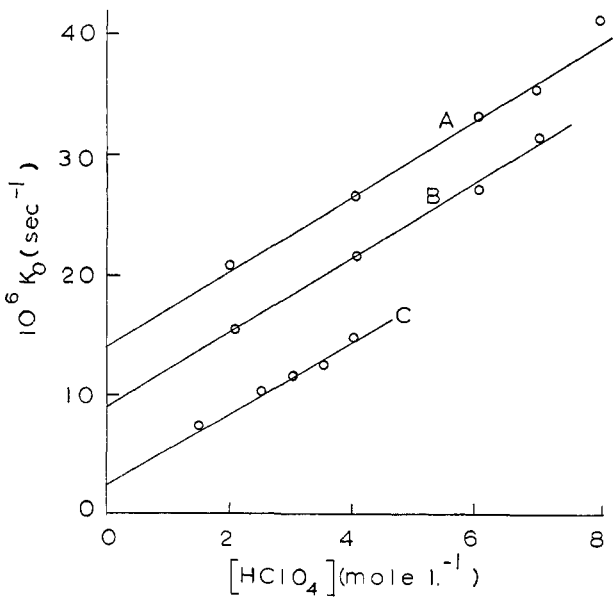


Fig. 9.—Acid-catalyzed hydrolysis of monomethyl phosphate at constant ionic strength at 100.1° (43): A, μ = 8.0, slope = 3.10×10^{-6}; B, μ = 7.0, slope = 3.12×10^{-6}; C, μ = 4.0, slope = 3.2×10^{-6}.

Isotopic analysis to determine the position of cleavage in the reaction of the neutral species is also complicated by the concurrent operation of more than one path in the acidity range over which the reaction can be observed. Within the considerable experimental error the reaction proceeds exclusively with carbon–oxygen fission; hence the predominant reaction of the neutral species (and perhaps the only one) is a substitution at carbon, rather than at phosphorus. Since separation of a methyl cation is energetically difficult, whereas water is a relatively good nucleophile toward saturated carbon, it may be concluded that the reaction is bimolecular (SN2), although the molecularity has not been established experimentally. Bunnett's criterion of mechanism (42) gives a result discordant with that arrived at by the above reasoning which Bunnett has interestingly rationalized.

If the path involving nucleophilic attack at carbon is made unfavorable, as, for example, by substituting for the methyl group an aromatic nucleus, reaction of the neutral species with phosphorus–oxygen cleavage is able to occur. Thus, the rate of reaction of the neutral species of phenyl phosphate is 2.6×10^{-5} sec.$^{-1}$ at 100° and the phosphorus–oxygen bond is cleaved exclusively. This rate is 50 times that of the reaction

of methyl phosphoric acid involving carbon–oxygen cleavage (5.0×10^{-7} sec.$^{-1}$) (22, 43).

Although the behavior of the monoanion of α-D-glucose-1 phosphate is unexceptional, the neutral species reacts 10^5 times as fast as that of methyl phosphate (3.03×10^{-2} compared with 5.0×10^{-7} sec.$^{-1}$, respectively, at 100°) (44). Here, as in the case of methyl phosphate, cleavage is between carbon and oxygen, but the molecularity of the reaction has not been established by experiment. Bunton and coworkers assign to this process a unimolecular mechanism on the basis of its rapid rate, and by analogy to the reactions of 2,3,4,6-tetra-O-methyl-α-glucopyranosyl chloride. In the solvolysis of the chloride, a bimolecular process could be observed only in solvents of low dielectric constant and in the presence of powerful nucleophiles such as thiophenoxide ions. The facile hydrolysis of *t*-butyl phosphates was briefly reported by Cramer (78). That this is also due to the separation of a carbonium ion from the neutral species of these compounds has been rigorously shown by Samuel (203) through a careful study of *t*-butyl phosphate. The pH–rate profile shows no minimum in the acidic region; labeling experiments show that the C–O bond is broken, and product studies and the demonstration of a large β-deuterium isotope effect are additional evidence for the SN1 reaction.

C. SOLVOLYSIS IN STRONG ACID

In 1 to 7 *M* perchloric acid the rate of hydrolysis of methyl phosphate increases in proportion to stoichiometric acidity (43). In more acidic media the rate of increase is somewhat greater. The linear portion of the curve gives a value for the second-order rate constant of the acid-catalyzed reaction, $k_A = 3.08 \times 10^{-6}$ l. mole^{-1} sec.$^{-1}$, in good agreement with that found in the experiments at different ionic strengths.

Isotopic tracer experiments revealed, however, that two processes, one involving carbon–oxygen cleavage and the other phosphorus–oxygen cleavage, occur with the conjugate acid. The specific rate constants for the two reactions were found to be (after correction for concomitant reactions) $k_A(C) = 0.65k_A$ and $k_A(P) = 0.35k_A$.

The formation of the conjugate acid is not rate-determining, since proton transfers in strong acids to oxygen are generally rapid, and also since change of solvent to 67.6% D_2O was associated with a small increase in reaction rate. The rate-determining steps of the acid-catalyzed reactions, therefore, involve equilibrium concentrations of the conjugate acid, and, since the rate is proportional to stoichiometric acid concentration, both reactions should by the Hammett–Zucker hypothesis be bimolecular. The reaction which proceeds by carbon–oxygen fission may therefore be represented in conventional manner as nucleophilic attack of water on the carbon atom of a protonated methyl phosphate molecule, the site of protonation probably being the ester oxygen, since in molecules such as phenyl phosphate, butyl thiophosphate, and N-benzenesulfonylphosphoramidic acid in which the basicity of this atom is reduced, no acid catalysis is observed up to high molar acid concentrations (22, 94a, 130). In dioxane–water solutions of acid, a medium of intrinsically higher proton donating ability than water alone, Chanley has observed acid catalysis of the hydrolysis of aromatic phosphates (53).

The hydrolysis of phosphoramidic acid and its derivatives is subject to acid catalysis (Table XII) provided the nitrogen atom is not substituted with strongly electron-withdrawing groups (53, 54, 129, 130, 132). The kinetic form of these acid-catalyzed hydrolyses varies considerably. The rate of hydrolysis of N-(4-chlorophenyl)phosphoramidic acid is linear in the stoichiometric acid concentration to 2 *M* HCl (53), while the rate of hydrolysis of phosphoramidic acid

TABLE XII

SECOND-ORDER RATE CONSTANTS FOR THE ACID-CATALYZED HYDROLYSIS OF MONOESTERS AND MONOAMIDES OF PHOSPHORIC ACID, $RXPO_3H_2$

$$v = k_A[H^+]e^{\beta\mu}$$

RX–	k_A, sec.$^{-1}$ (mole/l.)$^{-1}$	β	Temp., °C.	Cleavage	Ref.
HO–	5.45×10^{-7}	0.212	100.1	P–O	45
CH_3O–	2.00×10^{-6}	Nil	100.1	C–O	43
CH_3O–	1.08×10^{-6}	Nil	100.1	P–O	43
α-D-Glucose-1–[a]	4.0×10^{-5}		100.1	Probably C–O	44
α-D-Glucosamine-1–[b]	4×10^{-5}		100	Probably C–O	198
$C_6H_5CH_2O$–	8.7×10^{-5}		75.6	Probably C–O	167
H_2N–[a]	1.3×10^{-3}		10	P–N	132
H_2N–[a]	9.15×10^{-4}		10	P–N	54
p-ClC_6H_4NH–	2.44×10^{-4}		0	P–N	53
C_6H_5CONH–	5.02×10^{-5}		37	P–N	129
H_3N^+COO–[c]	1.6×10^{-3}	Positive salt effect	37	P–N	131, see also 9

[a] The log of the reaction rate appears to be linear in H_0, rather than in log $[H_3O^+]$; the rate quoted is for 1 *M* acid (see text). [b] The rate constant quoted is the specific rate constant for the protonated species. [c] $\Delta E^* = 26$ kcal./mole; $\Delta S^* = +12.3$ e.u./mole.

itself is proportional to the Hammett acidity function, H_0, with slope = 1 (132). These results would by the Hammett–Zucker hypothesis support the suggestion that the hydrolysis of the conjugate acid of the aromatic compound requires bimolecular attack of water, while the conjugate acid of phosphoramidic acid fragments to metaphosphoric acid and ammonia. The rate of hydrolysis of N-benzoylphosphoramidic acid also follows H_0 (129).

The composition of the product mixtures from solvolysis of these compounds in water–methanol mixtures does not seem easily reconciled with this hypothesis, however. Upon solvolysis in 1 *M* acid in a mixture of 29.2 mole % of methanol at 25°, there was produced from phosphoramidic acid 9.9% of methyl phosphate, while from N-(4-chlorophenyl)phosphoramidic acid there was produced under the same conditions 33.2% of methyl phosphate (54). Since methyl phosphate is formed in a quantity in slight excess of the mole % of methanol in the solvent mixture in the solvolysis of other compounds thought to produce metaphosphate, this evidence suggests that the solvolysis of the conjugate acid of phosphoramidic acid is bimolecular and that of the aromatic amide is unimolecular.

Obviously neither criterion affords an unambiguous answer; nor do the results shed much light on the important current question of when the Hammett–Zucker hypothesis may be safely applied.

The reaction by which oxygen exchange of methyl phosphate with water occurs prior to hydrolysis *via* an acid-catalyzed process may be assumed to be bimolecular by analogy with the hydrolysis sequence. In this case one of the hydroxyl groups is protonated to form a conjugate acid isomeric with that which undergoes hydrolysis, with the result that water is displaced rather than alcohol. The possibility exists that bond making and bond breaking are not concerted, but that a true intermediate is formed by addition of water to the phosphoryl group; the intermediate may decompose by elimination of water, to reform starting material which may now be labeled, or alcohol, to form products. At present no experimental test has been devised to distinguish between these possibilities; additional information bearing on this point is discussed in the section on the acid-catalyzed hydrolysis of ethylene phosphate (see section IIIB).

D. SUMMARY OF REACTIONS OF MONOESTERS

Four hydrolytic mechanisms, therefore, have been firmly identified by Bunton and co-workers (43) for monomethyl phosphate; their characteristics are summarized in Table XIII.

The rate constant for hydrolysis at 100° at any acidity within the range pH 7.5 to 7 *M* perchloric acid is given by Eq. 18.

$$10^6 k_{obsd}\ \text{sec.} = 8.23(C_M/C_P) + 0.50(C_N/C_P)e^{0.423\mu} + 3.08(C_{H^+}/C_P) \quad \text{(Eq. 18)}$$

The fit of Eq. 18 is very good, except in the region pH 1 to 0, where the uncertainties associated with the dissociation constants become relatively more important.

TABLE XIII

SUMMARY OF REACTION MECHANISMS FOR HYDROLYSIS OF MONOMETHYL PHOSPHATE (43)

Form	$10^6 \times$ specific rate constant (100°)	Bond fission	Molecularity	Salt effect
Monoanion	8.23[a]	P–O	?	Nil
Neutral species	0.50[a,c]	C–O	2	Large +
Conjugate acid	2.00[b]	C–O	2	Nil
Conjugate acid	1.08[b]	P–O	2	Nil

[a] Sec.$^{-1}$. [b] L. mole^{-1} sec.$^{-1}$. [c] At $\mu = 0$.

The most striking difference between the data obtained for monomethyl phosphate and for dimethyl phosphate is the very high reactivity of the monoanion of the monoester. This reactivity has been interpreted in terms of the special mechanism available to the monoester, which is not available to the monoanion of the diester.

The other observed differences are relatively small and do not lend themselves to ready interpretation. Bunton, *et al.* (46), have pointed out that although the over-all rate of hydrolysis of dimethyl phosphate in acid solution is greater than that of monomethyl phosphate, that of the true acid-catalyzed reaction (reaction of the conjugate acid) at zero ionic strength is smaller. This arises because the reaction of the diester exhibits a large positive salt effect, while that of the monoester does not. A mechanistic interpretation of this difference has not yet been advanced.

These reactions may be taken as characteristic in type of the reactions which any monoderivative of phosphoric acid will undergo. (No implication is intended that these are the only ones possible; nevertheless, they are so far the only well-characterized ones reported.) Variations in structure will produce variations in the relative importance of the contribution of each of these to the total rate. Thus, increasing the bulk of the substituent group should tend to suppress the bimolecular substitution reactions at carbon, while the presence of a tertiary alkyl group would be expected to lead to unimolecular reactions of the conjugate acid and of the neutral species, in which case the minimum in the pH–rate profile near pH 0 may disappear. Bunton, *et al.*, ascribe the shape of the pH–rate profile of glucose-1 phosphate to this reason (44). Benzyl phosphate shows high reactivity in the region of moderate acidity which similarly obscures the expected rate minimum (167), and since the derivatives of the benzyl group are known to exhibit high reactivity in both bi- and unimolecular substitution reactions at

TABLE XIV

RATE CONSTANTS FOR THE HYDROLYSIS OF THE DIFFERENTLY PROTONATED SPECIES OF PYROPHOSPHORIC ACID (190)

Temp., °C.	60.03
Ionic strength	0.44
Rate constants, hr.$^{-1}$	
$H_5P_2O_7^+$	115
$H_4P_2O_7$	0.037
$H_3P_2O_7^-$	0.024
$H_2P_2O_7^{-2}$	0.0112
$HP_2O_7^{-3}$	0.00036

carbon, either or both types of process may be operative in this case.

It should be emphasized that the foregoing analysis is valid only for solutions from which strong nucleophiles such as halide ions have been excluded. For example, ethyl phosphate is known to be hydrolyzed by halogen acids much more rapidly than by equivalent concentrations of perchloric or sulfuric acid. This is due to incursion of bimolecular reactions at carbon involving halide ions and the neutral or conjugate acid species.

By kinetic studies coupled with evaluation of the dissociation constants for the various species formed by successive loss of protons, Osterheld (190) and Campbell and Kilpatrick (50) have shown that the hydrolysis of pyrophosphoric acid can be fitted by an equation of the form

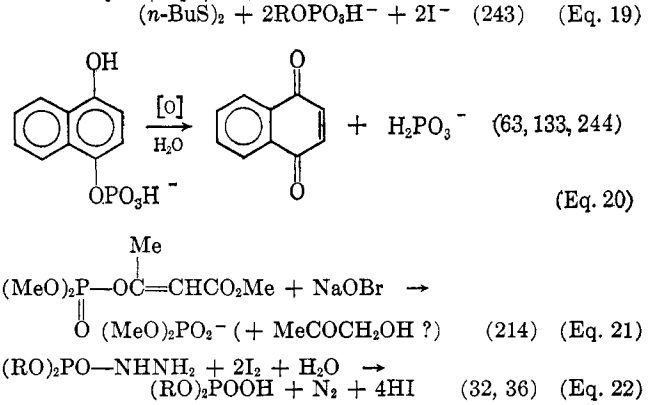
$$k_{obsd} = k_5x_5 + k_4x_4 + k_3x_3 + k_2x_2 + k_1x_1 + k_0x_0$$

where k_5 is the specific rate constant for the hydrolysis of the species $H_5P_2O_7^+$, k_4 that for the species $H_4P_2O_7$, k_3 that for $H_3P_2O_7^-$, etc. The rate constants found by Osterheld at 60° and ionic strength = 0.44 are listed in Table XIV. As is the case with other derivatives, the conjugate acid and the neutral species both undergo reaction, the monoanionic species is of rather high reactivity, and the progressively more ionized species grow much less reactive.

The only isolable monohalide, fluorophosphoric acid, is relatively stable near pH 7, but is hydrolyzed in either acidic or basic solution to fluoride ion and phosphate ion (89). The details of the processes involved appear not to have been studied. It should be noted that the attack of hydroxide ion on the doubly charged dianion of a monoester of phosphoric acid results in carbon–oxygen cleavage, rather than in nucleophilic substitution at phosphorus (123, 124).

V. OXIDATIVE REACTIONS RESULTING IN PHOSPHORUS–OXYGEN CLEAVAGE

In esters or other derivatives of phosphoric acid in which an easily oxidizable group is attached to phosphorus, very rapid hydrolysis frequently accompanies oxidation. In addition, if the oxidation is performed in the presence of an alcohol, the alcohol may be phosphorylated. The following equations are examples.

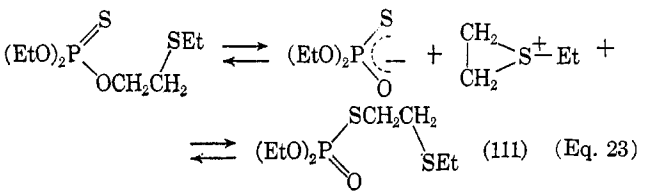


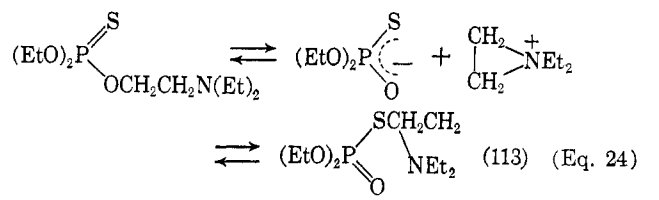
The mechanisms of these processes have not been intensively studied. It is tempting to formulate the first two of these sequences (Eq. 19 and 20) as producing monometaphosphate anion, and evidence consistent with this view has been presented (61).

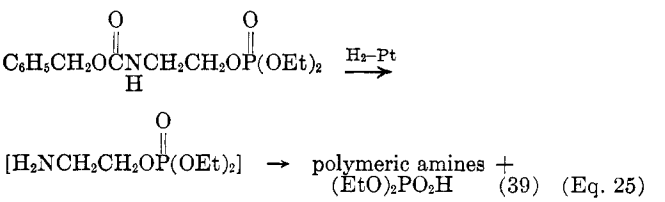
VI. FURTHER PROCESSES RESULTING IN CARBON–OXYGEN CLEAVAGE

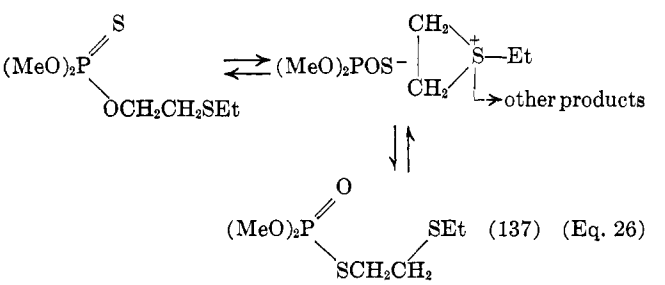
In addition to the SN1 and SN2 reactions at carbon previously described, certain other mechanisms lead to fission of the C–O bond of phosphate esters.

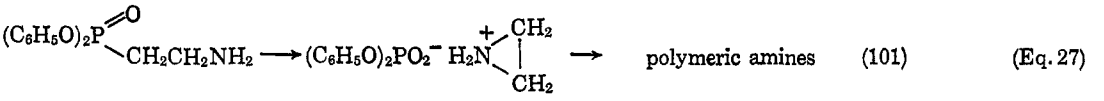
(1) A neighboring group on the β-carbon atom of the ester may displace a phosphate or substituted phosphate anion.



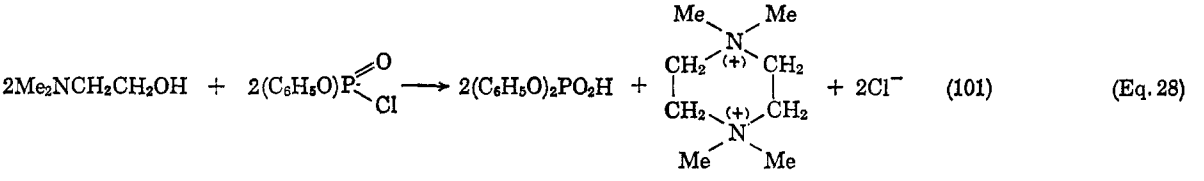




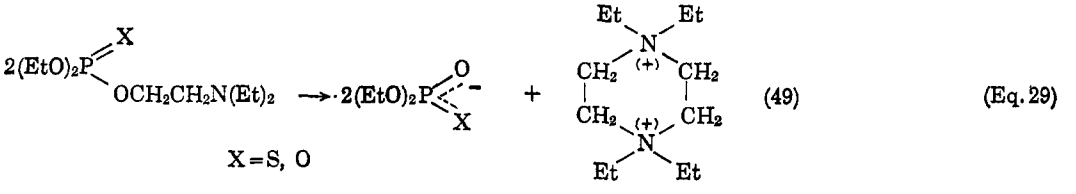




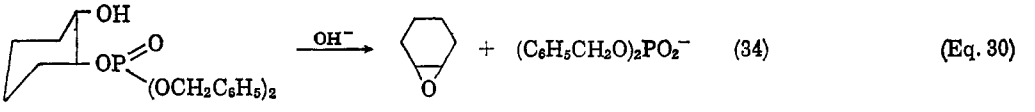
(Eq. 27)



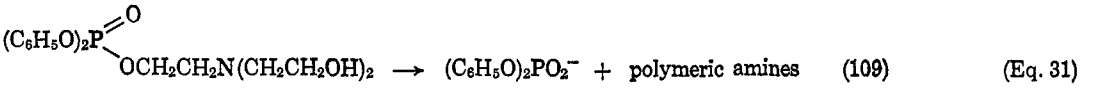
(Eq. 28)



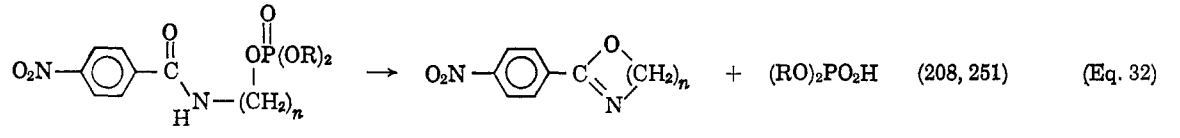
(Eq. 29)



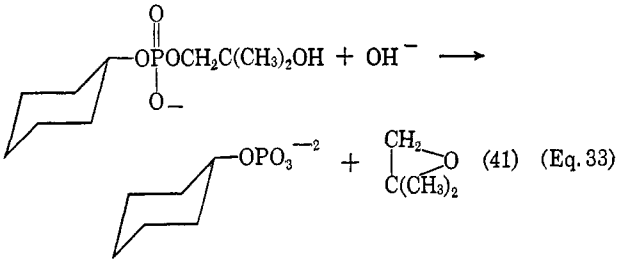
(Eq. 30)



(Eq. 31)

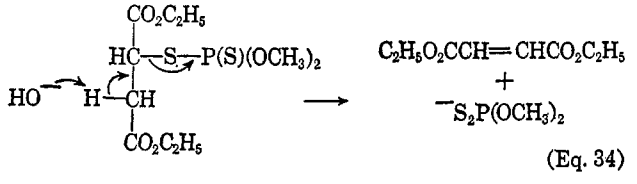


(Eq. 32)



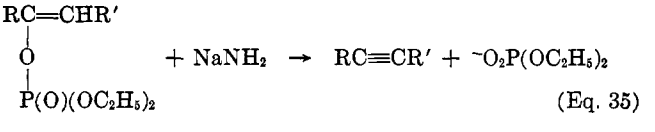
(Eq. 33)

(2) A base-catalyzed elimination may occur, leading to formation of a compound with a multiple bond and a phosphate anion. This kind of reaction has been reviewed by Lapidot, Samuel, and Silver (169). To the examples therein cited, there may be added the basic "hydrolysis" of O,O-dimethyl S-[(1,2-diethoxycarbonyl)ethyl] phosphorodithionate ("Malathion"). The rate of acid production from Malathion in acetone is first order in hydroxide ion as well as in the ester. The principal reaction product is O,O-dimethylphosphorodithioate ion (155). Since all other phosphate esters of thioalcohols which have been studied hydrolyze with P–S bond fission, it seems best to formulate the reaction of Malathion with base as an elimination activated by the carbethoxy group (Eq. 34).



(Eq. 34)

In similar fashion, vinyl phosphates are acted upon by sodium amide in liquid ammonia to afford acetylenes (Eq. 35) (76).



(Eq. 35)

VII. Catalytic Processes Related to Mechanisms of Enzymatic Hydrolysis of Phosphate Esters

One of the chief aims of the investigations of mechanisms of nucleophilic substitution in derivatives of phosphoric acid is the understanding of the mechanisms of enzymatic transformation of these compounds. So far this review has focused principally upon the variation of mechanisms of hydrolysis with variation in structure of the substrate. Here we wish to consider some types of catalytic processes which may have pertinence to the enzymatic catalyses.

A. METAL ION CATALYSIS

More than 50 references to metal ion catalysis of the hydrolysis of phosphate esters have appeared in the literature. It is beyond the scope of this review to discuss these critically. In general, the phosphate may be considered to be coordinated to the metal ion, thus increasing its susceptibility to nucleophilic attack. The metal ion is in effect a general acid

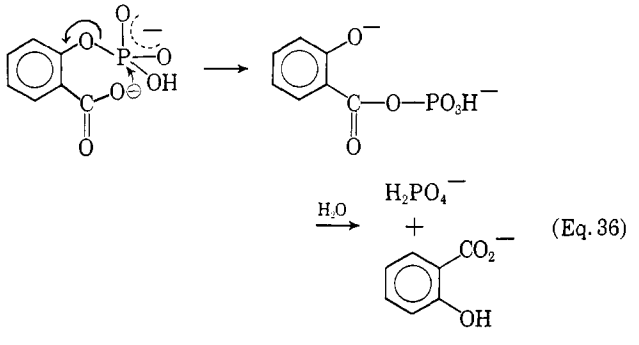
catalyst. Some representative examples of this sort of catalysis may be found (19, 20, 47, 67, 85, 100, 107, 156, 168, 206, 234).

Since many enzymatic reactions of phosphate derivatives require a metal ion, usually magnesium, it is possible that a portion of the enzymatic catalysis derives from complexation of the substrate with the metal ion. Alternatively, the metal ion might serve to bind the substrate to the enzyme. Some interesting speculations about these possibilities are to be found (24b).

B. INTRAMOLECULAR CATALYSIS

The most carefully studied example of intramolecular catalysis of hydrolysis, the utilization of a hydroxyl function to displace internally with formation of a five-membered ring, has been discussed in connection with ribonucleic acid hydrolysis, section IIIB. Sulfur and amino substituents located on the carbon generally displace a phosphate anion, forming an aziridine (see section VI), but one example of displacement by nitrogen at phosphorus with formation of a five-membered ring phosphoramidate ester has been reported (101).

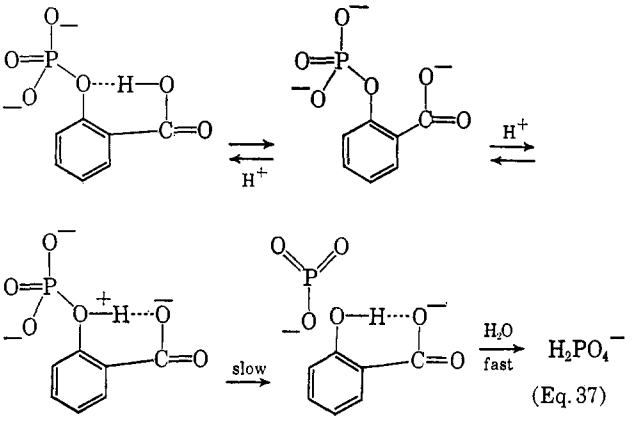
Chanley and his associates showed in a series of very interesting observations that the dianion of an *o*-carboxyaryl phosphate is hydrolyzed very much faster than is the monoanion of phenyl phosphate, but that *m*- and *p*-carboxyaryl phosphates do not exhibit the same enhanced reactivity (10, 52, 55, 56). The large rate effect must, then, be due to a direct interaction between the carboxyl group and the phosphate group, rather than to resonance or inductive effects. It was suggested that the carboxylate anion of salicyl phosphate, for example, attacks internally, Eq. 36, producing salicyloyl phosphate, which then hydrolyzes rapidly.



(Eq. 36)

Bender and Lawlor (25) have offered evidence that the catalysis is not nucleophilic in nature, but instead is general acid. Salicyloyl phosphate was synthesized and found to give a hydroxamic acid on treatment with hydroxylamine. Hydrolysis of salicyl phosphate in the presence of hydroxylamine gave no hydroxamic acid. Furthermore, when salicyl phosphate was hydrolyzed in H_2O^{18}, no excess O^{18} appeared in the carboxyl group of the salicylic acid formed, although the hydrolysis of acetyl phosphate under similar conditions yielded acetic acid in which approximately 10% of the oxygen was derived from the medium. These data seem to rule out nucleophilic attack by the carboxylate ion.

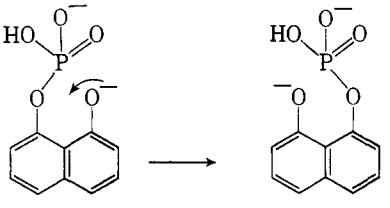
An alternate, and preferable, explanation of the effect of the *o*-carboxyl group as an internal, general acid catalyst is formulated in Eq. 37. Bender offers argu-



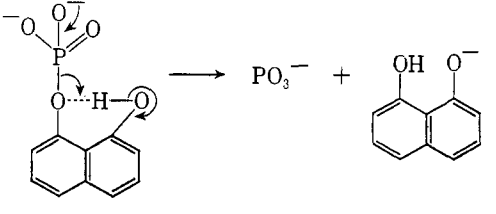
(Eq. 37)

ments in support of this mechanism based upon the solvent isotope effect. Very little change of rate was observed on going from water to D_2O.

Bender and Lawlor also found that the dianion of 8-hydroxy-1-naphthyl dihydrogen phosphate hydrolyzes about ten times as fast as the dianion of the 8-methoxy ester. Since, if the phenoxide ion were to attack phosphorus, the result would merely be formation of starting material, Eq. 38, a nucleophilic attack mechanism could not account for the observed rate acceleration. Possibly the observed rate acceleration is due to general acid catalysis by the phenolic proton, Eq. 39.

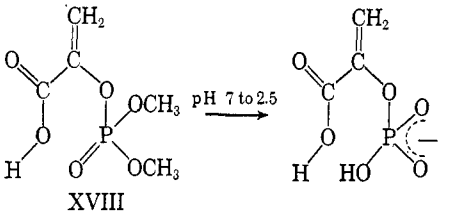


(Eq. 38)



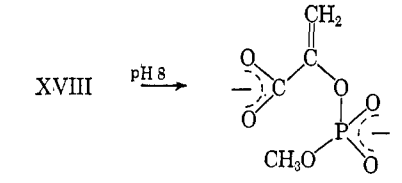
(Eq. 39)

A very similar case of neighboring group acceleration of phosphate ester hydrolysis has recently been reported by Clark and Kirby (62). The ester XVIII was found to be hydrolyzed rapidly to free phosphoenol pyruvate under mildly acidic conditions, Eq. 40, while in bicarbonate buffer at pH 8 the P-monomethyl ester is formed quantitatively, Eq. 41.



(Eq. 40)

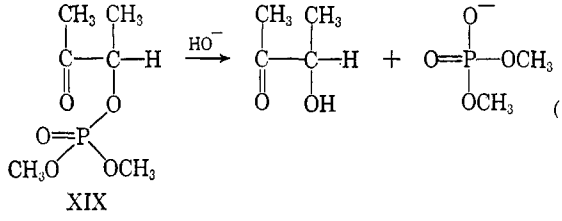
XVIII



(Eq. 41)

Clark and Kirby suggest that the carboxyl group is responsible for nucleophilic attack at phosphorus to form a five-membered ring cyclic anhydride, which by analogy with the cyclic diesters would be expected to be readily attacked by nucleophiles at phosphorus. By analogy with the behavior of salicyl phosphate, it would seem equally possible that internal protonation of the leaving oxygen atom by the carboxyl group might explain the observed catalysis.

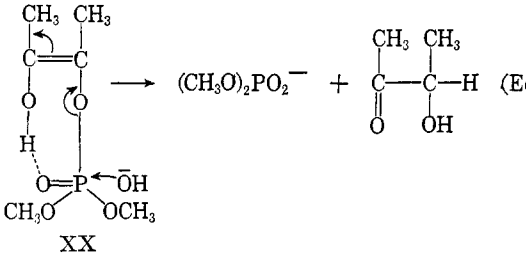
Ramirez and co-workers have reported that the rate of hydrolysis of dimethyl phosphoacetoin (XIX) is at least 10^6 times that of trimethyl phosphate in basic solution (195). The products of the hydrolysis were acetoin and dimethyl phosphate, Eq. 42. Two pos-



(Eq. 42)

XIX

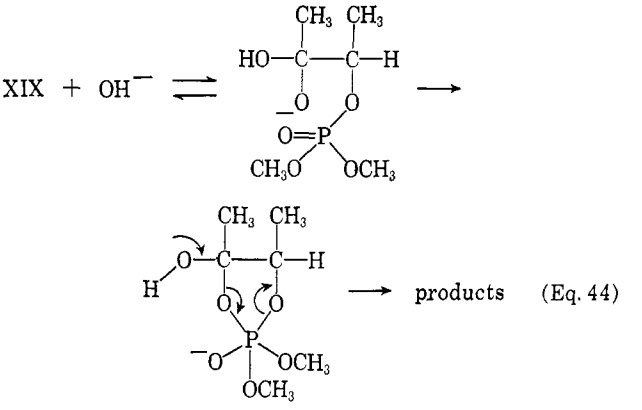
sible pathways were considered for this reaction: (a) The reactive species might be the enediol phosphate resulting from enolization of the carbonyl function (XX), in which case the rate enhancement would be due to the unsaturated nature of the leaving group and to the ability of the hydroxyl group to assist by hydrogen bonding to the phosphoryl oxygen, Eq. 43.



(Eq. 43)

XX

(b) The alternate suggestion that the hydroxide ion adduct of the carbonyl group attacks phosphorus to form an oxyphosphorane, Eq. 44, is not in accord with



(Eq. 44)

the behavior of β-hydroxyethyl phosphate derivatives, to which the analogy would seem close. In the latter case an alkoxide ion is expelled to give a cyclic phosphate ester.

Pathway a (Eq. 43) is consistent with the finding by Cox and Farmer that the dimethyl phosphate ion produced in the reaction contains one oxygen atom derived from the solvent (71).

C. POLYFUNCTIONAL CATALYSIS

Since at least a major portion of the rate accelerations occasioned by enzymes is believed to be due to the apposite disposition of several functional groups, each of which is able to contribute simultaneously to the catalysis, it has been of much interest to find or to devise systems in which such cooperative behavior could be demonstrated. Wagner-Jauregg and Hackley discovered that 3-hydroxypyridine is a much more efficient catalyst for the hydrolysis of diisopropyl phosphorofluoridate than is an equimolar mixture of pyridine and phenol (233). Since both pyridine and 2-methylpyridine are about equally good as catalysts (233), very likely the hydroxypyridine functions as a general base–general acid catalyst. 2-Hydroxypyridine had little effect in the system. Epstein, Rosenblatt, and Demek showed that the rapid attack of catecholate monoanion on isopropyl methylphosphonofluoridate can be well accounted for by general acid catalysis by the hydroxyl group or nucleophilic attack by the oxide anion (108). Cramer has shown that cyclodextrins which are of the proper size that the ester "fits" into the cavity catalyze the decomposition in aqueous solution of symmetrical diaryl pyrophosphates. A hydroxyl group on the dextrin becomes phosphorylated in the process (77).

Dudek and Westheimer demonstrated that calcium ion, acting as a general acid catalyst, and 2,6-lutidine, acting as a general base catalyst, independently increase the rate of 2-propanolysis of tetrabenzyl pyrophosphate. Furthermore, when both are present in solution at the same time, each enhances the effect of the other, thus effecting concerted general acid–general base catalysis (100).

D. ENZYMATIC CATALYSIS

In spite of the large amount of work which has been invested in the study of phosphate ester hydrolysis, the intimate details of the processes by which such enzymes as phosphoglucomutase and phosphorylases, and such other catalytic proteins as myosin function, remain unelucidated. The state of our knowledge of ribonuclease has recently been reviewed by Westheimer (242) and by Scheraga (207).

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