# Hydrolytic Mechanisms of Phosphoramidates of Aromatic Amino Acids

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**Abstract:** The pH–rate profile for the hydrolysis of *o*-carboxyphenyl phosphoramidate reveals intramolecular protonated carboxyl group interaction that serves to accelerate the rate of hydrolysis of the monoanionic and neutral species. Hydronium ion catalyzed hydrolysis of *o*-carboxyphenyl phosphoramidate also is more rapid than the reference compound, *p*-carboxyphenyl phosphoramidate. Solvolytic experiments in dioxane–water reveal the importance of the zwitterionic form in the hydrolysis of the various phosphoramidate species, in particular the neutral form. Product composition studies in alcohol–water mixtures (methanol, ethanol, 2-propanol, and *t*-butyl alcohol) reveal: (1) that the product composition in methanol–water (alkyl phosphate:orthophosphoric acid) is independent of the state of ionization of the phosphoramidate; (2) that the product composition is unaffected by the presence of the neighboring carboxyl group; (3) that the phosphorylating species exhibits a marked selectivity for alcohol rather than water; and (4) that steric factors in both substrate and alcohol affect the product composition obtained with the more branched alcohols. These results are discussed in terms of the metaphosphate hypothesis and a bimolecular alternative.

The present investigation was initiated in order to study the possibility of catalyzing intramolecularly the transfer of a phosphoryl moiety to hydroxylic acceptors. Phosphoramidates generally differ from phosphate monoesters in their greater lability at ambient temperatures and thus have been recognized for their potential as biochemical phosphorylating agents[1–6] as well as reagents of synthetic utility.[7–10] To choose one example, an enzyme-catalyzed phosphoryl transfer involving phosphoramidate and hexose has been demonstrated in microsomal preparation from rat liver and kidney.[3] Synthetically the preparation of symmetrical and unsymmetrical esters of pyrophosphoric acid has been achieved with phosphoramidate derivatives.[7–10] It is of interest that attempts to phosphorylate alcohols with monoesters of phosphoramidates (*e.g.*, methyl hydrogen N-cyclohexylphosphoramidate) are generally unsuccessful unless pyridine is present, whereas pyrophosphate formation occurs almost quantitatively in the absence of pyridine.[10] Several quantitative studies have dealt with the hydrolysis of phosphoramidate itself and various N-acyl and N-aryl derivatives.[11,12] Chanley,[11] Jencks,[13] and their co-workers also have shown that the hydrolysis of phosphoramidate monoanion is subject to nucleophilic catalysis by various amines, especially pyridines, and furthermore that the hydrolysis of phosphoramidate is accelerated by electrophilic catalysts including formaldehyde and nitrous and hypochlorous acid. The above studies, however, differed in their conclusions and described the hydrolysis of phosphoramidate monoanion either as strictly bimolecular[11] or in terms of a borderline mechanism not involving a "free metaphosphate" intermediate, the initial product of a unimolecular pathway. Both interpretations were drawn from similar experimental evidence which indicated that the hydrolysis proceeded *via* species that showed a degree of selectivity for nucleophilic reagents. This behavior is not anticipated for a reactive species such as metaphosphate. Moreover, Chanley and Feageson who extended their product composition studies to low pH, thus including the neutral and protonated species of phosphoramidate, encountered a change in product composition. This result was interpreted on the basis of a change to a metaphosphate mechanism. It therefore became important to investigate what effect intramolecular catalysis, the state of ionization of the phosphoramidate, and the nature of the nucleophile might have on product composition, and ultimately to attempt a formulation of a general mechanism for phosphoramidate hydrolysis.

## Experimental Section

All melting points are uncorrected. Microanalyses were performed by Midwest Microlabs. Infrared spectra were obtained with a Perkin-Elmer 237B spectrophotometer. Kinetic solutions were prepared from freshly boiled distilled water tested for inorganic ions with EDTA. In alcohol–water experiments all alcohols were either reagent or spectrophotometric grade (Fisher). Reagent grade salts and acids (Fisher, Baker) were used without further purification. Dioxane was purified by the method of Fieser,[14] stored over sodium, and distilled prior to use.

**Materials. Dicyclohexylammonium *o*-Carboxyphenyl Phosphoramidate.** Synthesis of the phosphoramidic dichloride precursor, (*o*-$ClCOC_6H_4NHPOCl_2$), was by the method of Uhlfelder,[15] mp 62° (lit.[15a] mp 62°); infrared absorption (Nujol) at

(1) A. Fujimoto and R. A. Smith, *Biochim. Biophys. Acta*, **56**, 501 (1962).
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(3) M. E. Holzer, K. D. Johnson, and R. A. Smith, *Biochim. Biophys. Acta*, **122**, 232 (1966).
(4) H. L. Auleb, M. J. Dowler, and H. I. Nakada, *Biochem. Biophys. Res. Commun.*, **23**, 280 (1966).
(5) A. Lapidot and D. Samuel, *Biochim. Biophys. Acta*, **111**, 537 (1965).
(6) W. Kundig, S. Ghosh, and S. Roseman, *Proc. Natl. Acad. Sci. U. S.*, **52**, 1067 (1964).
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(9) J. G. Moffatt and H. G. Khorana, *ibid.*, 80, 3756 (1958).
(10) N. K. Hamer, *J. Chem. Soc.*, 46 (1965).
(11) J. D. Chanley and E. Feageson, *J. Am. Chem. Soc.*, **85**, 1181 (1963); **80**, 2686 (1958).
(12) M. Halmann, A. Lapidot, and D. Samuel, *J. Chem. Soc.*, 3158 (1961), and references therein.
(13) W. P. Jencks and M. Gilchrist, *J. Am. Chem. Soc.*, **86**, 1410 (1964); **87**, 3199 (1965).
(14) L. F. Fieser, "Experiments in Organic Chemistry," D. C. Heath and Co., Boston, Mass., 1957, p 284.
(15) (a) E. Uhlfelder, *Chem. Ber.*, **36**, 1824 (1903). (b) The structures of the precursors merit further investigation, especially in view of the elucidation of the structure of Couper's compound [A. G. Pinkus,

3.14, 5.68, 5.85, 622, 6.33, 7.73 (doublet), 8.34, 10.5, 11.4, 13.1, and 13.5 $\mu$. The phosphoramide dichloride (1.9 g, $7.0 \times 10^{-3}$ mole) was added over a 15-min period to a vigorously stirred ice-cold slurry of $Ag_2CO_3$ (3.4 g, $1.2 \times 10^{-2}$ mole) in 30 ml of 50% v/v acetone–water. The mixture was filtered and the precipitate washed with 10 ml of water. To the combined filtrate and washings (maintained ice cold), cyclohexylamine was added dropwise until the initially formed precipitate disappeared (*ca.* pH 8).[16] The resultant clear solution was diluted with acetone until turbid, then chilled for 1 hr in ice. Filtration of the white, crystalline salt gave 0.65 g ($1.6 \times 10^{-3}$ mole) of dicyclohexylammonium *o*-carboxyphenyl phosphoramidate, mp 135–137°. The compound was stored at 0° in the dark. No attempts were made to maximize the yield; infrared absorption (Nujol) at 3.00 (broad), 3.20, 3.80 (broad), 6.20, 6.32 (broad), 7.83 (broad), 8.70, 9.30 (broad), 10.6 11.1, and 13.3 $\mu$. Recrystallization was achieved from acetone–water solutions.

*Anal.* Calcd for $C_{19}H_{34}N_3O_5P \cdot 0.5H_2O$: C, 53.76; H, 8.31; N, 9.90; P, 7.30. Found: C, 53.94; H, 8.54; N, 10.02; P, 6.30; P, 7.12 (method of Martin and Doty, see below).

**Tricyclohexylammonium *p*-Carboxyphenyl Phosphoramidate.** Synthesis of the phosphoramidic dichloride precursor (*p*-ClCO-$C_6H_4NHPOCl_2$) was by the method of Michaelis,[17a] mp 156–157°,[17b] softening and resolidification noted at 134–135° (lit.[17a] mp 168°); infrared absorption (Nujol) at 3.19, 5.66, 5.76, 6.24, 6.62, 7.70, 8.11 (multiplet), 8.60, 10.5, 11.3, 11.8, 12.7, and 13.9 $\mu$. The phosphoramidic dichloride[18] (2.0 g, $7.3 \times 10^{-3}$ mole) was added over a 15-min period to a vigorously stirred, ice-cold slurry of $Ag_2CO_3$ (3.5 g, $1.2 \times 10^{-2}$ mole) in 30 ml of 83% v/v acetone–water. The mixture was filtered and the precipitate washed with 10 ml of water. The combined filtrate and washings (maintained ice cold) were then treated with Norit and filtered, and the clear solution was brought to *ca.* pH 10 by rapid dropwise addition of cyclohexylamine. The resultant clear solution was diluted with acetone until turbid, then chilled in ice overnight. Collection of the white crystalline salt gave 0.57 g ($1.1 \times 10^{-3}$ mole) of tricyclohexylammonium *p*-carboxyphenyl phosphoramidate, mp 153–155°. The compound was stored at 0° in the dark; infrared absorption (Nujol) at 2.98 (broad), 3.14, 3.80 (broad), 6.12, 6.20, 6.35 (broad), 7.63, 8.76, 9.45 (broad), 10.3, 11.1, 12.7, and 13.9 $\mu$.

*Anal.* Calcd for $C_{25}H_{48}N_4O_5P \cdot 2H_2O$: C, 54.42; H, 9.13; N, 10.15; P, 5.61. Found: C, 54.68; H, 9.28; N, 10.44; P, 4.64; 5.73 (method of Martin and Doty).

**Apparatus.** Instrumentation used in this study has previously been described.[20] Kinetic runs of greater than 12-hr duration and product composition studies were carried out in Kimax (No. 45066) screw-cap tubes maintained at reaction temperature (±0.1°) by immersion in a circulating water bath. Shorter runs were conducted in thermostated, 2-cm stoppered cuvettes.

**Kinetics.** The hydrolysis of *o*-carboxyphenyl phosphoramidate was monitored at 245 m$\mu$ following the decrease in absorption due to fission of the P–N bond or by analysis for orthophosphate by the method of Martin and Doty[21] as modified by Jencks.[13] Reactions (spectrophotometric) were initiated by the addition of *o*-carboxyphenyl phosphoramidate (*ca.* 1 mg) by means of a microspatula directly into the preequilibrated buffer solution (pH >0.5, $\mu$ = 0.2, KCl). The cuvette was inverted and allowed to reequilibrate for 5 min before absorbance readings were taken. Reactions (orthophosphate analysis) were initiated by the addition of *o*-carboxyphenyl phosphoramidate (3–4 mg) to 10 ml of the preequilibrated buffer solutions, and 1-ml aliquots were withdrawn at the desired time intervals. Buffers employed were hydrochloric acid (pH <2.5),[22] formate (0.2 *M*, pH 3.0–3.8), acetate (0.2 *M*, pH 4.0–5.4), succinate (0.067 *M*, pH 5.7–6.1), imidazole (0.01 *M*, pH 6.7–7.0), and Tris (0.2 *M*, pH 7.6–8.1). No acceleratory buffer effects were noted although higher concentrations of imidazole (up to 0.2 *M*) had a slight retarding effect on the observed rate. The pH of the kinetic runs was measured at 35° (glass electrode) upon initiation and after completion of the runs; those exhibiting pH drift greater than ±0.02 unit were discarded. The rate of hydrolysis of the *p*-carboxyphenyl phosphoramidate was followed by analysis for the liberated orthophosphate.

The observed first-order rate constants (spectrophotometric or phosphate analysis)[23] for hydrolysis of *o*-carboxyphenyl phosphoramidate were calculated from slopes of plots of log $[(OD_\infty - OD_0)/(OD_\infty - OD_t)]$ or log $[OD_\infty - OD_t]$ against time. Plots were generally linear to at least three half-lives. Rate constants determined by the two methods agreed within ±3%. Duplicate runs by a given method agreed within ±3%. No buffer effects (formate, 0.06–0.2 *M*, pH 3.08) were noted, Kinetic runs at pH <0.5 were carried out at $\mu$ = 1.0, KCl; those in 1.0–3.0 *N* HCl at $\mu$ = 3.0, KCl.

The observed first-order rate contants for hydrolysis of *p*-carboxyphenyl phosphoramidate were measured by phosphate analysis and calculated as discussed above. No buffer effects (formate, 0.1–0.2 *M*, pH 3.08) were noted. Kinetic runs at pH <0.5 were carried out at $\mu$ = 1.0, KCl.

Kinetic runs for solvolysis of *o*-carboxyphenyl phosphoramidate conducted in mixed solvents (methanol–water, dioxane–water) were followed spectrophotometrically. The compositions of the solvent mixtures were obtained by mixing measured volumes of the nonaqueous and aqueous components. The desired pH was maintained by the above buffers; all solutions were at $\mu$ = 0.2, KCl.

The ionization constants of *o*- and *p*-carboxyphenyl phosphoramidate were determined by the method of half-neutralization in both aqueous and mixed solvents. The accuracy of these measurements is impaired by the lability of these compounds; reported p$K_a$'s have an estimated error of ±0.2 p$K_a$ unit (Table I).

**Table I.** Dissociation Constants of *o*- and *p*-Carboxyphenyl Phosphoramidates ($\mu$ = 0.2, 35°)

| Solvent | *o*-Carboxy p$K_1$[a] | *o*-Carboxy p$K_2$ | *o*-Carboxy p$K_3$ | *p*-Carboxy p$K_1$[a] | *p*-Carboxy p$K_2$ | *p*-Carboxy p$K_3$ |
|---|---|---|---|---|---|---|
| $H_2O$ | 2.17 | 4.43 | 7.25 | 2.63 (2.37)[b] | 5.14 (5.04)[b] | 6.99 |
| 53% v/v $CH_3OH$–$H_2O$[c] | 3.06 | ... | 8.14 | 2.86 | ... | 7.72 |

[a] No hydrogen ion correction applied. [b] Duplicate values, values not in parentheses used in actual calculation of rate constants. [c] pH measurements are uncorrected for solvent effects on the glass electrode.

**Products.** Spectrophotometric scanning (240–340 m$\mu$) at $t_\infty$ of *o*- and *p*-carboxyphenyl phosphoramidate kinetic solutions of known concentration disclosed an ultraviolet spectra identical with quantitative liberation of anthranilic and *p*-aminobenzoic acids, repectively. Exclusive P–N bond cleavage is the general mode of bond fission for phosphoramidates.[24] The absence of detectable reaction intermediates in the hydrolysis of *o*-carboxyphenyl phosphoramidate at significant concentrations is implied by the lack of any observable lag phase in the kinetics and the identical rate constants as measured by substrate disappearance and product appearance. Paper chromatography of the hydrolysis products of *o*-carboxyphenyl phosphoramidate by the method of Karl Kroupa[25] utilizing pyrophosphate and orthophosphate as reference standards revealed only orthophosphate, no pyrophosphate being detected.

In alcohol–water solvents the mole fraction of orthophosphate was calculated from the ratio of orthophosphate concentrations

P. G. Waldrep, and S. Y. Ma, *J. Heterocyclic Chem.*, **2**, 357 (1965)]. The analytic, infrared, and kinetic data, however, indicate that the desired phosphoramidates are formed upon partial hydrolysis.

(16) Isolation of the initial precipitate, presumably the monocyclohexylammonium salt, yielded a compound which rapidly decomposed upon drying over $CaCl_2$ at room temperature.

(17) (a) A. Michaelis, *Ann.*, **326**, 129 (1903). (b) The compound appears to fuse upon heating and an accurate melting point is difficult to determine.

(18) The synthesis of the above phosphoramidic dichlorides is accomplished under differing reaction conditions (solvent, temperature) than utilized in the synthesis of phosphoazoaryls from arylamines and $PCl_5$.[19] Furthermore, $POCl_3$ is generated in the above reactions. See, however, 15b.

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(20) S. J. Benkovic and P. A. Benkovic, *J. Am. Chem. Soc.*, **88**, 5504 (1966).

(21) J. B. Martin and D. M. Doty, *Anal. Chem.*, **21**, 965 (1949).

(22) No correction was applied for the small error incurred in reading pH values between pH 0 and 1.

(23) Development procedure for analysis of orthophosphate resulted in less than 5% hydrolysis of the phosphoramidate substrate.

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(25 E. Karl-Kroupa, *Anal. Chem.*, **28**, 1091 (1956).

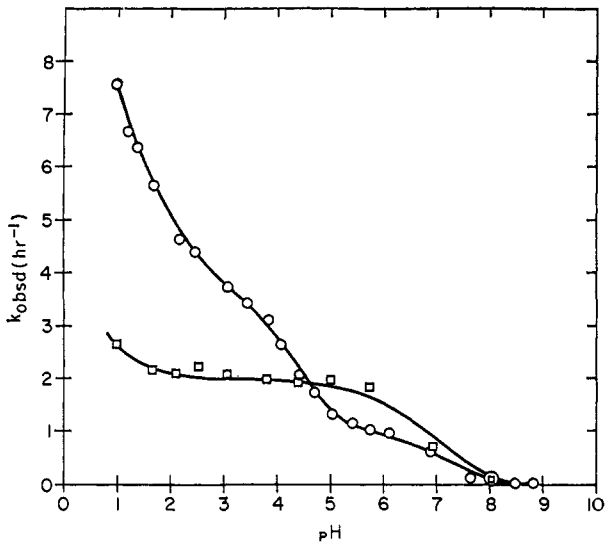



Figure 1. The pH–rate profiles for the hydrolysis of *o*-carboxyphenyl phosphoramidate, O, and *p*-carboxyphenyl phosphoramidate, □, at 35°, $\mu$ = 0.2. Solid line is theoretical curve calculated from values listed in Table II.

measured at $t_\infty$ in these solvents to the known value of orthophosphate concentration at $t_\infty$ in aqueous solution. In practice this was accomplished by measuring the OD at $t_\infty$ of aliquots of the alcohol–water mixture containing known concentrations of the phosphoramidates by the development procedure of Martin and Doty. Additional aliquots were withdrawn at *ca.* 1–2-hr intervals until successive readings agreed within experimental error. The method of Martin and Doty is specific for orthophosphate; phosphate monoesters are not hydrolyzed by the development conditions. The $OD_\infty$ for hydrolysis of an identical concentration of phosphoramidate in aqueous solution was determined from standard curves of optical density *vs.* concentration. The ratio of the above $OD_\infty$ (alcohol–water : water) is, therefore, the mole fraction of orthophosphate as the solvolysis product. At the temperature and pH's employed the solvolyses of the phosphoramidates are much faster than either of the possible competing reactions, solvolysis of products or esterification of phosphoric acid.[26] The mole fraction alkyl phosphate formed in each solvent mixture was calculated from 1.0 − mole fraction of orthophosphate.

Paper chromatography by the method of Chanley and Feageson[11] of the solvolyses of *o*-carboxyphenyl phosphoramidate in methanol–water utilizing orthophosphate, pyrophosphate, and methyl phosphate as reference standards revealed methyl phosphate and orthophosphate as the solvolytic products. The pH of the chromatographed solutions was 2.28, 3.76, and 6.25.

The presence of isopropyl phosphate in the solvolysis of *p*-carboxyphenyl phosphoramidate in 30% v/v 2-propanol–water was demonstrated by (1) the development procedure described above and (2) acidification of aliquots of the solvolysis solution to pH 0.3 and incubation at 75° for 8 days at which time the development procedure indicated *ca.* 50% of an acid-hydrolyzable phosphate had disappeared. Paper chromatography revealed no pyrophosphate in the original solvolytic media at $t_\infty$.

## Results

**Kinetics.** The pH–rate profiles for the hydrolysis of *o*-carboxyphenyl phosphoramidate (**1**) and *p*-carboxyphenyl phosphoramidate (**2**) are exhibited in Figure 1. Pertinent kinetic data are summarized in Table II. A generalized kinetic equation applicable to both **1** and **2** may be developed by assuming all species to be subject to hydrolysis excluding the phosphoramidate trianion (experimentally shown as relatively stable). The following dissociation constants may be defined and identified with those measured experimentally.[27]

(26) By comparison to the data of ref 11.

(27) $K_1$, $K_2$, and $K_3$ because of the multiple protonation sites are macroscopic dissociation constants. Consequently the actual and/or hydrolytically important species may not be those bracketed. This is, of course, also true for the kinetic expression 2; later, the various kinetically indistinguishable cases will be examined.

$$K_1 = \frac{[a_H][HOOC\text{-}R\text{-}NHPO_3H^-]}{[HOOC\text{-}R\text{-}NHPO_3H_2]}$$

$$K_2 = \frac{[a_H][^-OOC\text{-}R\text{-}NHPO_3H^-]}{[HOOC\text{-}R\text{-}NHPO_3H^-]}$$

$$K_3 = \frac{[a_H][^-OOC\text{-}R\text{-}NHPO_3^{2-}]}{[^-OOC\text{-}R\text{-}NHPO_3H^-]} \quad (1)$$

The over-all rate of hydrolysis at any pH is given by

$$v = k_{H^+}[a_H][HOOC\text{-}R\text{-}NHPO_3H_2] + k_1[HOOC\text{-}R\text{-}NHPO_3H_2] + k_2[HOOC\text{-}R\text{-}NHPO_3H^-] + k_3[^-OOC\text{-}R\text{-}NHPO_3H^-] \quad (2)$$

where $k_{H^+}$ is the second-order rate constant associated with hydronium ion catalyzed hydrolysis of the neutral species; $k_1$, $k_2$, and $k_3$ are defined as first-order rate constants for hydrolysis of the neutral, mono- and dianionic species, respectively. Inclusion of a hydronium ion term is justified by the general observation of such catalysis in the hydrolysis of phosphoramidates,[24] except in N-acylphosphoramidates where the acyl group is strongly electron withdrawing. No assumptions have been made, at this point, as to the order of solvent participation in the hydrolysis of any species. Since

$$v = k_{obsd}[\text{phosphoramidate}]_T \quad (3)$$

where

$$[\text{phosphoramidate}]_T = \sum[\text{species}]$$

substitution of the relationships of eq 1 into 2 and 3 then gives

$$k_{obsd} = \frac{a_H^2(k_{H^+}a_H + k_1) + K_1(k_3K_2 + k_2a_H)}{K_1K_2[1 + K_3/a_H] + a_H[K_1 + a_H]} \quad (4)$$

The values of $k_{obsd}$ calculated from eq 4 utilizing the rate constants listed in Table II and the experimentally determined dissociation constants are in satisfactory agreement with the experimental points for both **1** and **2**.

The pH–rate profile of **1** indicates that successive protonation of the substrate results in an increase in

**Table II.** Rate Constants for the Hydrolysis of *o*- and *p*-Carboxyphenyl Phosphoramidates (35°, $\mu$ = 0.2)

| Compd | $k_{H^+}$, $M^{-1}$ hr$^{-1}$ | $k_1$,[a] hr$^{-1}$ | $k_2$,[a] hr$^{-1}$ | $k_3$,[a] hr$^{-1}$ |
|---|---|---|---|---|
| *o*-COOH | 17.6 | 6.00<br>(10.0)[b]<br>(7.5)[c] | 3.55 | 0.90 |
| *p*-COOH | 5.4 | 2.10 | 1.99 | 1.63 |
| *p*-Cl[d] | 0.88 | 0.23 | | 0.034[f] |
| $NH_2PO_3H_2$[e] | 33.3 | 0.420 | | 0.252[f] |

[a] May also be expressed as second-order rate constants by dividing by 55.5. [b] Extrapolated intercept value of $k_{obsd}$ *vs.* (0–3.0 *M*) HCl, $\mu$ = 3.0. [c] Extrapolated intercept value of $k_{obsd}$ *vs.* (0–1.0 *M*) HCl, $\mu$ = 1.0. [d] From the data of Chanley and Feageson[11] (0°, $\mu$ = 2.0). [e] From the data of Chanley and Feageson[11] (36.8°, $\mu$ = 0.2). [f] Monoanion species.

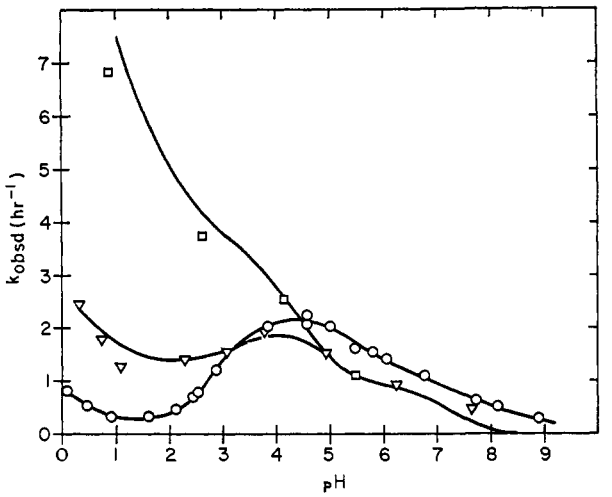



Figure 2. The pH–rate profiles for the solvolysis of *o*-carboxyphenyl phosphoramidate in 50% v/v dioxane–water, ○; 53% v/v methanol–water, △; and 5% v/v methanol–water, □, at 35°, $\mu$ = 0.2. Values of $k_{obsd}$ determined in 5% v/v methanol–water are plotted in relation to the pH–rate profile described in Figure 1.

$k_{obsd}$. The order of rate constants is $k_{H^+} > k_1 > k_2 > k_3$. This contrasts with the pH–rate profile of 2 which exhibits a broad pH-independent region (pH 1.5–6) and rate constants in the order $k_{H^+} > k_1 \approx k_2 \approx k_3$.

Solvolysis of **1** in mixed solvents, 50% dioxane–water and 53% methanol–water, results in a dramatic change both in the magnitude and pH dependency of $k_{obsd}$ (Figure 2). Only at pH >5 does any semblance remain to the original pH–rate profile. Allowing for the shift of p$K$'s to higher values in both mixed solvents (see also ref 11) it appears that only $k_3$ is essentially unperturbed. The rapid hydrolysis of the neutral and the monoanionic species is severely repressed in both solvents. Participation by the *o*-carboxyl group is no longer manifested by the appearance of a titration curve between pH 3 and 5.

Marked repression of hydrolysis of the neutral species by dioxane–water mixtures has been observed by Chanley and Feageson in the solvolysis of *p*-chlorophenyl phosphoramidate. In contrast the solvolysis of phosphoramidate itself is accelerated at all pH's in 50% methanol–water relative to purely aqueous media. In the present case the solvolysis of **1** in 5% methanol–water describes a pH–rate profile that closely approximates that observed in purely aqueous solution.

**Products.** Results of solvolysis experiments on **1** and **2** in alcohol–water mixtures are listed in Table III. Findings for methanol–water mixtures are presented graphically in Figures 3 and 4. The important features of these plots are that (1) the mole fraction of methyl phosphate produced is greater than the mole fraction of methanol in the solvent mixture and (2) the mole fraction of methyl phosphate produced is independent of pH. This is true for both **1** and **2**. This behavior is not readily rationalized at all pH values in terms of a simple bimolecular rate expression where

$$\text{mole fraction of } CH_3OPO_3H_2 = \frac{k_{CH_3OH}[CH_3OH]}{k_{CH_3OH}[CH_3OH] + k_{H_2O}[H_2O]} \quad (5)$$

because of the decrease in $k_{obsd}$ in 53% methanol–water relative to purely aqueous media which exceeds at pH <3 the decrease in the concentration of water.

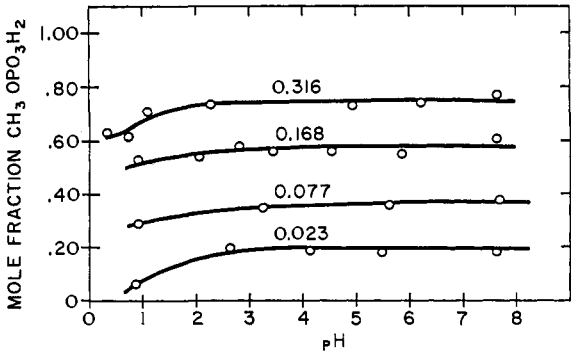



Figure 3. Plots of the mole fraction $CH_3OPO_3H_2$ *vs.* pH for solvolysis of *o*-carboxyphenyl phosphoramidate ($\mu$ = 0.2, 35°). Values above the curves are the mole fraction of methanol in the methanol–water solvent.

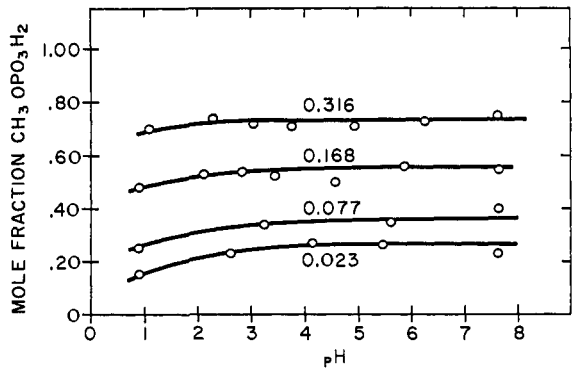


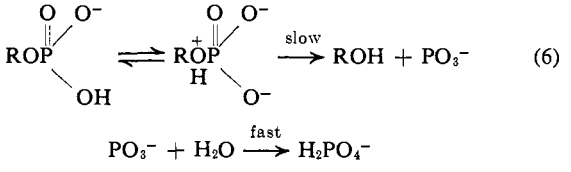

Figure 4. Plots of the mole fraction $CH_3OPO_3H_2$ *vs.* pH for solvolysis of *p*-carboxyphenyl phosphoramidate ($\mu$ = 0.2, 35°). Values above the curves are the mole fraction of methanol in the methanol–water solvent.

With more substituted alcohols, ethanol, 2-propanol, and *t*-butyl alcohol the mole fraction of alkyl phosphate produced decreases but generally exceeds the mole fraction of alcohol in the solvent. This decrease is also somewhat sensitive to the nature of the phosphoramidate; **1** yields less alkyl phosphate than **2**. With *t*-butyl alcohol, alkyl phosphate is only detected with **2**.

## Discussion

It is convenient to analyze the results in terms of each ionic species.

**Anions [$^-$OOC–R–NHPO$_3$H$^-$] and [HOOC–R–NHPO$_3$H$^-$].** Phosphate monoester monoanions have been postulated to undergo hydrolysis *via* a unique mechanism generating monomeric metaphosphate.[24,28-30] Evidence has been reported recently that suggests that this pathway probably involves a zwitterionic intermediate.[29]




(6)

(28) J. R. Cox and O. B. Ramsay, *Chem. Rev.*, **64**, 317 (1964), and references therein.
(29) A. J. Kirby and A. G. Varvoglis, *J. Am. Chem. Soc.*, **89**, 415 (1967).
(30) W. P. Jencks, *Brookhaven Symp. Biol.*, **15**, 134 (1962).

**Table III.** Variation of Mole Fraction of Alkyl Phosphate with pH upon Solvolysis of *o*- and *p*-Carboxyphenyl Phosphoramidates (35°, $\mu$ = 0.2)

| Alcohol (mole fraction) | pH | Mole fraction | Alcohol (mole fraction) | pH | Mole fraction | Alcohol (mole fraction) | pH | Mole fraction |
|---|---|---|---|---|---|---|---|---|
| | | | | *ortho* | | | | |
| $CH_3OH$ | 0.34[a] | 0.63 | $CH_3OH$ | 0.92 | 0.29 | $C_2H_5OH$ | 0.95 | 0[b] |
| (0.316) | 0.74[a] | 0.62 | (0.077) | 3.25 | 0.35 | (0.016) | 2.58 | 0[b] |
| | 1.10 | 0.71 | | 5.61 | 0.36 | | 4.18 | 0[b] |
| | 2.28 | 0.75 | | 7.64 | 0.38 | | 5.52 | 0[b] |
| | 3.06 | 0.73 | $CH_3OH$ | 0.87 | 0.06 | | 7.64 | 0.16 |
| | 3.74 | 0.72 | (0.023) | 2.62 | 0.20 | $C_3H_7OH$ | 0.98 | 0[b] |
| | 4.95 | 0.73 | | 4.14 | 0.19 | (0.094) | 2.88 | 0[b] |
| | 6.23 | 0.74 | | 5.47 | 0.18 | | 4.61 | 0.15 |
| | 7.65 | 0.77 | | 7.63 | 0.18 | | 5.96 | 0.14 |
| $CH_3OH$ | 0.91 | 0.53 | $C_2H_5OH$ | 1.00 | 0.27 | | 7.56 | 0.22 |
| (0.168) | 2.06 | 0.54 | (0.116) | 2.84 | 0.27 | $C_4H_9OH$ | 1.00 | 0[b] |
| | 2.83 | 0.58 | | 4.61 | 0.22 | (0.098) | 4.61 | 0[b] |
| | 3.45 | 0.56 | | 5.95 | 0.18 | | | |
| | 4.55 | 0.55 | | 7.59 | 0.35 | | | |
| | 5.86 | 0.55 | | | | | | |
| | 7.64 | 0.61 | | | | | | |
| | | | | *para* | | | | |
| $CH_3OH$ | 1.09 | 0.70 | $C_2H_5OH$ | 0.99 | 0.25 | $C_4H_9OH$ | 0.98 | 0.21 |
| (0.316) | 2.28 | 0.74 | (0.116) | 2.85 | 0.31 | (0.098) | 5.95 | 0.19 |
| | 3.04 | 0.72 | | 4.60 | 0.28 | | | |
| | 3.76 | 0.71 | | 5.94 | 0.35 | | | |
| | 4.95 | 0.71 | | 7.59 | 0.35 | | | |
| | 6.25 | 0.73 | $C_2H_5OH$ | 0.95 | 0[b] | | | |
| | 7.65 | 0.75 | (0.016) | 2.59 | 0.11 | | | |
| $CH_3OH$ | 0.90 | 0.48 | | 4.18 | 0.10 | | | |
| (0.168) | 2.10 | 0.53 | | 5.52 | 0.10 | | | |
| | 2.83 | 0.54 | | 7.64 | 0.16 | | | |
| | 3.45 | 0.52 | $C_3H_7OH$ | 0.98 | 0.15 | | | |
| | 4.56 | 0.50 | (0.094) | 2.88 | 0.16 | | | |
| | 5.86 | 0.56 | | 4.61 | 0.20 | | | |
| | 7.64 | 0.55 | | 5.96 | 0.15 | | | |
| $CH_3OH$ | 0.88 | 0.25 | | 7.56 | 0.18 | | | |
| (0.077) | 3.25 | 0.34 | | | | | | |
| | 5.61 | 0.35 | | | | | | |
| | 7.63 | 0.40 | | | | | | |
| $CH_3OH$ | 0.88 | 0.15 | | | | | | |
| (0.023) | 2.62 | 0.23 | | | | | | |
| | 4.14 | 0.27 | | | | | | |
| | 5.47 | 0.26 | | | | | | |
| | 7.63 | 0.23 | | | | | | |

[a] $\mu$ = 1.0. [b] Zero within experimental error ±4%.

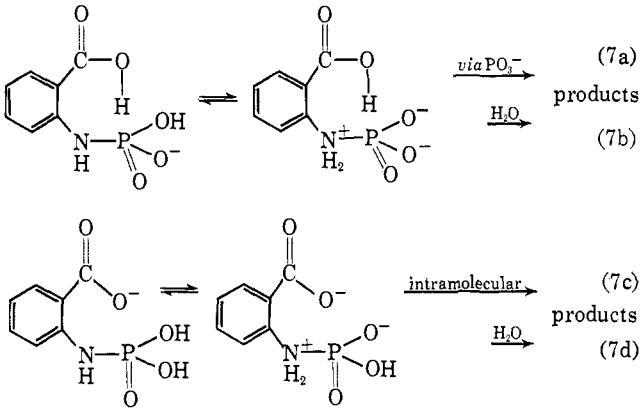
It is possible that zwitterion formation may become partially rate determining depending on the nature of the RO group. Solvolysis of phosphate monoanion species in alcohol–water mixtures generally gives rise to a product distribution that closely approximates the mole fraction solvent composition.[11,29] Such a reactive species as metaphosphate is anticipated to be insensitive to the relative nucleophilicity of the various solvent components. This, in a strict sense, is only true for a solvent mixture where both nucleophiles are sterically similar (methanol–water), and it is not expected to be valid in a solvent mixture such as 2-propanol- or *t*-butyl alcohol–water mixtures. The methanol–water results, along with much other experimental evidence,[24,28,30] have been cited in support of the intermediacy of metaphosphate.

The hydrolysis of phosphoramidates is more complex. The monoanion of phosphoramidate exists as the zwitterion in the crystalline state.[31,32] a fact revealed by X-ray crystallographic studies. It is also probably zwitterionic in aqueous solution since the second dissociation constant of phosphoramidate (p$K_2$ = 8.2) is significantly smaller than the reference compounds, N-benzoylphosphoramidate (p$K_2$ = 5.67, 6.42) and ethyl N,N-diethylphosphoramidate (p$K_2$ = 7.2).[11,33–35] In contrast the N-aryl derivatives, *e.g.*, *p*-chlorophenyl phosphoramidate (p$K_2$ = 6.8) and compounds **1** (p$K_3$ = 7.25) and **2** (p$K_3$ = 6.99), have second dissociation constants that appear to be normal; thus the nonzwitterionic form is probably the extant species. One may arrive at the same conclusion qualitatively by considering the phosphoramidate as a bimolecular species comprised of orthophosphoric acid and the corresponding amine. In the case of the aromatic amines p$K_a$ < p$K_2$ (7.2) of orthophosphoric acid; hence the thermodynamically favored site of protonation is one of the phosphoryl oxygens. In the case of the aliphatic amines

(31) E. Hobbs, D. E. C. Corbridge, and B. Raistrick, *Acta Cryst.*, **6** 621 (1953).
(32) D. E. C. Corbridge and E. J. Lowe, *J. Chem. Soc.*, 493 (1954).
(33) C. Zioudrou, *Tetrahedron*, **18**, 197 (1962).
(34) M. Halmann, A. Lapidot, and D. Samuel, *J. Chem. Soc.*, 4672 (1960).
(35) E. W. Crunden and R. F. Hudson, *ibid.*, 3591 (1962).

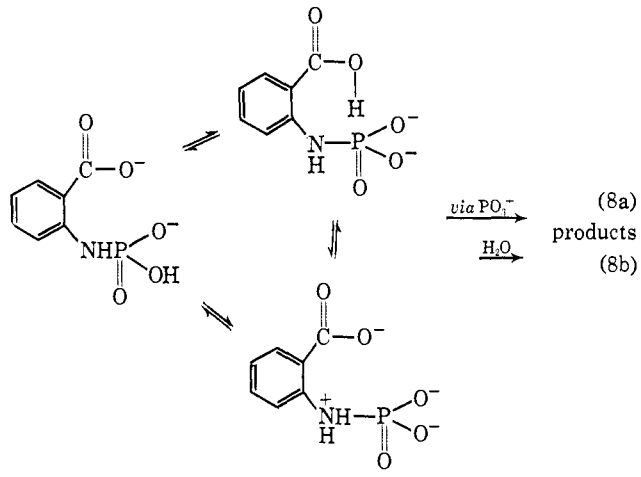
$pK_a > pK_2$ of orthophosphoric acid; thus zwitterion formation is anticipated.

Comparison of $k_2$ and $k_3$ for **1** and **2** shows that $k_3(\mathbf{2}) > k_3(\mathbf{1})$ whereas $k_2(\mathbf{1}) > k_3(\mathbf{1})$ and $k_2(\mathbf{2})$. It appears that carboxyl group participation may be important in $k_2(\mathbf{1})$ and may be occurring through one of several possible mechanisms.

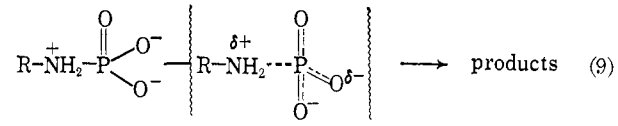



The available experimental evidence does not allow one to distinguish between the above and other possibilities which include: (1) formation of metaphosphate (7a) possibly assisted by intramolecular proton transfer which would be subsequent or concurrent to P–N bond fission; (2) bimolecular pathways b and d involving water as a participating nucleophile with the possibility of proton transfer or electrostatic stabilization respectively; (3) intramolecular phosphoryl transfer (7c) to yield an acyl phosphate[36] which then undergoes hydrolysis. Not shown are possible mechanisms featuring hydronium ion as the proton donor or intramolecular general base catalysis by the carboxylate anion. It seems reasonable on chemical grounds to assume that a zwitterionic species is that subject to hydrolysis. Pathway 7a is similar to but not identical with those postulated in salicyl phosphate[37] and sulfate[38] hydrolysis, which appear to involve a preequilibrium proton transfer, and analogous to the prior observed electrophilic-catalyzed hydrolysis of phosphoramidate itself. A related example of carboxyl group facilitation of hydrolysis may be present in N-phosphorylcreatine.[5] The acceleration due to the neighboring carboxyl group is much less in the present system than in salicyl phosphate where a factor of roughly $10^2$ is observed and may be restricted simply to some form of electrostatic interaction, the effects of which are usually less pronounced.[24] It is difficult to rationalize $k_3(\mathbf{1}) < k_3(\mathbf{2})$, since attractive pathways are available for the hydrolysis of this species. No unequivocal explanation can be offered at this time.

Additional mechanistic information may be gained from rate studies in mixed solvents (53% methanol–water, 50% dioxane–water) that indicate $k_3(\mathbf{1})$ is effectively unperturbed while $k_2$ is depressed (*ca.* 30%). It is known that zwitterion equilibrium is solvent sensitive,




decreasing approximately linearly with increasing alcohol and dioxane molarity in the solvent mixture.[39] From considerations discussed above, the mono- and dianions should predominantly exist as the nonzwitterionic species. Insofar as it is applicable, *p*-aminobenzoic and anthranilic acids are effectively nonzwitterionic in aqueous solution.[39] Furthermore it is thought that the hydrolysis of phosphoramidate and *p*-chlorophenyl phosphoramidate monoanions apparently does not involve proton transfers in the rate-determining step due to the absence of substantial deuterium solvent isotope effects.[11] Thus one anticipates that $k_{obsd}$ is directly proportional to $K_{zw}$ where $K_{zw}$ is defined as an equilibrium constant between nonzwitterionic and zwitterionic forms.[40] If one accepts this hypothesis it becomes necessary to postulate that the reaction of the $[^-OOC\text{–}RNH_2^+\text{–}PO_3^{2-}]$ species is kinetically accelerated by solvents of decreasing polarity to compensate for the decrease in $K_{zw}$. Assuming the hydrolysis involves a transition state with charge dispersal or neutralization relative to the ground state, then the qualitative theory of Hughes and Ingold[41] or simple electrostatic theory[42a] predicts rate enhancement in less polar solvents.[42b] Such a transition state is depicted in eq 9. Related reactions which appear to proceed *via* mechanisms that show predominant unimolecular character as in the hydrolysis of phosphate monoester dianions[29] and the




hydronium ion catalyzed hydrolysis of sulfate monoesters are accelerated by organic solvents.[43] The effect of mixed solvents on $k_{obsd}$ should vary, however, with the nature of the phosphoramidate since $K_{zw}$ and the degree of charge neutralization are expected to roughly

(36) Recent studies by Hamer [N. K. Hamer, *J. Chem. Soc.*, 404 (1966)] on monoesterified phosphoramidic acids derived from N-phenylethylenediamine and 2-aminobenzylamine have failed to disclose intramolecular attack by the vicinal amino group. These results have been interpreted to signify the need for a linear transition state which cannot be accommodated through five- or six-membered ring formation. However, it appears premature to rule out (7c) on this basis at present.

(37) M. L. Bender and J. M. Lawlor, *J. Am. Chem. Soc.*, **85**, 3010 (1963).

(38) S. J. Benkovic, *ibid.*, **88**, 5511 (1966).

(39) J. T. Edsall and M. H. Blanchard, *ibid.*, **55**, 2337 (1933).

(40) Since $v = k_r[RNH_2^+\text{–}PO_3^{2-}]$, where $[RNH_2\text{–}PO_3H^-] = [RNH_2^+\text{–}PO_3^{2-}] + [RNH\text{–}PO_3H^-]$ and $K_{zw} = [RNH_2^+\text{–}PO_3^{2-}]/[RNH\text{–}PO_3H^-]$, $k_{obsd} = k_rK_w/(K_{zw} + 1)$. In the above case $K_{zw} < 1$ is assumed. A similar postulation has been offered by Chanley and Feageson in the hydrolysis of N-arylphosphoramidates.

(41) C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p 346.

(42) (a) K. Wiberg, "Physical Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1964, p 374. (b) The above solvent theories must be applied with caution. Solvolyses of phosphate monoester dianions which are to a high degree unimolecular generally, as predicted, are accelerated by nonpolar solvents. Exceptions, however, are known.[29]

(43) B. D. Batts, *J. Chem. Soc.*, 547, 551 (1966).

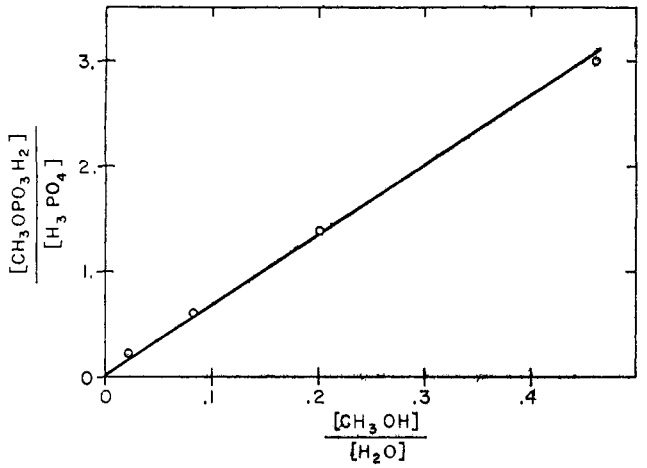



Figure 5. Plot of the ratio of methyl phosphate–orthophosphoric acid concentration against the ratio of methanol–water concentration for *o*- and *p*-carboxyphenyl phosphoramidates at $\mu$ = 0.2, 35°. Ordinate values are averages of mole fractions $CH_3OPO_3H_2$ observed over pH 2–8 at various methanol concentrations for both compounds.

correlate with the $pK_a'$ of the amino group. Since $K_{zw} > 1$ for phosphoramidate, $k_{obsd}$ is independent of a solvent effect on $K_{zw}$[11] and presumably only reflects a kinetic solvent effect which results in *ca.* a 20% increase in $k_{obsd}$ in 50% dioxane–water. The species [$HOOC-RNH_2^+-PO_3^{2-}$] may hydrolyze, at least partially, *via* a bimolecular pathway and reflect both a change in $K_{zw}$ and the decrease in water concentration. Consistent also with a pathway unimolecular in character is the fact that $\Delta S^{\pm}$ for both *p*-chlorophenyl phosphoramidate and phosphoramidate itself is −6.3 and −1.6, respectively.

It is pertinent at this point to consider the product composition in alcohol–water mixtures. In the solvolysis of both anionic species for either **1** or **2**, a marked preference for the alcohol component is shown by the solvolytically important species. This may be expressed quantitatively by plotting the ratio of the mole fractions of alkyl phosphate to orthophosphoric acid against the ratio of mole fraction of alcohol to mole fraction of water. The slope, assuming the nonzwitterionic species does not undergo solvolysis, is simply the relative selectivity of the phosphoramidate for alcohol. Figure 5 is such a plot for the methanol–water mixtures, and applies to *all* species for both **1** and **2**, since the mole fraction methyl phosphate is invariant over the pH range 2–8. The selectivity for methanol is eightfold greater than that for water. Similar calculations for ethanol, 2-propanol, and *t*-butyl alcohol show the selectivity for alcohol is *ca.* four-, two-, and twofold, respectively.[44] On chemical grounds it seems reasonable to assume that the solvolysis products arise from the reactions of the zwitterionic species. One cannot rule out at present a contribution from solvolysis of the nonzwitterionic form on the basis of the above pH–rate profiles in mixed solvents. However, experiments in 5% methanol–water where the solvolytic contribution of the nonzwitterionic species should be minimal yield the same ratio of methyl phosphate:orthophosphoric acid as mixed solvents 53% by volume in methanol. Assuming that the partitioning of the nonzwitterionic species to solvolytic products differs from the zwitterionic form, one may argue that the zwitterionic species is the active species in the solvolysis reactions.

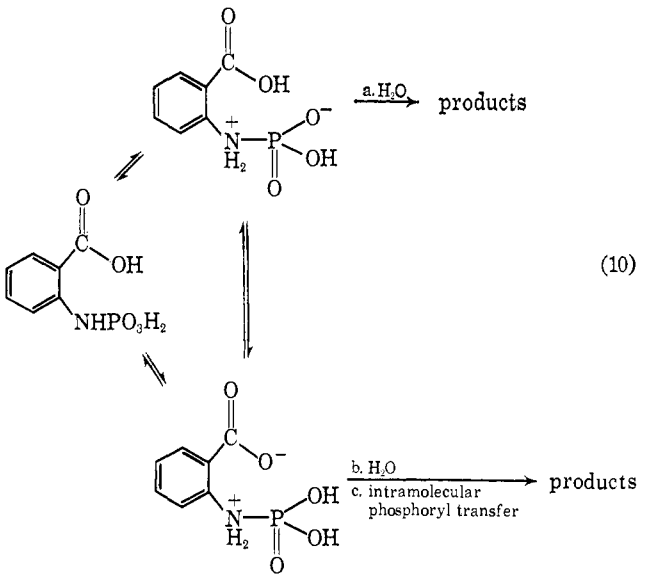
It appears, therefore, that the collective data do not support a "free metaphosphate" intermediate in the hydrolysis of the anionic species since the former is predicted to be nonselective. A similar conclusion has been reached on the basis of solvent composition studies in the electrophilic-catalyzed hydrolysis of phosphoramidate monoanion,[13] whereas its hydrolysis has been viewed as being bimolecular.[11] The bimolecular postulation was based on the fact that both $k_{obsd}$ and the product composition in methanol–water mixtures could be correlated with a bimolecular rate expression since $k_{obsd}$ increased with increasing methanol concentration in these mixtures. Indeed it is possible that the noncatalyzed hydrolysis of phosphoramidate has a greater degree of bimolecular character due to a more basic leaving group. In **1** and **2** no such correlation is observed, the leaving group is less basic, and this bimolecular aspect disappears. The selectivity of the monoanionic species of both phosphoramidate and *p*-chlorophenyl phosphoramidate for methanol is *ca.* eightfold and therefore similar to that observed for **1** and **2**. It is noteworthy that catalysis by the neighboring carboxyl group does not alter the product composition; the same is true in the catalyzed and noncatalyzed hydrolysis of phosphoramidate. This phenomena is most readily explained in unimolecular terms. On the other hand the partial bimolecular character of the solvolysis of **1** and **2** is manifested by the phosphorylation of 2-propanol, a synthesis which is not observed in the solvolysis of aromatic monoester monoanions where nonselective phosphorylation is the generally observed case. Thus it appears that aryl phosphoramidate monoanions represent a situation where the transition state has both unimolecular and bimolecular character, and we prefer to view the transition state as featuring a long P–N bond with little bond formation between phosphorus and the incoming nucleophile. The unusual features of this transition state are probably in part due to the availability of vacant d orbitals on phosphorus. Insofar as the attack of nitrogen nucleophiles is applicable to alcohols, the slope of the Brønsted plot (0.22) for the reaction of a series of pyridines with phosphoramidate monoanion indicates little bond formation between the incoming nucleophile and phosphorus.

**Neutral Species.** Inspection of the pH–rate profiles for **1** and **2** indicate that $k_1(\mathbf{1}) > k_1(\mathbf{2})$. This may result from the operation of one of several possible mechanistic modes that include general acid catalysis, intramolecular phosphoryl transfer, or electrostatic catalysis (eq 10a, c, and b, respectively). In addition to these and other related variations suggested for the anionic species, a steric acceleration of a bimolecular mechanism as noted in other *ortho*-substituted reagents is possible.[45]

It seems reasonable to postulate the zwitterionic species as the active hydrolytic form, a hypothesis supported by the marked depression (*ca.* fivefold) of $k_1$ in mixed solvents. A similar phenomenon has been ob-

(44) The value of the selectivity ratio for ethanol–water mixtures was calculated from the average product composition at pH >2 for solvolysis of 1 and 2; for 2-propanol and *t*-butyl alcohol–water mixtures the value was computed from the average product composition at pH >2 for solvolysis of 2.

(45) R. W. Taft, "Steric Effects in Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1956, Chapter 3.




(10)

served by Chanley in the solvolyses of N-arylphosphoramidates which may be attributed to a decrease in $K_{zw}$ and water concentration. On chemical grounds the formation of metaphosphate seems unlikely; thus the mechanisms in eq 10 are written as bimolecular. It is possible to form a protonated metaphosphate which should be at least as reactive as metaphosphate, but this is not borne out by the product composition studies.

Product composition studies in alcohol–water mixtures reveal that the selectivity of the neutral parallels that of the anionic species.[46] Although $k_{obsd}$ is greater for the former, the ratio of $k_{CH_3OH}/k_{H_2O}$ must be similar, again exhibiting a *ca.* eightfold selectivity for methanol. The data of Chanley and Feageson may be interpreted in this manner with the result that the neutral species of phosphoramidate and *p*-chlorophenyl phosphoramidate exhibit a selectivity for methanol similar to **1** and **2**.[46] It appears, therefore, that the relative insensitivity of phosphate and phosphoramidate monoanions may extend to the neutral species with the result that an anticipated small increase in $\beta$ would be difficult to detect experimentally at low $\beta$ values. It is also possible that the increased reactivity of the neutral relative to the monoanionic species may be generally due to a change in the Brønsted intercept rather than the slope as is found in the attack of nucleophiles on *p*-nitrophenyl phosphate dianion. This hypothesis remains to be tested further experimentally. Solvolysis of the neutral species, however, is more sensitive to probable steric effects since *t*-butyl alcohol and 2-propanol–water mixtures phosphorylate **2** but not **1** at pH's where the neutral species predominates. Thus, a bimolecular mechanism involving attack of the nucleophile on the zwitterion is favored. It is anticipated that the transition state for the neutral species features a shorter P–N bond than in the monoanionic species. This is evidenced by an increased sensitivity to the above steric effect and the decrease in $\Delta S^{\pm}$ (−18.2 eu) found for phosphoramidate.

**Hydronium Ion Catalysis.** Both substrates are subject to hydronium ion catalysis, a general phenomena for phosphoramidates. The greater value of $k_{H^+}$(**1**) may be due to steric acceleration as found in the hydronium ion catalyzed hydrolysis of salicyl sulfate or general acid catalysis. Hydronium ion catalysis as expected is greater with the more basic substrate, phosphoramidate itself.

With the advent of hydronium ion catalysis, a slight drop in the mole fraction of methyl phosphate is observed. This parallels the observation of Chanley and Feageson[11] who extended their studies to higher acidities and demonstrated that the product composition for N-arylphosphoramidates approached that of the solvent. It seems doubtful that this arises from the formation of metaphosphate since a chemical driving force is lacking; on experimental grounds the solvolysis of the highly reactive triester, diisopropyl phosphorochloridate, in mixed ethanol–water solvents reveals that the relative reactivity of ethanol to water is only 1.22.[47] Thus we favor a bimolecular pathway involving attack by solvent on the protonated zwitterion.


(11)

It is possible that solvent sorting is in some measure responsible for orthophosphoric acid formation since the proton donor is hydronium ion, and protonation and hydrolysis may be approaching a concerted process.

## Summary

The hydrolysis of the *o*- and *p*-carboxyphenyl phosphoramidates appears to occur through a mechanistic gradation from a transition state predominantly unimolecular in character with the dianions to increasing bimolecular character with successive protonation. Kinetic studies reveal acceleration by the *o*-carboxyl group in the hydrolysis of several of the species. Product composition studies give no indication of free monomeric metaphosphate formation in the solvolysis of any of the species of either **1** or **2**. Although the hydrolysis of the di- and monoanionic species appears to involve considerable unimolecular character, the intermediate species is a selective rather than nonselective phosphorylating agent. Thus the zwitterionic species involved in the solvolysis of **1** and **2** appears to be chemically similar to the presumed N-phosphorylpyridinium phosphorylating agent generated by the addition of pyridine to alcoholic solutions of phosphoramidate monoesters.[48] However, the synthetic utility of phosphoramidate monoesters to yield pyrophosphates in the presence of hydroxyl groups (solvent, dimethylformamide, no pyridine) may arise from a general acid catalyzed nucleophilic displacement of $HPO_4^{2-}$ on the nonzwitterionic species of the phosphoramidate, the latter being greatly favored under the synthetic conditions employed.[49]

(46) There seems to be no reason to postulate that the products from alcohol–water mixtures at pH 2–3.5 do not arise from solvolysis of the neutral species. Calculations based on p$K$ measurements in 53% methanol–water indicate that the neutral species will be present at *ca.* 90% at pH 2. Although the rate of solvolysis of the neutral species is repressed in mixed solvents, it is still competitive with the solvolysis of the monoanions.

(47) I. Dostrovsky and M. Halmann, *J. Chem. Soc.*, 502 (1953).

(48) The finding that solvolysis of methyl hydrogen cyclohexylphosphoramidate and phosphoramidate itself in the absence of pyridine yield predominantly orthophosphate in ethanol–water mixtures at 100° may arise from a difference in activation energies for ethanolysis *vs.* hydrolysis. This may favor the former at elevated temperatures. See ref 10.

(49) A. Todd, *Proc. Chem. Soc.*, 199 (1962).

Recently Kirby has postulated that the hydrolysis of aromatic phosphate dianions appears to proceed *via* formation of free monomeric metaphosphate.[29] It is important to note that the dianions in methanol–water mixtures yield consistently a product composition favoring methyl phosphate, whereas the monoanionic species more closely approximates the solvent composition. The authors rationalize this on the basis of selective solvation of the dianion. An alternate explanation is the possibility that the transition state does not involve free metaphosphate, although predominantly unimolecular in character. Treatment of the reported data in terms of the plot of Figure 5 reveals that 2,4-dinitrophenyl phosphate dianion is *ca.* fourfold more reactive with methanol than with water, consistent with considerable P–O fission in the transition state. Selective solvation cannot be completely eliminated nevertheless as a competing factor in the above and present study. It appears, however, that the nonselectivity criterion for metaphosphate is only satisfied at present in the solvolysis of phosphate monoester monoanions in methanol–water mixtures where there exists in addition a considerable body of data supporting the metaphosphate hypothesis. In conclusion it should also be noted that the zwitterionic intermediates in phosphate monoester monoanion hydrolysis constitute the best leaving groups in terms of $pK_a'$ arguments and probably delineate one end of the scale from bimolecular to unimolecular hydrolysis in phosphorus-containing esters. Moreover, in phosphoramidate hydrolysis in particular, one definitely appears to encounter transition states in which the extent of bond formation and fission are not directly proportional, which suggests that pentacovalent intermediates may be important in the hydrolysis of several of these species. From a biological standpoint the lability of phosphoramidates and their selectivity toward hydroxylic nucleophiles other than water indicate their utility as biochemical phosphorylating agents.

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# Chromic Acid Oxidation of Allyl Alcohols[1]

**Sumner H. Burstein and Howard J. Ringold**

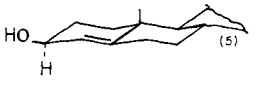
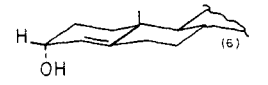
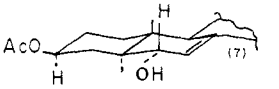
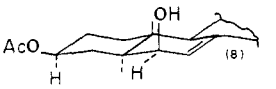
*Contribution from the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545.* *Received March 6, 1967*

**Abstract:** The rate-limiting step in the chromic acid oxidation of allyl alcohols is the cleavage of the carbon–hydrogen bond. In the absence of substantial strain factors equatorial alcohols are oxidized faster than the axial isomer, which may be rationalized on the basis of better overlap of the departing axial hydrogen. The rapid oxidation rate of unsaturated alcohols indicates a substantial contribution from $\alpha,\beta$-unsaturated ketone resonance in the transition state.

In a previous publication,[2] evidence was presented that the conversion of allyl alcohols to the corresponding $\alpha,\beta$-unsaturated ketones by dichlorodicyanoquinone (DDQ) oxidation proceeds *via* a rate-determining abstraction of hydride ion from carbon. In the case of a sterically compressed axial allyl alcohol, for example, the $\Delta^7$-6$\beta$-ol **8**, DDQ oxidation of the axial alcohol was faster than oxidation of the equatorial counterpart **7**. However, the situation was reversed when the alcohols were relatively unhindered such as the $\Delta^4$-3-ols **5** and **6** (Table I). These findings prompted us to determine the effect of unsaturation on chromic acid oxidation rates with these two isomeric alcohol pairs.

It has been demonstrated quite conclusively[3] that saturated secondary axial alcohols are oxidized more rapidly by chromic acid than the equatorial isomers and that these differences are magnified in sterically crowded situations. It is generally accepted[3,4] that rate enhancement in the latter case is due primarily to strain relief in the transition state which proceeds concomitantly with the development of carbonyl (trigonal) character. The primary deuterium isotope effect with

**Table I.** Relative Oxidation Rates of Allyl Alcohols with Dichlorodicyanoquinone[a]

| | $k_{rel}$ | $\frac{k_{eq}}{k_{ax}}$ |
|---|---|---|
|  | 33.7 | |
| | | 6.4 |
|  | 5.2 | |
|  | 1.0 | |
| | | 0.086 |
|  | 11.7 | |

[a] Reported in ref 2.

(1) Supported in part by Grant T-185, American Cancer Society.
(2) S. H. Burstein and H. J. Ringold, *J. Am. Chem. Soc.*, 86, 1952 (1964).
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