# The Anomalous Hydrolytic Behavior of 1-Phenylvinyl Phosphate

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The kinetics of hydrolysis of 1-phenylvinyl phosphate, **1**, were studied over a pH range of 1 to 8.3 and over a pD range of 1 to 5.6 at 25°C and $\mu = 0.5$ *M* with sodium chloride. The hydrolytic behavior of **1** was found to differ, in many respects, from that of alkyl and aryl phosphomonoesters. First, the rates of hydrolysis of **1** were extremely rapid and, in the hydronium ion-catalyzed region, gave a solvent deuterium isotope effect ($k_H/k_D$) of 3.20. Also, the $^1$H-NMR spectrum of acetophenone formed upon complete hydrolysis of **1** in $D_2O$ (pD 1.2) revealed that only one deuterium atom was incorporated into the methyl group. Hence, the evidence was consistent with a rate-limiting and nonreversible proton transfer from the solvent to **1**. In addition, using an $H_2{}^{18}O$ labeling study in conjunction with $^{31}$P-NMR analysis, the hydrolytic mechanism appeared to involve nucleophilic attack by water at both the α-carbon and the phosphorus atom with concurrent C–O and P–O bond fission. Second, in the pH region where the monoanionic species of **1** predominated, buffers had a pronounced catalytic effect on the hydrolysis rate; there appeared to be a normal solvent deuterium isotope effect; and the rate constant, $k'_o$, showed a positive deviation from the established Brønsted relationship. The dissimilarities between **1** and alkyl and aryl phosphomonoesters supported the involvement of an alternate dephosphorylation pathway. One potential mechanism for the hydrolysis of **1**, consistent with the experimental findings, might be rate-limiting and nonreversible protonation of the β-carbon of the olefinic bond, resulting in the formation of a rapidly hydrated carbonium ion intermediate, a mechanism similar to that proposed for the more acidic pH region. Alternatively, a concerted mechanism involving proton transfer with expulsion of a monomeric metaphosphate anion might be operating.

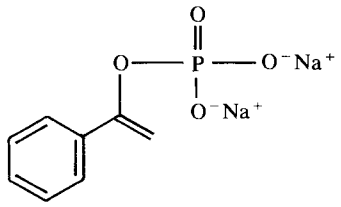
KEY WORDS: acetophenone; 1-phenylvinyl phosphate; enol phosphate; phosphate ester; hydrolysis; prodrug.

## INTRODUCTION

One of the principal problems encountered in the field of pharmaceutics is the poor solubility of a drug molecule in an aqueous environment. This property may manifest itself by causing a number of undesirable characteristics which often severely limit the therapeutic potential of the drug entity. For sparingly water-soluble compounds containing an enolizable keto group, the potential exists for phosphorylation of the enol form of the given drug. The resulting enol phosphate (or vinyl ester of phosphoric acid) might be useful as a prodrug.

Enol phosphates are difficult to synthesize, and therefore their hydrolysis kinetics have not been extensively studied. Cyanovinyl phosphate was found to be reasonably stable in the pH range of 7 to 11, where the hydrolysis rate was independent of the pH (1). In this pH region, the predicted half-life at 25°C would be about 2 years. Vinyl phosphate was rapidly hydrolyzed at room temperature and further esterification of the phosphate group enhanced the hydrolytic stability (2). The monoanilinium salt of 1-phenylvinyl phosphate was described as being an unstable compound (3). This claim was later disputed by Satterthwait and Westheimer, who stated that the monoanilinium salt degraded 10% in 1 month at 4°C (4). Phosphoenolpyruvate, the most extensively studied enol phosphate, was relatively stable, with half-lives varying between 0.75 and 12 hr over the pH range of 0 to 7, respectively, at 75°C (5).

Because of the paucity of kinetic data and the interest as to whether enol phosphates might be useful as prodrugs of enolizable ketones, the object of this study was to evaluate the reactivity and the mechanism(s) of hydrolysis of the enol phosphate of acetophenone, disodium 1-phenylvinyl phosphate, **1** (4), a model enol phosphate for other ketone-containing molecules.



**1**

## MATERIALS AND METHODS

### Materials

Bromoacetophenone, deuterium oxide (99.8 mol% $D_2O$), deuterium chloride (37 wt% solution in $D_2O$, 99 atom% deuterium), and silica gel (70–270 mesh, 60 Å) were obtained from the Aldrich Chemical Co. (Milwaukee, WI). $H_2{}^{18}O$ was obtained from MSD Isotopes (St. Louis, MO). The water was deionized and charcoal filtered prior to distillation from an all glass still. All other chemicals were of reagent or analytical grade and were used without further purification.

### Analysis

The HPLC system consisted of a Kratos Spectroflow-757, variable-wavelength, UV detector operating at a fixed wavelength; an Altex model 110A pump; a Rheodyne six-port injection valve with a 20-μl external loop; and a Shimadzu C-R3A integrator. The analytical column was an ASI Phenyl (30 cm × 3.9 mm) with a mean particle diameter of 10 μm. The mobile phase was composed of acetonitrile and 25 m*M*, pH 4, phosphate buffer (5:95, v/v). A detection wavelength of 240 nm was employed. At an eluent flow rate of 1.5 ml/min, **1** had a retention volume of 5.1 ml, and acetophenone had a retention volume of 27.4 ml.

All pH measurements were determined using a Corning-155 pH meter and an Orion semimicro-Ross combination

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glass electrode. The proton magnetic resonance ($^1$H-NMR) spectra were recorded on a Varian FT-80A spectrometer. The chemical shifts in $D_2O$ were relative to the sodium salt of 3-(trimethylsilyl)-1-propane-sulfonic acid, which was used as an internal standard. The $^{31}$P-NMR and the $^{13}$C-NMR spectra were recorded on a Varian XL-300 spectrometer operating at 121.4 MHz for $^{31}$P and at 75 MHz for $^{13}$C. The chemical shifts on the $^{13}$C-NMR spectra were relative to the methyl resonance of TMS, which was used as an internal standard.

### Synthesis

Compound **1** was synthesized by a modification of the procedure used by Borowitz *et al.* (6). A solution of bromoacetophenone (0.02 mol) in 4.6 ml of acetic acid was added dropwise with stirring to trimethyl phosphite (0.02 mol). After stirring for 22 hr at room temperature, the acetic acid and unreacted trimethyl phosphite were removed *in vacuo*. The residual liquid was chromatographed on silica gel using ethyl acetate:hexane (1:1) as the eluent. The fraction containing the desired product, 1-phenyl dimethylphosphate (**2**), was dried over sodium sulfate. This was followed by filtration and removal of the residual eluent *in vacuo*.

Fresh bromotrimethylsilane ($9.5 \times 10^{-3}$ *M*) and **2** ($5 \times 10^{-3}$ *M*) were allowed to react in a dry, stoppered test tube for 4 hr at room temperature. To this mixture was added, with stirring, a solution of aniline (1 equiv) in 20 ml of ethanol. The resulting crystals were filtered and dried.

Compound **1** was formed by mixing **3** with 0.1 *M* NaOH (2 equiv). This solution was extracted twice with an equal volume of ether, and the aqueous phase was then lyophilized, leaving the solid product. $^1$H-NMR ($D_2O$): δ 5.0 (d of d, 1H), 5.1 (d or d, 1H), 7.35 (m, 3H), and 7.65 ppm (m, 2H).

No attempts were made to purify the product by recrystallization; therefore, inorganic impurities may have been present. However, following the synthetic procedure, HPLC and $^1$H-NMR analyses confirmed the presence of only **1**. In addition, HPLC, $^1$H-NMR and $^{31}$P-NMR analyses of the hydrolysis products revealed the formation of only acetophenone and inorganic phosphate.

### Hydrolysis Studies

The pH or pD was maintained at 25°C with aqueous hydrochloric acid solutions, with aqueous deuterium chloride (in $D_2O$) solutions, or with solutions of varying buffer concentration (25–100 m*M*). The pD values were attained by adding a factor of 0.4 to the glass electrode pH values (7). The pH or pD values were attained by mixing equimolar amounts of the acidic and basic components at one of the ratios (v/v) 1:3, 1:1, or 3:1, leading to fixed and known fractions of the acidic component, $f_{HA}$. For the studies performed in $D_2O$, the acidic component of the acetate buffer was prepared by dissolving the required amount of acetic acid in $D_2O$. The ionic strengths of these solutions were adjusted to 0.5 *M* with sodium chloride.

The hydrolysis reactions were initiated by the addition of 100 μl of an aqueous solution of **1** (2 mg/ml) to 10 ml of the reaction mixture, which was preequilibrated at 25°C in a circulating water bath. Aliquots were withdrawn at appropriate time intervals and analyzed by HPLC for the disappearance of **1** and for the appearance of the parent compound, acetophenone. The pseudo-first-order rate constants, $k_{obs}$, were obtained by following the disappearance of the peak height of the prodrug with time for at least 1.5 half-lives.

From linear regression analysis of the plots of $k_{obs}$ versus the total buffer concentration at a fixed pH (or pD) and ionic strength, the intercepts yielded the first-order, buffer-independent rate constants, $k'_{obs}$; and the respective slopes yielded the second-order, buffer-dependent (or buffer-catalyzed) rate constants, $k_{buff}$. The buffer-independent pH–rate profile was curve fit to a semiempirical equation [Eq. (1)], which was chosen based on scientific precedent established for the majority of primary alkyl phosphate monoesters (8).

### Determination of the Site of Bond Cleavage (C–O and/or P–O)

To determine the site(s) of bond cleavage or hydration, the hydrolysis of **1** was carried out in 97% $H_2{}^{18}O$, and the $^{31}$P-NMR and $^{13}$C-NMR spectra of the products of hydrolysis, inorganic phosphate and acetophenone, were examined. The complete hydrolysis of **1** was carried out in 400 μl of $H_2{}^{18}O$, which was adjusted to pH 1.0 with 38% HCl. The samples were then adjusted to pH 7 with 10% (w/v) NaOH and diluted with 400 μl of $D_2O$. Additionally, the samples for $^{31}$P-NMR analysis were made 1 m*M* with respect to EDTA to enhance the resolution between inorganic phosphate peaks containing different isotopic compositions. The incorporation of the $^{18}$O label into the product inorganic phosphate and acetophenone was examined by $^{31}$P-NMR and $^{13}$C-NMR spectroscopy, respectively.

To determine if oxygen exchange between the solvent and the hydrolysis products was occurring, two control experiments, under the same reaction conditions, were performed using inorganic phosphate and acetophenone as the starting materials. Once again, the incorporation of the $^{18}$O label into the inorganic phosphate and the acetophenone was examined by $^{31}$P-NMR and $^{13}$C-NMR spectroscopy, respectively.

## RESULTS AND DISCUSSION

### Hydrolysis Kinetics of 1

The kinetics of hydrolysis of **1** were studied at 25°C and $\mu = 0.5$ *M* as a function of pH and buffer concentration. The resulting kinetic data are shown in Table I. The dependence of the buffer-independent rate constants, $k'_{obs}$, on the pH and on the pD is shown in Fig. 1. Over the pH range studied, the rates of hydrolysis were described by the following equation,

$$k'_{obs} = \frac{k_H[H^+]^3 + k_0[H^+]^2 + k'_0K_{a1}[H^+]}{[H^+]^2 + [H^+]K_{a1} + K_{a1}K_{a2}} \quad (1)$$

which assumes that the neutral and monoanionic species are hydrolytically reactive. In this equation, $k_H$ is the second-order rate constant for hydronium ion-catalyzed hydrolysis of the neutral phosphate species, and $k_o$ and $k'_o$ are the

Table I. Apparent First-Order Rate Constants for Hydrolysis of 1 at 25°C and $\mu$ = 0.5 $M$

| Buffer | Buffer conc. × $10^2$ ($M$) | pH | $k_{obs}$ × $10^4$ ($sec^{-1}$) |
|---|---|---|---|
| HCl | 10.0 | 1.02 | 79.2 |
| HCl | 8.0 | 1.10 | 64.9 |
| HCl | 6.0 | 1.23 | 50.7 |
| HCl | 4.0 | 1.42 | 33.5 |
| HCl | 2.0 | 1.74 | 19.8 |
| HCl | 1.0 | 2.02 | 12.4 |
| HCl | 0.75 | 2.15 | 8.69 |
| HCl | 0.50 | 2.34 | 5.70 |
| Formate | 5.0 | 3.06 | 3.00 |
| Formate | 7.5 | 3.05 | 3.10 |
| Formate | 10.0 | 3.05 | 3.15 |
| Formate | 2.5 | 3.53 | 2.17 |
| Formate | 5.0 | 3.51 | 2.38 |
| Formate | 7.5 | 3.51 | 2.50 |
| Formate | 10.0 | 3.52 | 2.87 |
| Formate | 2.5 | 4.00 | 1.93 |
| Formate | 5.0 | 4.00 | 2.03 |
| Formate | 7.5 | 4.00 | 2.41 |
| Formate | 10.0 | 4.00 | 2.68 |
| Acetate | 2.5 | 4.10 | 1.70 |
| Acetate | 5.0 | 4.10 | 1.83 |
| Acetate | 7.5 | 4.10 | 1.90 |
| Acetate | 10.0 | 4.09 | 2.03 |
| Acetate | 2.5 | 4.57 | 1.62 |
| Acetate | 5.0 | 4.57 | 1.95 |
| Acetate | 7.5 | 4.59 | 2.12 |
| Acetate | 10.0 | 4.57 | 2.33 |
| Acetate | 2.5 | 5.04 | 1.54 |
| Acetate | 5.0 | 5.04 | 1.82 |
| Acetate | 7.5 | 5.04 | 2.00 |
| Acetate | 10.0 | 5.05 | 2.48 |
| Phosphate | 2.5 | 6.00 | 1.53 |
| Phosphate | 5.0 | 6.00 | 2.30 |
| Phosphate | 7.5 | 6.00 | 3.37 |
| Phosphate | 10.0 | 6.00 | 4.20 |
| Phosphate | 2.5 | 6.50 | 0.98 |
| Phosphate | 5.0 | 6.50 | 1.72 |
| Phosphate | 7.5 | 6.50 | 2.55 |
| Phosphate | 10.0 | 6.50 | 3.02 |
| Tris | 2.5 | 8.30 | 0.0241 |
| Tris | 5.0 | 8.30 | 0.0372 |
| Tris | 7.5 | 8.30 | 0.0618 |
| Tris | 10.0 | 8.30 | 0.0820 |

first-order rate constants for water-catalyzed or spontaneous hydrolysis of the neutral and monoanionic phosphate species, respectively. The constants $K_{a1}$ and $K_{a2}$ represent the first and second dissociation constants of the phosphate group of 1.

The pH–rate profile in Fig. 1 could be adequately described by fitted values for $k_H$ of 7.55 × $10^{-2}$ $M^{-1}$ $sec^{-1}$, $k_o$ of 1.28 × $10^{-3}$ $sec^{-1}$, $k'_o$ of 1.46 × $10^{-4}$ $sec^{-1}$, $K_{a1}$ of 8.26 × $10^{-3}$ ($pK_{a1}$ 2.08), and $K_{a2}$ of 1.43 × $10^{-6}$ ($pK_{a2}$ 5.84). A comparison of the rate constant for the hydronium ion-catalyzed hydrolysis of the neutral species of 1, $k_H$, with that for the hydronium ion-catalyzed ketonization of the enol of acetophenone at 25°C (9), 1 × $10^3$ $M^{-1}$ $sec^{-1}$, reveals that phosphorylation stabilizes, as expected, the enol form.

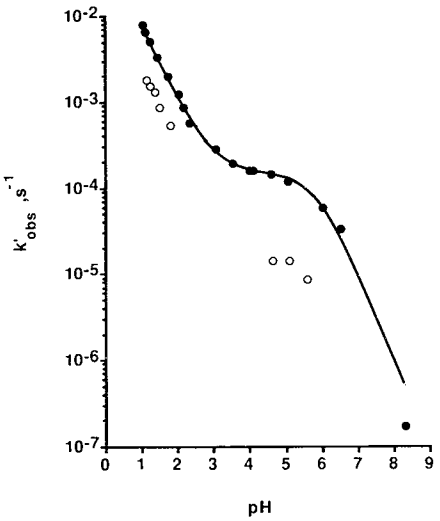



Fig. 1. The dependence of the buffer-independent rate constants for the hydrolysis of 1 on the pH (●) in $H_2O$ and on the pD (○) in $D_2O$ at 25°C and $\mu$ = 0.5 $M$. The solid curve represents the theoretical pH-rate profile.

The reactions defined by $k_o$ and $k'_o$ are kinetically equivalent to hydronium ion-catalyzed hydrolysis of the mono- and dianions of 1. If this were the mechanism, then the reactions would be described by Eq. (2),

$$k'_{obs} = \frac{k_H[H^+]^3 + k'_H K_{a1}[H^+]^2 + k''_H K_{a2} K_{a1}[H^+]}{[H^+]^2 + [H^+]K_{a1} + K_{a1}K_{a2}} \quad (2)$$

where $k'_H$ and $k''_H$ are the hydronium ion-catalyzed rate constants for the mono- and dianionic phosphate, respectively. The constant $k'_H$ is numerically equivalent to $k_o/K_{a1}$ and $k''_H$ is equivalent to $k'_o/K_{a2}$. Values of 1.55 × $10^{-1}$ $M^{-1}$ $sec^{-1}$ for $k'_H$ and 1.02 × $10^2$ $M^{-1}$ $sec^{-1}$ for $k''_H$ adequately describe the pH–rate profile.

In dilute DCl solutions, the observed first-order rate constants, corrected for the fraction of 1 present as the neutral species ($f_{ne}$), were found to be proportional to the DCl concentration (Table II). Therefore, the hydrolytic mechanism occurring in this region of the pD–rate profile was second order overall. Linear regression analysis of this kinetic data gave a value for $k_D$ of 2.36 × $10^{-2}$ $M^{-1}$ $sec^{-1}$, resulting

Table II. Rates of Hydrolysis of 1 in Dilute Acid (DCl) Solutions at 25°C and $\mu$ = 0.5 $M$ (NaCl)[a]

| $f_{ne}$ | $10^2$ [acid] ($M$) | $10^3$ $k_{obs}$ ($sec^{-1}$) |
|---|---|---|
| | $D_2O$ | |
| 0.93 | 7.2 | 1.85 (±0.09) |
| 0.92 | 5.8 | 1.56 (±0.07) |
| 0.89 | 4.3 | 1.32 (±0.07) |
| 0.85 | 2.9 | 0.884 (±0.026) |
| 0.75 | 1.5 | 0.532 (±0.028) |

$10^3$ $k_{obs}$ = (23.6 ± 1.6) × $f_{ne}$ × [DCl] + (0.312 ± 0.070)

[a] The error limits are standard errors and $f_{ne}$ represents the fraction of 1 existing as the neutral species at the given acid concentration.

in an apparent, kinetic, solvent deuterium isotope effect, $k_H/k_D$, of 3.20.

In the above relationship, the fraction, $f_{ne}$, was approximately by Eq. (3),

$$f_{ne} = \frac{[H^+]}{[H^+] + K_{a1}} \quad (3)$$

In $D_2O$, the fractions were calculated with a $K_{a1}$ of $5.1 \times 10^{-3}$ ($pK_a$ 2.29). This value was used for the $K_{a1}$ in $D_2O$ based on the assumption that the ionization behavior of 1 is comparable to that of phosphoric acid. For the first ionization constant of phosphoric acid, $\Delta pK_a$, the difference between the $pK_a$ value found in $D_2O$ and that found in $H_2O$, was determined to be approximately 0.21 (10). Hence, since in $H_2O$ the $pK_{a1}$ was found to be 2.08, then, by analogy, the value in $D_2O$ should be 2.29.

Determination of the apparent rate constant for water-catalyzed or spontaneous hydrolysis of the neutral or monoanionic species, defined by $k_o$ and $k'_o$, in $D_2O$ was not attempted since only a partial pD–rate profile was generated. However, a normal solvent deuterium isotope effect should be observed based on a qualitative comparison of the $k_{obs}$ values observed in the pH and pD regions of 4 to 6 (Fig. 1).

## Mechanism of Hydrolysis of 1

### *Hydronium Ion-Catalyzed Hydrolysis of Fully Protonated 1*

Of the phosphomonoesters which have been studied in the past, three distinct mechanisms have been postulated to account for a hydronium ion-catalyzed pathway involving the neutral species. Two of the mechanisms involved rate-limiting, nucleophilic attack by water occurring at either the phosphorus atom or the carbon atom of the ester linkage of the rapidly formed conjugate acid species (8). The third mechanism involved rate-determining, unimolecular, carbon–oxygen bond fission of the conjugate acid species, resulting in the formation of a rapidly hydrated carbonium ion intermediate. The latter mechanism has been observed with alkyl phosphates possessing carbonium ion-stabilizing substituents (e.g. benzyl-, isopropyl-, *t*-butyl-, and glucose 1-phosphate) (11,12).

The hydrolysis of 1 via a hydronium ion-catalyzed pathway differed from that of phosphomonoesters, which were hydrolyzed by the aforementioned bimolecular mechanisms in the following ways: (i) the rate was dramatically faster for 1 compared to other phosphomonoesters and (ii) a kinetic solvent deuterium isotope effect ($k_H/k_D$) of 3.20 was observed for 1, which was inconsistent with the bimolecular mechanisms and the unimolecular mechanism (11).

A comparison of the rates of hydrolysis in $H_2O$ versus $D_2O$ is a useful mechanistic probe to elucidate if a proton is being transferred in the rate-limiting step (13,14). An inverse solvent isotope effect, $k_H/k_D < 1$, is consistent with a non-rate-determining proton transfer from $H_3O^+$ (not $H_2O$) before, not after, the rate-limiting step. A normal solvent isotope effect, typically in the range of 2–4, is indicative of rate-determining proton transfer from the solvent or the acidic species to the reactant. The typical mechanisms for phosphate ester hydrolysis are catalyzed by the hydronium ion in a preequilibrium proton-transfer step; therefore, these reactions normally display inverse kinetic solvent isotope effects. For example, the hydronium ion-catalyzed hydrolysis of the phosphomonoesters of methanol, which was postulated to hydrolyze by the bimolecular mechanism, and *t*-butanol which was postulated to hydrolyze by the unimolecular mechanism, had $k_H/k_D$ values of 0.83 and 0.56, respectively (11).

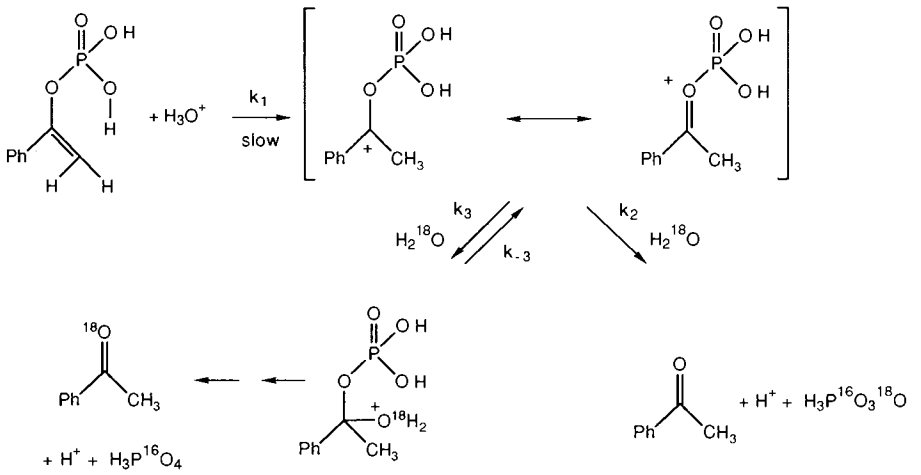
The unimolecular mechanism proceeding through a carbocation can also be ruled out by a comparison of the hydrolysis rates of benzyl phosphate and 1. Since the benzyl cation has been found to be considerably more stable than the 1-phenylvinyl cation (15), the hydrolysis of benzyl phosphate by the unimolecular mechanism should be significantly faster than the hydrolysis of 1 by the same mechanism. However, the hydronium ion-catalyzed rate constant for benzyl phosphate hydrolysis was $8.70 \times 10^{-5}\ M^{-1}\ sec^{-1}$ at 75.6°C and $\mu = 1.0$ (16), whereas the mechanistically comparable rate constant for 1 was $7.55\ M^{-1}\ sec^{-1}$ at 25.0°C and $\mu = 0.5$.

When the complete hydrolysis of 1 was carried out in $D_2O$ at pD 1.2, only one deuterium was incorporated into the methyl group of the acetophenone product, as evidenced by integration of the $^1H$-NMR spectrum. A control experiment conducted with acetophenone alone under the same conditions revealed no deuterium exchange into the methyl group. These findings implied that electrophilic addition of a proton to 1 was rate-limiting and nonreversible.

A mechanism consistent with all these experimental findings is rate-controlling and nonreversible protonation of the β-carbon of the olefinic bond by hydronium ion, resulting in the transient formation of a carbonium ion intermediate, which would be resonance stabilized through an oxonium ion (Scheme I). Further reaction of this unstable intermediate, leading to the formation of the parent compound, acetophenone, and inorganic phosphate, would follow. A similar mechanism has been proposed for the hydronium ion-catalyzed hydrolysis of enol phosphates where the phosphate group is fully esterified (17) and for the hydrolysis of vinyl ethers (18,19).

Breakdown to the phosphoryloxycarbocation may proceed via attack of water at the α-carbon atom to form a hemiacetal ($k_3$) or attack of water at the phosphorus atom ($k_2$). Both pathways would lead to the formation of the desired products acetophenone and inorganic phosphate. The use of an $^{18}O$-labeling study in combination with $^{31}P$-NMR or $^{13}C$-NMR spectroscopy is a useful mechanistic probe for substitution or exchange of an $^{18}O$ for $^{16}O$ in phosphorus and carbon containing compounds (20). Replacement of an $^{16}O$ in phosphate with an $^{18}O$ results in an upfield shift of about 0.02 ppm in the $^{31}P$-NMR spectrum (21). Replacing the $^{16}O$ in *t*-butyl alcohol with an $^{18}O$ results in an upfield shift of about 0.035 ppm (22). This technique was used to elucidate the site(s) of hydration and, ultimately, the site(s) of bond cleavage for the hydronium ion-catalyzed hydrolysis of 1.

The hydrolysis of 1 at pH 1.0 was examined in $H_2{}^{18}O$. One of the products of hydrolysis, inorganic phosphate, was analyzed for its isotopic composition by measuring the $^{18}O$-isotope-induced shift in the $^{31}P$-NMR spectra. As can be seen in Fig. 2A, the $^{31}P$-NMR spectrum showed the presence



Scheme I. Proposed scheme for the hydronium ion-catalyzed hydrolysis of 1 via carbon–oxygen and/or phosphorus–oxygen bond fission.

of a mixture with some $^{18}O$ incorporation in the formed inorganic phosphate along with some fully [$^{16}O$]phosphate. The isotope-induced shift was 0.015 ppm upfield. Under similar reaction conditions, no solvent exchange occurred when inorganic phosphate was used in place of 1. These observations were consistent with nucleophilic attack by water at the phosphorus atom and at the α-carbon atom with concurrent P–O and C–O bond scission.

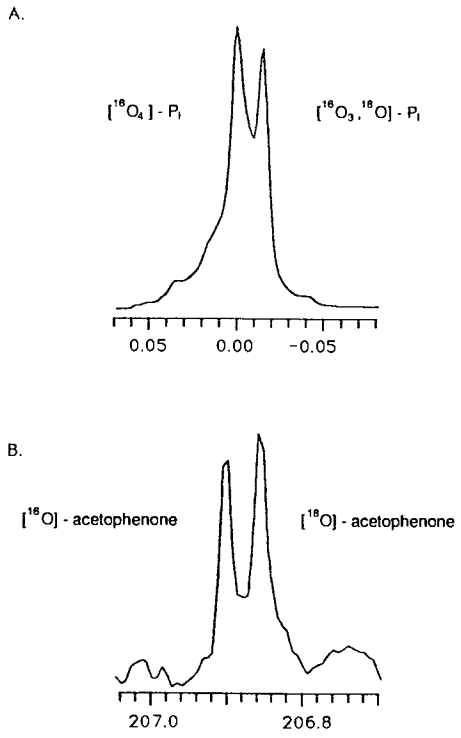



Fig. 2. (A) The proton-decoupled $^{31}P$-NMR spectrum of the inorganic phosphate, $P_i$, formed upon complete hydrolysis of 1 in pH 1.0 $H_2{}^{18}O$ (arbitrarily set at 0 ppm). Spectrometer conditions included the following: sweep width, 6000 Hz; pulse width, 17.3 μsec; tip angle, 26°; number of transients, 80; $T$ = 20°C. (B) The proton-decoupled $^{13}C$-NMR spectrum of acetophenone in pH 1.0 $H_2{}^{18}O$. Spectrometer conditions included the following: sweep width, 2400 Hz; pulse width, 3.5 μsec; tip angle, 18.5°; number of transients, 30,000; $T$ = 20°C.

Similar attempts using $^{13}C$-NMR spectroscopy to follow the incorporation of the $^{18}O$ label into acetophenone under the reaction conditions failed because of rapid incorporation, probably due to solvent exchange, of the $^{18}O$ label into acetophenone formed upon hydrolysis of 1 (Fig. 2B).

The site of bond cleavage and hydration has been determined for other enol phosphates. The acid-catalyzed hydrolysis of diethyl 1-phenylvinyl phosphate was shown to proceed by attack of water at the carbon atom (17) [although it was originally believed that attack occurred at the phosphorus atom of dialkyl vinyl phosphates (23)]. In contrast, hydrolysis of phosphoenolpyruvate was shown to proceed with P–O bond fission over the pH range of 1.3 to 5.5 (5), consistent with attack of water at phosphorus.

Another mechanistic possibility in the hydrolysis of 1 was the potential for hydronium ion-catalyzed, rate-limiting proton transfer via intramolecular general acid catalysis. A rapid exchange of an acidic phosphoryl proton with the deuterated solvent may occur, followed by rate-controlling, intramolecular transfer of the exchanged deuterium from the phosphoryl moiety to the carbon–carbon double bond of the conjugate acid species. This transfer would proceed through an energetically favorable six-membered ring transition state. Such a transition state has been previously proposed for the hydrolysis of phosphoenolpyruvate and ethyl phosphoenolpyruvate (5). The second-order rate constant for the hydronium ion-catalyzed hydrolysis of diethyl 1-phenylvinyl phosphate was $1.31 \times 10^{-5}\ M^{-1}\ sec^{-1}$ at 25°C (24), whereas the analogous rate constant for the hydrolysis of 1, the corresponding dihydrogen ester, was $7.55 \times 10^{-2}\ M^{-1}\ sec^{-1}$ at 25°C. Hence, the rate was about $5.8 \times 10^3$ times more rapid when the phosphoryl group contained protons instead of ethyl groups. This comparison supports the possibility of the intramolecular mechanism.

### *Hydrolysis of the Monoanionic Species*

Buffer systems were employed to maintain the pH (or pD) value constant over the pH range of 3 to 8.3. Unlike

most alkyl and aryl phosphomonoesters, buffers had a pronounced catalytic effect on the observed rates of hydrolysis of **1** in $H_2O$ or $D_2O$. The hydrolysis rates increased as the buffer concentrations were increased while maintaining a constant pH, temperature, and ionic strength. The first-order, buffer-independent rate constants, $k'_{obs}$, and the second-order, buffer-dependent rate constants, $k_{buff}$, were determined from the intercepts and slopes by linear regression analysis of plots of $k_{obs}$ versus total buffer concentration, respectively. The values for $k'_{obs}$ and $k_{buff}$ are listed as a function of pH (or pD) in Table III.

Due to the complexity of the reaction, multiple ionic species of substrate and buffer, and limited number of $k_{buff}$ values determined for a given buffer, no attempt was made to isolate the individual, apparent general base- and general acid-catalyzed rate constants. For example, the data in Table III indicate that formate and acetate anions are better catalysts than the corresponding acids, suggesting that general base catalysis might be operating, while dihydrogen phosphate is a better catalyst than monohydrogen phosphate, suggesting that the reaction is subject to general acid catalysis. It should be noted, however, that general base catalysis of the neural phosphate species is kinetically equivalent to general acid catalysis of the monoanionic species.

Unlike other alkyl and aryl phosphomonoesters, the rate of hydrolysis of the monoanion of **1** is not predicted by the established Brønsted relationship defined by Eq. (4) (25),

$$\log k'_o = 0.91 - 0.27\ pK_a \qquad (4)$$

where the $pK_a$ value is a measure of the acidity of the corresponding alcoholic or phenolic leaving group. This assumes that the reaction is defined adequately to be $k'_o$ rather than the kinetically equivalent reaction, acid-catalyzed breakdown of the dianion. The equilibrium constant for the ionization of the enol of acetophenone as the oxygen acid, $K_a^E$, was found to be $4.57 \times 10^{-11}$ ($pK_a^E$ of 10.34) (26). Using this value and Eq. (4), the predicted rate constant for hydrolysis of the monoanionic species of **1**, by the normal phosphate monoester cleavage mechanism, was $2.18 \times 10^{-4}$ $sec^{-1}$ at 100°C. In contrast, the experimentally determined rate constant for hydrolysis of the monoanion of **1** was $1.46 \times 10^{-4}$ $sec^{-1}$ at 25°C; therefore, the latter rate should be significantly greater than the predicted value when determined at 100°C. A positive deviation from a Brønsted relationship is consistent with a change in the reaction mechanism. A similar deviation from the linear free energy relationship of Eq. (4) was seen with the hydrolysis of the monoanions of phosphoenolpyruvate and ethyl phosphoenolpyruvate (5).

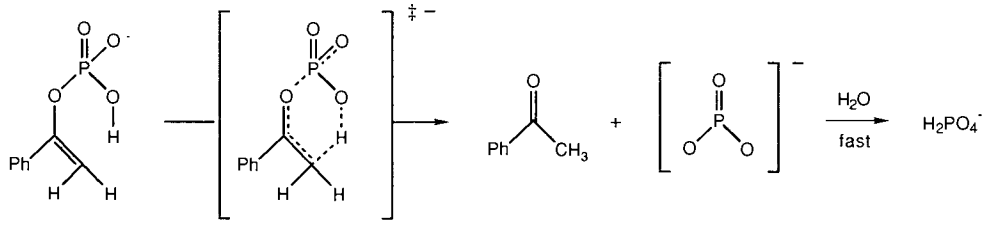
In the pH regions where the monoanionic species of **1** predominated, a hydrolytic mechanism similar to that shown in Scheme I can be proposed. For the monoanionic species, as in the case of the neutral species, there is the possibility for intramolecular, general acid catalysis proceeding via a six-membered ring transition state with transfer of the lone phosphoryl proton to the β-carbon of the olefinic bond. This possibility is difficult to reconcile when the relative values of $k_H$, $k'_H$, and $k''_H$ are compared. Alternatively, the rate-limiting proton-transfer step can occur via a bimolecular route with transfer of a proton from the acidic component of the buffer system or from the solvent to the double bond. The former route would be consistent with the observation of strong buffer catalysis for the reaction and a greater-than-unity kinetic solvent isotope effect.

The controversy associated with the mechanism of hydrolysis of the monoanionic and dianionic species of typical alkyl and aryl phosphomonoesters, whether the reaction proceeds through an associative or a dissociative transition state, could also pertain to the mechanism of hydrolysis of unesterified enol phosphates. In this case, the rate-determining proton-transfer step could be followed by either hydration of the carbonium ion intermediate (Scheme I) or unimolecular decomposition of this intermediate, resulting in the formation of the monomeric metaphosphate anion (Scheme II). With this mechanism, the apparent positive deviation from the established Brønsted relationship cannot be explained by a change in mechanism. However, the deviation can be reasonably accounted for by the leaving group having a lower "effective" $pK_a$ value. If the proton-transfer step occurs concomitantly with expulsion of the leaving group (Scheme II), the acidity of the leaving group would be increased in the transition state (27), thereby enhancing the

**Table III.** The Effect of pH on the Buffer-Independent Rate Constant, $k'_{obs}$, and on the Buffer-Catalyzed Rate Constant, $k_{buff}$, for Hydrolysis of **1** at 25°C and $\mu = 0.5\ M$[a]

| Buffer | pH | pD | $k'_{obs} \times 10^6$ ($sec^{-1}$) | $k_{buff} \times 10^4$ ($M^{-1}\ s^{-1}$) |
|---|---|---|---|---|
| Formate | 3.05 | | 287 (±0.04) | 3.00 (±0.58) |
| Formate | 3.52 | | 193 (±0.09) | 8.87 (±1.37) |
| Formate | 4.00 | | 161 (±0.11) | 10.5 (±1.5) |
| Acetate | 4.10 | | 160 (±0.02) | 4.27 (±0.38) |
| Acetate | 4.57 | | 143 (±0.06) | 9.23 (±0.94) |
| Acetate | 5.04 | | 121 (±0.12) | 12.0 (±1.7) |
| Acetate | | 4.60 | 14.4 (±0.004) | 0.90 (±0.06) |
| Acetate | | 5.07 | 14.3 (±0.004) | 1.17 (±0.06) |
| Acetate | | 5.57 | 8.60 (±0.007) | 1.97 (±0.10) |
| Phosphate | 6.00 | | 58.2 (±0.107) | 36.3 (±1.6) |
| Phosphate | 6.50 | | 33.0 (±0.147) | 27.8 (±2.2) |
| Tris | 8.30 | | 0.175 (±0.440) | 0.793 (±0.064) |

[a] The error limits are standard errors.



Scheme II. Proposed scheme for the hydrolysis of the monoanion of 1 via a monomeric metaphosphate intermediate.

rate of hydrolysis. Such a mechanism has been previously proposed with the hydrolysis of phosphoenolpyruvate (5) and the monoanion of acyl phosphates (28).

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