VOLUME 115, NUMBER 23
NOVEMBER 17, 1993

JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Rate-Determining Carbonyl Hydration in the Intramolecular Hydrolysis of Phenacyl Phosphonate Esters: Isotopic Probes and Activation Parameters

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Received June 21, 1993[⊗]

Abstract: 4-Nitrophenyl 4-substituted-phenacyl methylphosphonate esters (CH_3, H, CH_3O, NO_2, and Cl) decompose in aqueous buffers 1–4 orders of magnitude faster than analogs with alkoxy substituents not containing a β-carbonyl group. This is consistent with an intramolecular displacement of 4-nitrophenol by the anion of the carbonyl hydrate. ^{18}O incorporation into the hydrolysis product of the 4-CH_3 derivative from $H_2{}^{18}O$ in alkaline solution is 50%. This indicates a rapid protonic equilibrium between the carbonyl hydrate and the medium but slower equilibration of the carbonyl O with the medium than cyclization. General base-catalyzed water attack in formation of the carbonyl hydrate is rate determining followed by cyclization with a rate constant $>10^6$ s^{-1}. Supportive evidence is provided by buffer dependence, small normal solvent isotope effects, effect of 4-substituents in the phenacyl group ($\rho = 1.83 \pm 0.15$), and activation parameters $\Delta H^{\ddagger} = 49 \pm 2$ kJ/mol and $\Delta S^{\ddagger} = -118 \pm 6$ J/(mol K) for the phosphate dianion-catalyzed reaction and $\Delta H^{\ddagger} = 21.5 \pm 0.8$ kJ/mol and $\Delta S^{\ddagger} = 183 \pm 3$ J/(mol K) for the Tris base-catalyzed reaction. Inverse dependence of the rate constants for the phosphate dianion-catalyzed reaction on increasing ionic strength and solvent polarity also support the proposed mechanism. The solvent isotope effect for nucleophilic addition of increasing concentrations of lyoxide ion decreases from ~2.0 to 0.9. The normal effect may indicate significant solvent restructuring as the negative charge is redistributed at the transition state in dilute solutions of lyoxide ion. The activation parameters for the reaction of hydroxide ion are $\Delta H^{\ddagger} = 35.9 \pm 0.1$ kJ/mol and $\Delta S^{\ddagger} = -59.8 \pm 0.4$ J/(mol K). The compounds have a built-in trap for the carbonyl hydrate formed with instantaneous release of a signal molecule, 4-nitrophenol, and thus are ideally suited for the measurement of hydration rates of aryl alkyl ketones.

Neighboring group participation in displacement reactions at carbonyl[1,2] and phosphoryl[2a3–6] centers in the condensed phase has attracted much attention in the past. Reactions in a subcategory that occur with the intervention of a five-membered ring intermediate are of particular interest in biochemistry and synthetic chemistry. Among these are the decompositions in

⊗ Abstract published in *Advance ACS Abstracts.* October 15, 1993.

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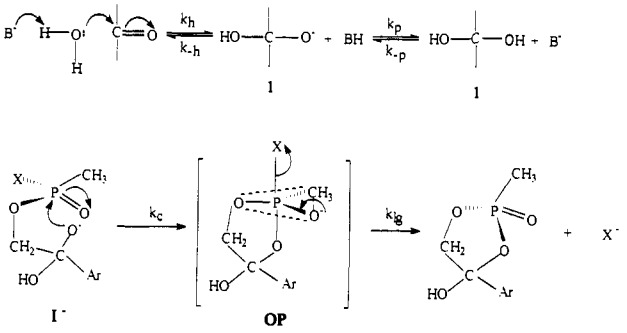
Scheme I



solution of phosphonates with β-keto[3] and β-hydroxy[4] in the alkoxy fragment and phosphates containing a β-keto group,[5,6] which have been reported to occur via intramolecular formation of a five-membered ring intermediate. Scheme I describes the initial phases of the reaction of nucleophiles, in general, with phosphonate or phosphate esters containing a β-keto group in an alkoxy ligand: hydration of the ketone, the related protonic equilibria, and a stepwise cyclization process from the carbonyl hydrate anion [I⁻] with the intervention of an oxyphosphorane [OP].

Substantial evidence has lately been provided for the mechanism of intramolecular catalysis via oxyphosphorane formation from the carbonyl hydrate of acetoin diethyl phosphate and its derivatives.[6] Results of these investigations discount the occurrence of intramolecular catalysis by intermediate enol formation or other previously suggested mechanisms.

The rate of cyclization of the adduct for acetoin diethyl phosphates was two- to multifold faster than the rate of hydroxide ion elimination from the carbonyl hydrate adduct, which was estimated to be 10^5 s^{-1}.[6] Thus, later events in the course of the reaction seem to determine the rate.

Nucleophilic addition to phosphoryl, in general, presents a greater variety of rearrangements along the reaction coordinate than does nucleophilic addition to carbonyl, due to the extra ligand and the potential participation of d-orbitals in accepting the electron pair.[7,8] Thus, rapid interconversion of permutational isomers of the pentavalent intermediates formed along the reaction coordinate in nucleophilic additions to phosphoryl, pseudorotation, is also observed if the electronic properties of the ligands are not too different, as in phosphate esters. This phenomenon was in fact observed and used to explain the product distribution in the intramolecular catalytic decomposition of acetoin diethyl phosphate and its derivatives.[6]

Phosphonate esters, however, are markedly different from phosphates in their electronic makeup, and the preference for orientation of the ligands in the pentacoordinate intermediates of phosphonates is more restrictive than that of phosphates. In cyclic oxyphosphoranes, where the ring oxygens have to span an axial and an equatorial position and the alkyl substituent of P prefers the equatorial position, the totality of restrictions greatly reduces the possibility for pseudorotation.[5,8] This is discernable from Scheme I. The contention is also supported by a completely different product distribution for the hydrolysis of analogous phosphate and phosphonate esters of acetoin.[5]

The phosphonate esters of our interest are 4-nitrophenyl 4-substituted-phenacyl methylphosphonates (PMNs) with a much better leaving group than ethoxyl and a keto group with an aromatic substituent. Since these compounds are potentially good candidates for reversible modification of serine hydrolase activity,[9] we investigated the molecular mechanisms of their nonenzymic hydrolysis. Here we wish to report that the extent of ^{18}O incorporation into product from the medium, activation parameters, substituent effects, buffer catalysis, solvent isotope effects, and medium effects all support a predominantly rate-determining nucleophilic attack at carbonyl followed by extremely rapid intramolecular displacement of 4-nitrophenolate, at $>10^6$ s^{-1}, from the phosphonate esters.

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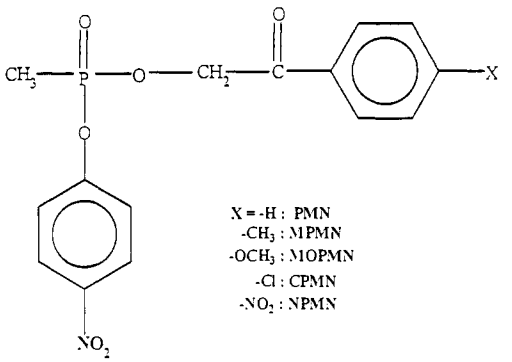
(9) (a) Kovach, I. M.; McKay, L. *BioMed. Chem. Lett.* **1992**, *2*, 1735–1740. (b) Zhao, Q.; Kovach, I. M.; Papathanassiu, A. *Biochemistry*, to be submitted.

Table I. Second-Order Rate Constants for the Hydrolysis of MPMN and of IMN[11] for Comparison at μ = 1.0 M (KCl) and 25.0 °C

	k, M^{-1} s^{-1}		
catalyst	MPMN	IMN	acceleration factor
phosphate	0.0131 ± 0.0016	2.65×10^{-5}	490
Hepes	0.09 ± 0.01		
borate	0.35 ± 0.03		
Tris	1.1 ± 0.3		
hydroxide	2593 ± 20	0.27	9600
water	(1.8 ± 0.5) × 10^{-6}	1.0×10^{-7}	18



Results

Kinetic Measurements. All reactions in this study obeyed good pseudo-first-order kinetics through 6 half-lives, and the release of 1 stoichiometric equiv of 4-nitrophenol was observed at 400 nm. Pseudo-first-order rate constants obtained in buffer systems can be described in general by buffer-dependent and buffer-independent components:

$$k_{obs} = k_{HOH}[H_2O] + k_{OH}[OH^-] + k_b[buffer] \quad (1)$$

Rate constants obtained from data reduction of the observed hydrolytic rate constants measured in phosphate, *N*-(2-hydroxyethyl)-*N'*-piperazine-2-ethanesulfonic acid (Hepes), tris(hydroxymethyl)aminomethane (Tris), borate, and carbonate buffers and in NaOH solutions at 25.00 ± 0.05 °C for 4-nitrophenyl 4-methylphenacyl methylphosphonate (MPMN) are listed in Tables IS–VS provided in the supplementary material. From a linear fit of the buffer-dependent rate constants to the fraction of base, the second-order rate constants for buffer catalysis, tabulated in Table I, were calculated. The catalytic contribution of phosphate monoanion as calculated from the intercept of the dependence on the fraction of phosphate dianion was found to be negligible. Figure 1 illustrates the pH dependence of the buffer-independent term of the observed rate constants ($k_0 = k_{HOH}[H_2O] + k_{OH}[HO^-]$) up to pH 9.3 and above that the rate constants obtained in carbonate buffer and in sodium hydroxide solutions. Buffer catalysis by carbonate and bicarbonate ions was insignificant. Data above pH 9.3 were obtained by fast reaction kinetic techniques (Table VS). The second-order rate constant for hydroxide ion-catalyzed hydrolysis of MPMN was calculated to be 2600 ± 30 M^{-1} s^{-1} (Table I), from a fit of the buffer-independent rate constants to the first two terms of eq 1. Solvent isotope effects at four different pH values for the hydrolysis of MPMN are listed in Table II. Data for the dependence of the rate of hydrolysis of MPMN on ionic strength are given in Table VIS

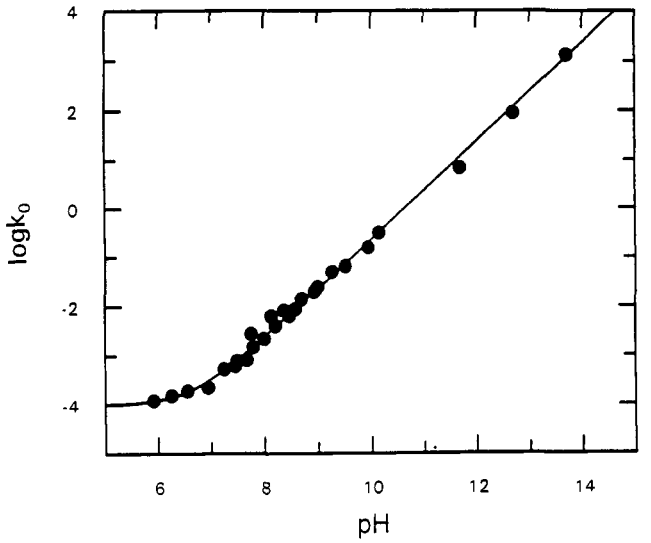


Figure 1. Log(rate)–pH profile for buffer independent hydrolysis of MPMN at 25.00 ± 0.05 °C. The line is the calculated least squares line.

Table II. Solvent Isotope Effects for the Hydrolysis of MPMN at μ = 1.0 M (KCl) and 25.0 °C

pH	buffer	k_0[a](HOH)/k_0(DOD)	k_b(HOH)/k_b(DOD)
7.45	phosphate	2.0 ± 0.3	2.1 ± 0.1
8.00	Hepes	1.8 ± 0.1	1.31 ± 0.04
8.93	borate	2.1 ± 0.1	1.3 ± 0.1
9.25	Tris	2.1 ± 0.1	1.7 ± 0.1
11.70	hydroxide	0.85 ± 0.02	
12.70	hydroxide	0.97 ± 0.02	
13.70	hydroxide	0.98 ± 0.17	

[a] k_0 is the intercept value of buffer plots.

Table III. Activation Parameters for the Hydrolysis of MPMN at μ = 1.0 M (KCl)

catalyst	$\Delta H^{\ddagger}$, kJ/mol	$\Delta S^{\ddagger}$, J/(mol K)
hydroxide ion	35.9 ± 0.1	−59.8 ± 0.4
phosphate dianion	49 ± 2	−118 ± 6
Tris base	21.4 ± 0.8	−183 ± 3

in the supplementary material. Temperature dependence of the second-order rate constants for the reaction of hydroxide ion, Tris base, and phosphate dianion (Tables VIIS–VIIIS in the supplementary material) was evaluated by data fitting to the Eyring equation (2), and the activation parameters calculated

$$k_{obs} = kT/h(\exp -\Delta H^{\ddagger}/RT)(\exp \Delta S^{\ddagger}/R) \qquad (2)$$

are tabulated in Table III. Figure 2 gives a representative example of an Eyring plot. Observed hydrolytic rate constants for five derivatives of 4-nitrophenyl 4-substituted-phenacyl methylphosphonates, H, CH_3, CH_3O, NO_2, and Cl, at pH 7.75 are tabulated in Table IV.

Isotopic Labeling. Both hydroxide ion-catalyzed and general base-catalyzed reactions of unlabeled MPMN were carried out in 28 or 78% $H_2{}^{18}O$-containing water, and the phosphonate hemiester product was analyzed for the extent of ^{18}O incorporation into P by observing the ^{18}O shift on the ^{31}P NMR signal.[10] At pH values 8.4, 8.8, 9.2, and 10.0 in carbonate buffers and at 13.4 in sodium hydroxide solutions, duplicate or triplicate experiments gave 50 ± 5% incorporation of label from the medium into P. The level of ^{18}O incoroporation was also 50% under general base-catalyzed conditions in Tris and Hepes buffers.

Discussion

All results of this study are completely consistent with the intramolecular displacement of 4-nitrophenol from the PMNs,

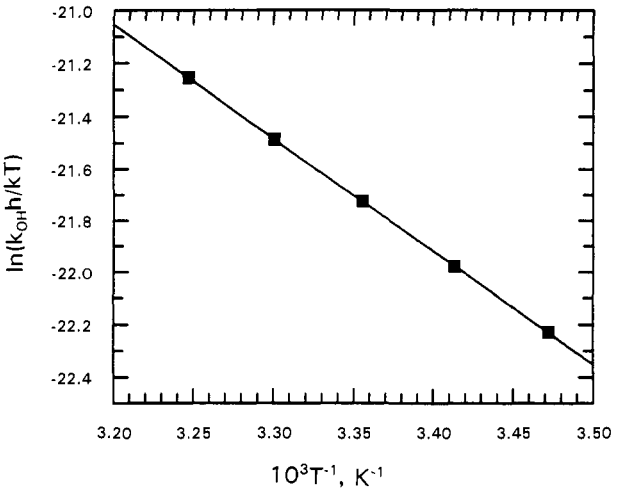


Figure 2. Eyring plot for the hydroxide ion-catalyzed hydrolysis of MPMN at μ = 1.0 M. The line was calculated by least squares.

Table IV. First-Order Rate Constants for the Hydrolysis of 4-Substituted PMNs in pH 7.75, 0.10 M Phosphate Buffer at μ = 0.3 M (KCl) and 25.00 ± 0.05 °C

4-X	10^3k_{obs}, s^{-1}	4-X	10^3k_{obs}, s^{-1}
H	6.40 ± 0.59	Cl	16.8 ± 0.5
CH_3	3.17 ± 0.27	NO_2	145 ± 13
OCH_3	2.18 ± 0.02		

because we observed (1) stoichiometric release of 4-nitrophenol and (2) several hundredfold faster hydrolysis than that for alkoxy-substituted phosphonate analogs. Table I shows a comparison of the second-order rate constants for the reaction of nucleophiles with MPMN to those of a close analog, 4-nitrophenyl 2-propyl methylphosphonate (IMN).[11] The reaction of hydroxide ion with MPMN is 9600 times faster than nucleophilic attack at P in IMN. Water attack on MPMN is only 18 times faster than that of P in IMN. The difference in susceptibility to hydroxide ion attack vs water attack is generally 100 times greater for carboxylic centers than for phosphoryl centers.[4] For the hydrolysis of MPMN the susceptibility to hydroxide ion vs water attack is ~500 times greater than that for IMN, supporting rate-determining nucleophilic attack at C rather than at P. The phosphate dianion-catalyzed water addition to MPMN is 490 times faster than nucleophilic attack at P in IMN by the phosphate dianion. Thus the acceleration factor is related to the basicity of the catalyst, which bears on the polarity of the incipient transition state.

On the basis of Scheme I, the observed rate constants can be expressed in terms of the microscopic rate constants as follows:

$$k_{obs} = \frac{k_c k_h[B]}{k_{-h} + k_c} \qquad (3)$$

Water, phosphate dianion, Hepes, Tris, borate, and hydroxide ion all catalyze the hydrolysis of MPMN, which may occur in the addition of water to the keto group.[12-14] Unless the newly formed anion is trapped through cyclization, there would be time for protonic equilibration of the adduct formed. We estimate the

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(11) (a) Kovach, I. M.; Bennet, A. J.; Bibbs, J. A.; Zhao, Q. *J. Am. Chem. Soc.* **1993**, *115*, 5138. (b) The comparison of the hydrolytic rates of MPMN and IMN might be justified by the fact that the steric effect introduced by the methyl group in the γ-C from the reaction center reduces the rate by ~20% and the electron withdrawing effect of the keto group in the γ position causes only a minor rate acceleration.

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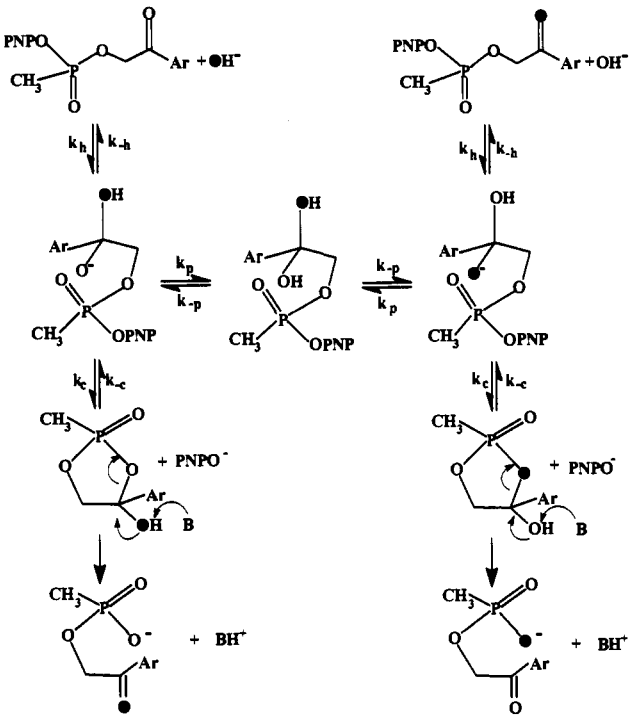
Scheme II



pK of the keto hydrate of the phenacyl compound to be 13.5. This value can be calculated both from the correlation of Hine and Koser,[15] pK = 14.19 − 1.32 (σ^*_{R1} + σ^*_{R2}) for the hydrates of aliphatic aldehydes and ketones, and from the pK of 10 reported[16] for trifluoroacetophenone hydrate using again ρ^*_{Ka} = 1.32 and σ^* = 2.58 for CF_3. Consequently, proton transfer from the bases used in this study to the anion of the carbonyl hydrate might occur at near the rate of diffusion. General base-catalyzed proton removal from the carbonyl hydrate, prior to or concurrent with attack on phosphoryl, should then also be considered, unless the cyclization step is comparable in rate to protonation of the anion of the carbonyl hydrate. Cyclization of the carbonyl hydrates of PMNs might be faster than that of acetoin phosphate derivatives (10^5 s^{-1})[6] but probably not exceeding the rate of proton transfer to the anion of the carbonyl hydrate.

Incorporation of ^{18}O from the Medium. The relative rates of formation and breakdown of the conjugate base of the carbonyl hydrate and the competing protonic equilibria were evaluated for MPMN from incorporation of ^{18}O into P in the course of hydroxide- and buffer-catalyzed reactions. It is discernable from Scheme II that three limiting results of the experiments can be expected for the intramolecular reaction: (1) If protonic equilibria are not rapid enough, the unlabeled O derived from carbonyl will be the attacking nucleophile at P, while the hydroxyl derived from the solvent remains protonated and no label will be incorporated into P. (2) If protonic equilibria are faster than covalent rearrangements, the labeled and unlabeled hydroxyl O will be unprotonated to the same extent and up to 50% label will incorporate into P. (3) If expulsion of hydroxide ion from the carbonyl hydrate adduct is faster than cyclization, then label incorporation into P will be greater than 50%, up to 100%, for complete equilibration of ^{18}O between carbonyl and the medium. *Direct nucleophilic attack of hydroxide at P*, either in MPMN or in the cyclic ester, *would also result in 100% incorporation of the label.*[11] In the reactions of MPMN with hydroxide ion, around 50% incorporation of label was observed, which reflects complete protonic equilibrium but faster cyclization of the conjugate base of the carbonyl hydrate than its collapse back to the keto form. Label incorporation into the product is the same for the Hepes- and Tris-catalyzed reactions. Carbonyl participation in the hydrolysis of carboxylic esters through the intervention of a four-membered ring intermediate also occurred with 50% label incorporation.[17]

Kluger and Taylor have lately provided evidence for the mechanism of intramolecular catalysis via oxyphosphorane formation from the carbonyl hydrate of acetoin diethyl phosphate and its derivatives.[6] Their ^{18}O-exchange experiments indicated that hydroxide ion expulsion from the adduct took place twice to multifold faster than oxyphosphorane formation. Then the rate-limiting process for these reactions is, most likely, cyclization and/or expulsion of the leaving group.

Because of the much greater leaving tendency of the 4-nitrophenol than that of the ethoxyl group and the greater susceptibility to nucleophilic attack of phosphonates than phosphates, we anticipated the rate-determining step(s) of the intramolecular reaction of MPMN to be in the initial phase of the reaction, namely, in formation or breakdown of the carbonyl hydrate intermediate rather than departure of the leaving group. Results of the ^{18}O-exchange experiments, the acceleration observed with respect to bimolecular reactions of phosphonate ester analogs of MPMN, and the buffer dependence of the hydrolytic rates are consistent with this proposition.

pH–Rate Profile. The pH–rate profile serves to probe the identity of the rate-determining elementary step. The rate of buffer-independent hydrolysis of MPMN increases with increasing hydroxide ion concentration in the phosphate buffer range, and at pH values above 8 the logarithm of the rate constants depends linearly on pH, with a slope of 1.01 ± 0.03. The reaction becomes too rapid at pH 10 for conventional measurements. Rate data obtained up to pH 13.7 with fast reaction kinetic techniques, however, fit exactly the log(rate)–pH profile without showing a plateau at the pK value of the carbonyl hydrate. *This finding negates the importance of proton removal from the neutral carbonyl hydrate in the rate-determining step* but indicates the continued involvement of hydroxide ion in the rate-determining process.

Hydroxide ion can then participate in rate-determining adduct formation as a nucleophile adding directly to carbonyl[6] or as a general base catalyst[12] in the removal of a proton from a water molecule adding to the carbonyl group. The exact role of hydroxide ion in reactions of this kind can be best determined by the use of isotope effects.

Solvent isotope effects were, therefore, measured at pH values 7.45, 8.00, 8.93, 9.25, 11.7, 12.7, and 13.7 to assess more precisely the role of lyoxide ions in the mechanisms of decomposition of MPMN. The solvent isotope effects were slightly inverse in sodium hydroxide (pH 11.7, 12.7, and 13.7) solutions as measured by stopped-flow techniques. These solvent isotope effects around 0.9 are similar to what has been observed for nucleophilic attack by lyoxide ion addition at P in IMN, a phosphonate ester analog of MPMN.[11] Since the reaction rates in this pH range are still about 2–3 orders of magnitude higher than rates of hydrolysis of 4-nitrophenyl alkyl methylphosphonates,[11] the intramolecular mode of decomposition of MPMN must be operative here too. Slightly inverse solvent isotope effects were also reported for carbonyl participation in the alkaline hydrolysis of methyl benzoate esters.[2d]

Inverse solvent isotope effects observed on lyoxide ion addition to carbonyl or phosphoryl compounds are customarily interpreted by the loss, at the transition state for nucleophilic attack, of some of the hydrate shell formed by three water molecules around the

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(16) Stewart, R.; Van der Linden, R. *Can. J. Chem.* **1960**, *38*, 400.
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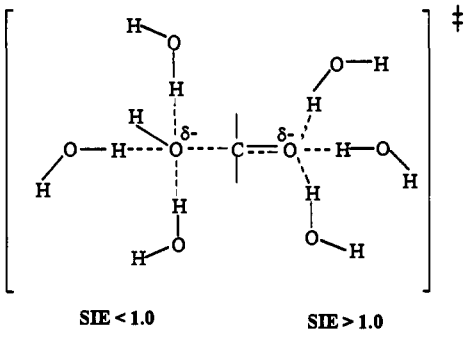
Scheme III



lyoxide ion.[11,18] However, we measured normal solvent isotope effects up to 2.0 for both general base catalysts from buffer salts and lyoxide ion at pH values below 10 (Table III). These could be indicative of base-catalyzed and partially rate-determining proton removal from water as nucleophile. Attack of a water molecule in the hydrate shell of lyoxide ion, which would act as a base catalyst, may occur. Similarly small normal solvent isotope effects were also reported by Bell[12b] for carbonyl hydration reactions in alkaline media. A completely different mode of catalysis by lyoxide ion below and above pH 10 would, however, most likely take place at different rates and thus would entail a break in the pH-rate profile. Since this is not the case, we would like to propose that the small normal solvent isotope effects observed for the reactions of lyoxide ion with MPMN arise from solvent reorganization resulting in an increase in the number of solvent hydrate at the transition state relative to the ground state for nucleophilic addition of lyoxide ion to carbonyl. Efficient protonic equilibrium between the two oxygens of the carbonyl hydrate adduct may be mediated by solvate water molecules. Scheme III illustrates a transition state with a less tightly bound solvate shell around the nucleophile as it bonds to carbonyl and tighter solvation of the developing negative charge at the anion of the carbonyl hydrate by proton-bridging water molecules. The scheme also accounts for enforcement of the expeditious protonic equilibrium between the two oxygens of the hydrate, which might be linked with a chain of hydrogen-bonding water molecules.[18a] At high lyoxide ion concentration, protonic solvation of the negative charge is lessened and the rate-determining process is dominated by the bond formation between lyoxide ion and carbonyl.

The enthalpies of activation for the reaction of MPMN were calculated to be 35.9 ± 0.1 kJ/mol for the buffer-independent, hydroxide ion-catalyzed reaction, 49 ± 2 kJ/mol for the phosphate dianion-catalyzed reaction, and 21.5 ± 0.8 kJ/mol for the Tris base-catalyzed reaction. **The entropies of activation** for the same processes were calculated to be −59.8 ± 0.4, −118 ± 6, and −183 ± 3 J/(mol K), respectively. Small enthalpic barriers and the quite large negative entropies, as observed for the hydroxide ion reaction, were considered excellent diagnostics for rate-determining carbonyl hydration.[2] Termolecular general base-catalyzed water attacks at carbonyl entail even more negative activation entropies.

Ionic strength dependence at pH 7.45 of the rate of, particularly phosphate-catalyzed, hydrolysis of MPMN is inverse, indicative of diminishing polarity from reactant state to transition state. This is to be expected if an anionic reactant interacts with an electrophile and dissipates the charge into the empty (p or d) orbitals at the transition state. The dependence of the reaction on cosolvents also supports this observation, since reduction of polarity of the medium enhanced the rate.[2,14]

Hammett Correlation. Rate-determining carbonyl hydration is expected to be more sensitive to electronic effects in the 4-substituted phenacyl derivatives than if other elementary steps are involved in the rate limiting process. The logarithms of the rate constants in Table IV for the phosphate dianion-catalyzed hydrolysis for five 4-substituted phenacyl derivatives were fit to Hammett σ para-coefficients, and the ρ value obtained was 1.83 ± 0.15. This reaction constant is similar to what has been reported (ρ^* = 1.7) for the hydrolysis of acetoacetate esters that also undergo carbonyl hydrate-promoted decomposition.[17] Equilibrium addition of hydroxide ion to para-substituted benzaldehydes gives ρ = 2.24.[14d] The similarity of the reaction constants corroborates the proposition for a predominantly rate-limiting ketone hydration followed by rapid cyclization to oxyphosphorane.

Rate-Determining Carbonyl Hydration. This investigation clearly establishes the rate-determining attack of nucleophile at carbonyl in MPMN; thus, the rate equation (3) further simplifies to $k_{obs} \sim k_h[B]$, and the second-order rate constant for hydroxide ion addition to carbonyl, 2600 M^{-1} $s,^{-1}$ equals k_h. Similarly, the rate of general base-catalyzed water addition to carbonyl is given by k_{obs} under the appropriate conditions. This information provides an extremely valuable estimate for the rate of hydration of 4-substituted-phenyl methyl ketones, for which direct measurement of k_h is fraught with technical problems due to the unfavorable equilibrium. In fact, the PMNs are uniquely suited for the measurement of rates of carbonyl hydration because they have a built-in trap for the hydrate and provide a simultaneous release of a signal molecule, 4-nitrophenol.

True Rate Acceleration of Nucleophilic Attack at P. This information can also be obtained if the rate of cyclization, k_c, can be evaluated. Results of the labeling experiments imply that k_c is at least 5 times greater than k_{-h}. Then, on the basis of Scheme I, from the equilibrium hydration of 4-methylacetophenone (K_h ~ 10^{-3} M^{-1}),[19] the ionization constant estimated for the carbonyl hydrate ($K_a \sim 10^{-13.5}$) and k_h, the value of $k_{-h} \sim 10^6$ s^{-1} can be calculated. Consequently, k_c must be $>10^6$ s^{-1}. The true acceleration factor is then given by the ratio of k_c/k_2, where k_2 ~ 0.27 M^{-1} s^{-1} for hydroxide ion attack at P in IMN which was used for comparison earlier.[11] The acceleration factor is then $>10^7$ M, a staggering magnitude. By comparison, the rate of alkaline hydrolysis of dimethyl phosphoacetoin was reported to be ca. 2×10^6-fold over that of trimethyl phosphate.[5,20] Expulsion of 4-nitrophenol might take place slower than or at best concerted with nucleophilic attack.

In summary, we propose rate-determining base-catalyzed carbonyl hydration followed by very rapid cyclization ($>10^6$ s^{-1}) and perhaps slower elimination of 4-nitrophenol from 4-nitrophenyl 4-susbstituted phenacyl methylphosphonates. The conclusion is derived from rate accelerations relative to analogs, results of ^{18}O incorporation into the product, general base catalysis of the reaction, a pH–rate profile for the buffer-independent hydration process, solvent isotope effects up to 2 for buffer-catalyzed reactions and for the buffer-independent reaction below pH 9.25, and the Hammett correlation. Solvent reorganization seems to contribute significantly to the rate-determining nucleophilic addition of hydroxide ion to carbonyl at low hydroxide ion concentration but to a much diminished extent at high hydroxide ion concentration where the solvent isotope effect becomes inverse. The activation parameters are also consistent with a rate-determining hydration of carbonyl. The ionic strength dependence is consistent with a greater charge delocalization in

(18) (a) Alvarez, F.; Schowen, R. L. In *Isotopes in Organic Chemistry*; Buncel, E., Lee, C. C., Eds.; Elsevier: New York, 1984; pp. 1–60. (b) More O'Ferrall, R. A.; Kresge, A. J.; Powell, M. F. In *Isotopes in Organic Chemistry*; Buncel, E., Lee, C. C., Eds.; Elsevier: New York, 1984; pp 177–276.

(19) K_h is calculated from K_h = 29 M^{-1} for 4-methyltrifluoroacetophenone at 31.4 °C (Stewart, R.; van Dyke, J. D. *Can. J. Chem.* **1970**, *48*, 3961.) divided by 3×10^4, which is the mean effect of trifluoro substitution in alkyl methyl ketones on K_h (Guthrie, J. P. *Can. J. Chem.* **1975**, *53*, 898–906).

(20) Similar magnitudes of rate enhancement in intramolecular reactions with the intervention of five-membered ring intermediates were reported by: Higuchi, T.; Eberson, L.; Herd, A. K. *J. Am. Chem. Soc.* **1966**, *88*, 3805. Gordon, M.; Notaro, V. A.; Griffin, C. E. *J. Am. Chem. Soc.* **1964**, *86*, 1898.

the transition state than in the ground state, where charge is more localized on the phosphate dianion catalyst.

Experimental Section

Materials. Inorganic salts, buffer components, and other reagents were analytical or reagent grade chemicals, which were used as purchased or dried, recrystallized, or distilled as necessary. Water was distilled from a copper-bottom still, passed through a Barnstead mixed-bed ion-exchange column, boiled for 20 min, and cooled suddenly. Deuterium oxide (Norell Inc., 99.9% deuterium) was used shortly after opening the bottle. ^{18}O-enriched water (Isotec, 98%, or EG & G Mound Applied Technologies, 30%) was stored under nitrogen in sealed ampules or vials and was used immediately after withdrawal with a syringe.

Synthesis of 4-Nitrophenyl Phenacyl Methylphosphonate (PMN), 4-Nitrophenyl 4-Chlorophenacyl Methylphosphonate (CPMN), 4-Nitrophenyl 4-Methylphenacyl Methylphosphonate (MPMN), and 4-Nitrophenyl 4-Nitrophenacyl Methylphosphonate (NPMN).[21a] Purification of the esters involved recrystallization from an acetone/methanol mixture. Analytical purity was 98% for PMN, 98% for MPMN, and 97% for NPMN based on basic hydrolysis to 4-nitrophenol (400 nm). The melting points were: 112–114 °C for PMN, 123–125 °C for CPMN, 111.5–112.5 °C for MPNN, and 146–47 °C for NPMN. Other properties (NMR signals, repetitive scans between 250 and 450 nm of the hydrolytic reactions, etc.) also agreed with the structures.[21]

Synthesis of 4-Nitrophenyl 4-Methoxyphenacyl Methylphosphonate (MOPMN). Bis(4-nitrophenyl) methylphosphonate[22] and 4-nitrophenyl methylphosphonochloridate[21a] were made by coupling methylphosphonic dichloride with 1 equiv of the Na salt of 4-nitrophenol in dry benzene. The NaCl and bis(4-nitrophenyl) methylphosphonate precipitated out of benzene and were filtered out. The 4-nitrophenyl methylphosphonochloridate was then coupled with 2-hydroxy-4′-methoxyacetophenone in benzene in the presence of dry pyridine. The pyridine hydrochloride was filtered out of the benzene solution; the benzene was then evaporated, and MOPMN was recrystallized from methanol/acetone (0.5 g). Analytical purity was 97% based on hydrolysis to 4-nitrophenol: mp 112–113 °C. Other properties are in agreement with previous reports.[21]

Synthesis of 2-Hydroxy-4′-methoxyacetophenone. It was synthesized from 2-bromo-4′-methoxyacetophenone (Aldrich). The 2-bromo-4′-methoxyacetophenone was refluxed in 20% water and *N*-methyl-2-pyrrolidinone for 10 h at 105 °C. The product was extracted with ethyl ether and dried over $MgSO_4$, the ether was evaporated, and the dry solid was recrystallized before use (0.8 g). The clear crystals gave a single spot on silca gel TLC: mp 98.5–100.5 °C; ^1H NMR ($CDCl_3$ 2% $(CH_3)_4Si$) δ 3.88 (s, 3H, C*H*$_3$), 4.82 (s, 2H C*H*$_2$), 6.94 (d, 2H, Ar*2,6*, $J_{2,3}$ = 5 Hz), 7.92 (d, 2H, Ar*3,5*, $J_{2,6}$ = 5 Hz).

Rate Measurements. Automated acquisition of 200–1000 data points at 400 nm with a Perkin Elmer Lambda-7 Spectrophotometer interfaced to a Zenith Z-100 microcomputer was used for monitoring 4-nitrophenol release. First-order rate constants were calculated for 4 half-lives by least squares fit of absorbance/time coordinates. The temperature was controlled with a Lauda K4/DR circulating water bath furnished with a thermistor probe and attached to a digital readout. Measurements of the pH of kinetic solutions before and after reaction were performed with a Radiometer PHM 84 pH meter.

Hydrolytic rates were measured for MPMN at buffer concentrations of 0.05–0.30 M total phosphate, 0.02–0.10 M Hepes, 0.06–0.30 M Tris, 0.01–0.05 M borate, 0.05 M carbonate, and 0.005–0.05 M NaOH at μ = 1.0 M (KCl). The buffer solutions were made by serial dilution of the most concentrated solutions with 1.0 M KCl to maintain the ionic strength. Temperature dependence of the second-order rate constants was determined in pH 7.45 phosphate buffer and pH 8.98 Tris buffer at μ = 1.0 (KCl). Ionic strength dependence of the second-order rate constants was studied in phosphate buffers at pH 7.45 and 25.00 ± 0.05 °C by varying the amount of added KCl. D_2O solutions were buffered with the same ratio of buffer acid to conjugate base, and the same amount of salt was added as in the corresponding H_2O solutions.

Kinetic Protocol. The 4-nitrophenyl phosphonate esters were used in $1–3 \times 10^{-3}$ M acetonitrile stock solutions. In a typical kinetic experiment the 990 μL of buffer was equilibrated within 0.05 °C of the working temperature (as monitored with a thermistor probe) in a quartz cuvette in the cell compartment of the instrument. Ten microliters of the substrate solutions were introduced into 1 mL total volume to initiate the reaction.

Fast Reaction Kinetics. An OLIS stopped-flow system and the OLIS software were used for rate measurements in carbonate buffers and sodium hydroxide solutions (faster than about 0.03 s^{-1} under pseudo first-order conditions). Equivalent volumes of phosphonate ester solutions in dilute HCl (pH 4.0) and 0.01–1.0 M hydroxide solutions were injected from the drive syringes, and 4-nitrophenol release was monitored at 400 nm. The pH was checked after the reaction.

^{18}O Incorporation into the Hydrolysis Product. Measurements of the extent of ^{18}O incorporation are based on mathematical curve fitting and the integration of the ^{31}P NMR resonances for the ^{18}O- and ^{16}O-containing products.[11] ^{31}P NMR spectra were recorded on a GE QE-300 or Bruker AM-400 FT-NMR spectrometer at 121.7 and 162.0 MHz, respectively. A 90° pulse was employed and the spectra were broad-band proton-decoupled. Inverse-gated experiments demonstrated that the NOE effects on peak integration during the proton decoupling are negligible. The sample was prepared as follows. To 600 μL of ^{18}O-enriched (28% or 78% ^{18}O, containing ~10% D_2O for deuterium lock) aqueous solution containing a calculated amount of NaOH or buffer salt was added 4.2 mg of MPMN (0.02 M final concentration), and the solution was stirred at room temperature until a clear solution was obtained (incubated at 55 °C when necessary). For control, ^{18}O incorporation into 4-nitrophenyl methylphosphonate via hydroxide attack at the P of bis(4-nitrophenyl) methylphosphonate was measured and found to be 100%.

Acknowledgment. This research was supported in part by the National Science Foundation Grant DMB 9003440. We are grateful to Dr. Yiu-Fai Lam, University of Maryland, College Park, MD 20742, for the assistance with analysis of the NMR data and to Mr. C. N. Lieske, US Army Medical Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010, for sharing experiences with the compounds.

Registry Number supplied by author: 4-Nitrophenyl phenacyl methylphosphonate, 6203-26-6; 4-nitrophenyl 4-chlorophenacyl methylphosphonate, 21070-23-5; 4-nitrophenyl 4-methylphenacyl methylphosphonate, 22739-60-2; 4-nitrophenyl 4-methoxyphenacyl methylphosphonate, 21070-22-4; 4-nitrophenyl 4-nitrophenacyl methylphosphonate, 21161-62-6.

Supplementary Material Available: Tables of primary kinetic data (3 pages). Ordering information is given on any current masthead page.

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(22) (a) Kovach, I. M.; Larson, M.; Schowen, R. L. *J. Am. Chem. Soc.* **1986**, *108*, 5490–5494. (b) The system of abbreviations for the compounds is described in this paper: It is for the general formula X–R(PO)–Y, where Y is a leaving group, R a substituent attached to P, and X a second ligand. N = 4-nitrophenoxy, M = methyl and I = isopropyl. (c) Kovach, I. M.; Schowen, R. L. In *Peptides and Proteins: Recent Advances*; Schowen, R. L., Barth, A., Eds.; Pergamon: Oxford, England 1987; pp. 205–212.