models has recently been questioned by a few authors,[19,20] who prefer to consider them as NAD^+ analogues. The 1-deazaisoalloxazines are certainly closer analogues to flavins than the 5-deazaisoalloxazines. In this study, we have demonstrated some interesting chemical properties of 1,5-dideazaisoalloxazines. These compounds resemble the 5-deazaflavins more than the 1-deazaflavins. From the properties of these three kinds of deazaisoalloxazines one may conclude that position 5 of the isoalloxazine ring system is the most important site for flavin-mediated chemical reactions. More specifically, the 1,4-pyrazine ring which incorporates the N(5) and N(10) members provides stability to the radical states of oxidation and this is not realized on replacement of the N(5) moiety by carbon. As we have previously pointed out,[21] the radical stability in isoalloxazines may be attributed to Hoffman orbital splitting,[22] a feature which has found experimental proof in the established stability of radicals generated from 1,4-dihydropyrazines.[23]

Acknowledgment. This work was supported by grants from the National Institutes of Health and the National Science Foundation. We thank Professors Neil Castagnoli (University of California at San Francisco) and George Popjack (University of California at Los Angeles) for mass spectral analysis and for discussion concerning their interpretation.

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Rates of Formation and Decomposition of Tetrahedral Intermediates in the Hydrolysis of Dimethyl Aroylphosphonates. Substituent Effects on a Model for Carboxylate Ester Hydrolysis

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Abstract: Dimethyl aroylphosphonates are known to undergo hydrolysis to form substituted benzoic acids and dimethyl phosphonate: $ArCOPO(OCH_3)_2 + H_2O \rightarrow ArCOOH + HPO(OCH_3)_2$. A detailed kinetic analysis of the reaction for a series of phenyl-substituted dimethyl benzoylphosphonates was performed using spectroscopic techniques. The reaction proceeds in two steps which under certain conditions can be observed separately. Initially, hydration of the carbonyl group occurs (eq 2). Below pH 5 the rate of establishment of this equilibrium can be measured without interference from the subsequent decomposition process, which is proportional to hydroxide concentration and slower than hydration under these conditions. Above pH 6, hydration (k_1) is slow compared to conversion to products. Thus, above pH 6 hydration becomes rate limiting and the observed rate constant approaches k_1. Between pH 5 and 6 the reaction steps are comparable in magnitude and complex kinetic behavior results. Thus a complete profile for a carbonyl cleavage reaction can be obtained. Linear free energy relationships have been used to estimate the values of K_a (eq 4) for the species studied (substituents: 3-bromo, 4-methyl, 4-methoxy, hydrogen). This provides values for k_3 (eq 5) ($\sim 10^4\ s^{-1}$). The equilibrium and kinetic data obtained in this way give enough information to determine substituent dependencies of each step, solvent isotope effects, and activation parameters. These fit into patterns established for other carbonyl cleavage and hydration reactions which have not been analyzed. It is suggested that since the phosphonate diester anion is about as basic as alkoxide, the reaction serves as a model for the breakup of intermediates in ester hydrolysis. The hydration reaction fits into reactivity schemes suggested for aldehydes. The observed features of acyl phosphate ester hydrolysis can be analyzed in terms of rate-limiting hydration as well.

A reaction sequence that many types of carbonyl group containing compounds undergo involves addition of water to the carbonyl group to form a hydrate followed by expulsion of one of the substituents attached to the hydrated carbon atom (eq 1). Members of this general class of reaction include ester hydrolysis, amide hydrolysis, and ketone cleavage. Although

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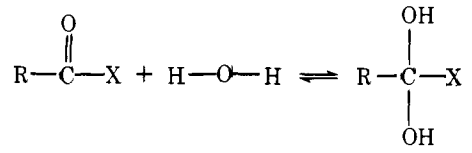
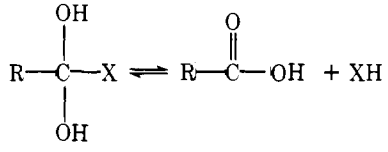
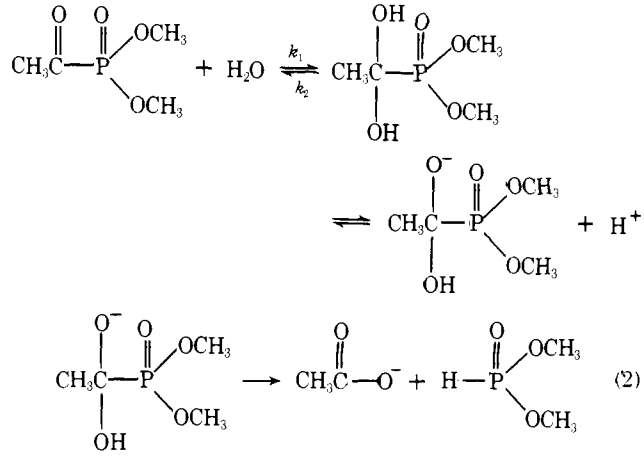


(1)



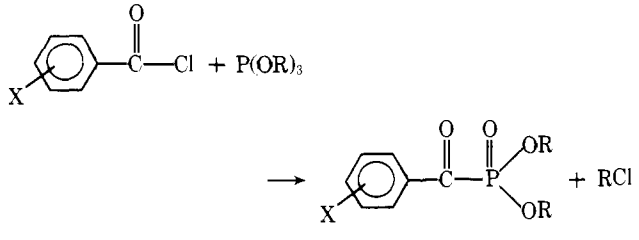
the overall reactions are often accessible for mechanistic analysis, the individual steps of the sequence are more difficult to analyze for a number of reasons. First, for esters and amides, the thermodynamic barrier to hydrate formation is so great that an observable amount of intermediate hydrate does not form.[1] Second, for compounds such as 2-ketocarboxylic acids, which are hydrated to an observable extent, the "leaving group" is poor (CO_2^-) and the decomposition reaction does not occur. Third, no systematic variation of substrates has been done for cases in which kinetically observable hydration is followed by measurable decomposition.[2] Therefore, a series of compounds that could be used to develop structure-reactivity relationships for both phases of carbonyl hydrolysis would provide a basis for obtaining general information for many reactions.

We recently showed that the rate of hydrolytic cleavage of 2-ketophosphonate diesters could be explained quantitatively by a two-step mechanism of hydration and cleavage.[3] Thus, dimethyl acetylphosphonate hydrolyzes in neutral solutions by the mechanism of eq 2. It is known that aroylphosphonate



(2)

diesters also undergo an analogous reaction[4-6] and it would appear that a similar mechanism should apply. Since the method of preparing aroylphosphonate diesters simply involves reaction between an aroyl chloride and a trialkyl phosphite,[4-6] a series of phenyl-substituted materials can easily be prepared.[5]



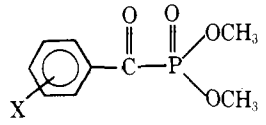
Analysis of reactions in terms of substituent parameters is easily accessible.

We have prepared a series of phenyl-substituted dimethyl benzoylphosphonates. These compounds undergo hydrolytic reactions which can be analyzed to obtain kinetic information about both hydration and decomposition reactions. These data have been used to analyze the behavior of related carbonyl group hydration-decomposition reactions.

Table I. Absorbance of Ketonic and Hydrated Forms of Substituted Dimethyl Benzoylphosphonates at 25 °C

substituent	3-bromo	none	4-methyl	4-methoxy
λ_{max}, nm, dioxane	260	262	272	298
$10^{-3}\,\epsilon$	9.63	12.76	13.19	15.96
λ_{max}, nm, water	264	268	282	310
$10^{-3}\,\epsilon$	2.17	5.46	8.53	13.76
background,[a] $10^{-3}\,\epsilon$ water	0.9	0.7	0.6	0.03
$10^{-3}\,\epsilon$ of substituted benzoate (water) at λ_{max} above[b]	0.921	0.694	0.641	≤.055

[a] Absorbance of hydrolysis product (substituted benzoate) taken as common portion of absorbance of both ketonic and hydrated starting material. [b] L. Láng, "Absorption Spectroscopy", Akadémiai Kiadó, Budapest, 1975.



1, X = Br
2, X = H
3, X = CH_3
4, X = OCH_3

Experimental Section

Materials. All chemicals for which preparations are not given were purchased and used as obtained from commercial suppliers. Liquids used for synthesis were distilled. Dimethyl aroylphosphonates were prepared from aroyl chlorides and trimethyl phosphite following published procedures.[4-6] Trimethyl phosphite (0.04 mol) was added to 0.03 mol of aroyl chloride (under nitrogen). Temperature was kept below 35 °C. The resulting pale yellow solutions were purified by vacuum distillation. Boiling points follow: dimethyl *m*-bromobenzoylphosphonate (87–90 °C, 0.05 Torr), dimethyl *p*-methylbenzoylphosphonate (197–111 °C, 0.05 Torr), dimethyl benzoylphosphonate (98–100 °C, 0.05 Torr), dimethyl *p*-anisylphosphonate (115–120 °C, 0.1 Torr). ^{1}H NMR spectra (CCl_4 relative to internal Me_4Si) consisted of methoxyl resonances (δ 3.93 d, J_{PH} = 11.0 Hz), signals due to aromatic-substituted protons (δ 6.90–8.3), and singlet absorptions due to methyl substituents of the *p*-methyl (δ 2.43) and *p*-anisyl (δ 3.87) compounds.

Ultraviolet spectroscopy of materials in dioxane was used to obtain absorbance due to the keto form of the substrates.[2] Spectra in water were due to a mixture of keto and hydrated forms. "Background" absorbance of hydrolysis products was assumed to be a constant for both hydrated and unhydrated forms. Equilibrium constants for hydrate formation were determined by the method described by Hine and Koser,[2] using the equation $K = (a_K - a_H)/(a_H - a_C)$, where a_K refers to the absorbance of the pure ketone (in dioxane), a_H is the observed absorbance when the ketone is dissolved, and a_C is background absorption. Wavelengths used and extinction coefficients are listed in Table I.

Product Analysis. The UV spectrum of the hydrolyzed substituted dimethyl benzoylphosphonate corresponded quantitatively to that of a benzoate with the same substituent (dimethyl phosphonate has no UV absorbance). The general reaction pattern to give benzoates and dimethyl phosphonate is well known.[4-6]

Kinetic Methods. A Unicam SP 1800A recording spectrophotometer, equipped with a Heto constant temperature circulator, was used to record the decrease in absorbance due to hydrolysis of the substituted dimethyl benzoylphosphonates. Five microliters of a 1% (v/v) substrate-acetonitrile solution was added with a syringe into 3 mL of a buffer solution which had been thermally equilibrated. Ionic strength was adjusted to 1.0 M with potassium chloride solution. At moderate acidities (pH ≤5), there was a rapid disappearance of starting material followed by a slower first-order reaction to yield the spectrum of the hydrolysis products. First-order rate constants were

Table II. Observed Rate Constants ($\times 10^4$ s^{-1}) for Hydrolysis of Phenyl-Substituted Dimethyl Benzoylphosphonates at 25 °C and Equilibrium Constants for Formation of Hydrates at 25 °C[a]

substituent	3-Br			none			4-CH_3			4-OCH_3		
K_1	6.00			1.53			0.59			0.16		
pH (or −log [HCl])	k'_{obsd}	k''_{obsd}	k'''_{obsd}	k'_{obsd}	k''_{obsd}	k'''_{obsd}	k'_{obsd}	k''_{obsd}	k'''_{obsd}	k'_{obsd}	k''_{obsd}	k'''_{obsd}
0	6880			4380			4160			5590		
0.34	6770			4050			2760			2630		
0.67	5330			2510			2040			1980		
1.00	4170			2060			1320			1100		
2.52	4500			1800	0.33		1200			100		
3.85	3850	15.8		1540	7.46		1000	3.85		924	1.67	
4.42	4080	48.1		1540	23.1		1070	12.0		924	5.78	
4.83		139			64.0			32.1			13.2	
6.16			2140			790			277			100
6.61			3250			950			445			160
7.10			4520			1600			611			236
7.65						5900			2050			703

[a] Ionic strength maintained at 1.0 with KCl.

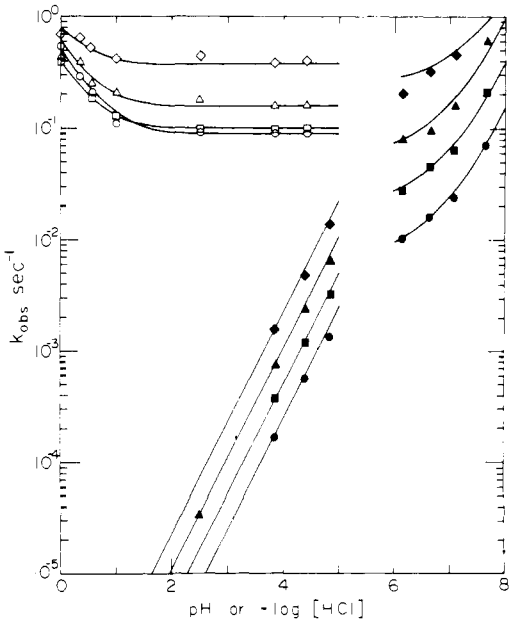


Figure 1. Observed rate constants for hydrolysis of phenyl-substituted benzoylphosphonates. Solid symbols are observed rate constants for formation of substituted benzoates. Open symbols are for attainment of equilibrium with hydrates. Substituents: ♦, 3-bromo; ▲, none; ■, 4-methyl; ●, 4-methoxy. Curves have been drawn according to equations discussed in text.

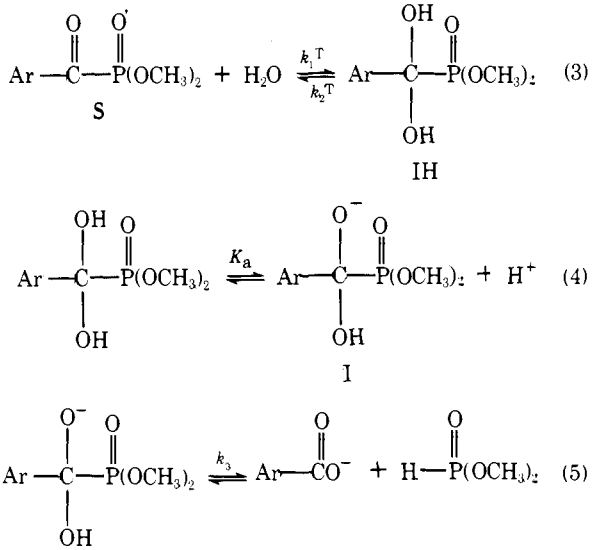
obtained from conventional first-order plots.[7] For reactions with half-lives of less than 10 s, a rapid mixing technique was used in which a plunger was used instead of a syringe. Between pH 5 and pH 6, two nonseparable consecutive reactions were observed.[7] Above pH 6, a single first-order process could be followed. Above pH 8, reaction rates were too rapid to follow by these methods.

For the pH 6–8 region, where hydration was shown to be rate determining, it was also expected that general base catalysis would be observed since the phenomenon has been observed for other carbonyl hydration reactions.[8,9] Such buffer catalysis was observed. Therefore, determination of the buffer-independent rates in this region involved extrapolation of rates determined at various buffer concentrations to zero buffer concentrations. Since buffer catalysis is well understood for this type of reaction,[9] we did not analyze the rate component due to buffers. Rate constants are reproducible to ±3%.

Results

Hydrolysis of Aroylphosphonate Diesters. The hydrolysis of dimethyl benzoylphosphonates is first order in substrate (**S**)

Scheme I



(3) (4) (5)

$$k_1^T = k_1^U + k_1^H[H^+] + k_1^{OH}[OH^-] \quad (6)$$

$$k_2^T = k_2^U + k_2^H[H^+] + k_2^{OH}[OH^-] \quad (7)$$

$$k_1^T/k_2^T = K_1 \quad (8)$$

over the acidity range we have studied (1 M hydrochloric acid to pH 7.5). Below pH 5, the reaction occurs as two separable first-order processes. The first process is conversion of the ketone to its hydrate[3] (IH) (absorbance due to substrate decreases before product appears) followed by base-catalyzed conversion of hydrate to the hydrolysis products. Thus, two observed first-order rate constants (k'_{obsd} and k''_{obsd}) can be obtained for the two pseudo-first-order processes. Between pH 5 and pH 6 the two processes become sufficiently close in rate to prevent separate rate constants from being estimated by conventional first-order procedures. Above pH 6, the second step becomes sufficiently fast to make hydration clearly rate determining. Therefore, a single first-order rate constant is observed for the conversion of **S** to products (k'''_{obsd}).

The observed rate constants for **1–4** are plotted as a function of pH in Figure 1. The lines have been plotted according to eq 9–11 derived from Scheme I, fitting eq 6–8, where $K_a \ll 1$.[2]

$$k'_{obsd} = k_1^T + k_2^T \quad (9)$$

$$k''_{obsd} = (K_1/K_1 + 1)(K_a k_3/[H^+]) \quad (10)$$

$$k'''_{obsd} = k_1^T K_a k_3/((k_2^T) + K_a k_3/[H^+])[H^+] \quad (11)$$

Here k'_{obsd} is the rate constant observed for attainment of

Table III. Rate Constants for Hydration of Phenyl-Substituted Dimethyl Benzoylphosphonates and for Dehydration of the Hydrates at 25 °C[a]

substituent	$10^2 k_1^H$, M^{-1} s^{-1}	$10^2 k_1^U$, s^{-1}	$10^{-5} k_1^{OH}$, M^{-1} s^{-1}
3-bromo	36.8	33.0	12.5
none	24.7	9.31	8.54
4-methyl	11.7	3.71	3.42
4-methoxy	5.73	1.27	1.38

[a] Ionic strength 1.0.

equilibrium between S and IH. The rate constant k''_{obsd} is for conversion of the S-IH equilibrium mixture to products (pH $\leq$5). The rate constant under conditions where IH is a true steady-state intermediate (pH $\geq$6) is k'''_{obsd}. Derived rate constants k^H refer to acid catalysis, k^U to water catalysis, and k^{OH} to hydroxide catalysis for each process.

Equilibrium Constants for Hydration. The degree of interconversion of S and IH of **1–4** at equilibrium was determined by the procedure described in the Experimental Section. Values of $K_1 = [IH]/[S]$ are summarized in Table II.

Kinetics of Hydration. The observed rate constants, k'_{obsd}, k''_{obsd}, and k'''_{obsd}, along with equilibrium constants for interconversion of S and IH, can be used to generate a pH-rate profile for the rate constant for conversion of S to IH, k_1^T. Since k'_{obsd} measures attainment of equilibrium, the observed rate constant is the sum of the rate constants k_1^T and k_2^T. Since K_1 is known, k_1^T and k_2^T can be determined. Furthermore, at high pH, k'''_{obsd} approaches k_1^T, since equilibrium is not achieved. The rate constants in Table II were used to obtain rate constants for acid-catalyzed, base-catalyzed, and uncatalyzed hydration and dehydration processes. The values obtained for these rate constants thus obtained are shown as pH-rate profiles in Figure 2, plotted according to eq 6. The values of rate constants k_1^H, k_1^U, and k_1^{OH} used to generate the curves in Figure 2 are listed in Table III. From knowledge of the equilibrium constant for hydration, K_1 (Table III), the rate constants, k_2, for dehydration can be obtained.

Decomposition Reaction. The conversion of substrates to products involves initial formation of hydrates, followed by decomposition of the hydrate to products.[3] The rate of the decomposition reaction step is known to be proportional to hydroxide ion concentration and to be buffer independent.[3] This specific base catalyzed process involves decomposition of the conjugate base of the hydrate in analogous reactions (Scheme I).

The observed rate constant (k''_{obsd}) for decomposition of IH (at low pH) can be used to determine the value of k_3 of Scheme I for **1–4**, if K_a for the various IH species can be estimated. Values of K_a can be estimated by the empirical linear free energy relationships of Greenzaid,[10] based on the data of Stewart and Van der Linden[11] and of Hine and Koser.[2]

The procedure involves using the measured pK_as of hydrates of substituted trifluoroacetophenones. It is known that these hydrate pK_as obey the expression[11]

$$pK_a = 10.0 - \rho\sigma \qquad (12)$$

where ρ has a value of 1.11. For aliphatic substituents, Hine and Koser[2] determined that a $\rho^*\sigma^*$ relationship holds:

$$pK_a = 14.19 - 1.32\,(\sigma^*_{R_1} + \sigma^*_{R_2}) \qquad (13)$$

with $\rho^* = 1.32$. Therefore, the difference in value of σ^* [12] for a trifluoromethyl group and a dimethylphosphono group can be used to modify eq 12. The σ^* value for $P(O)(OCH_3)_2$ is approximately 2.18.[13] The unknown σ^* value for trifluoromethyl can be estimated by a proportionality relation among remaining known quantities:[12]

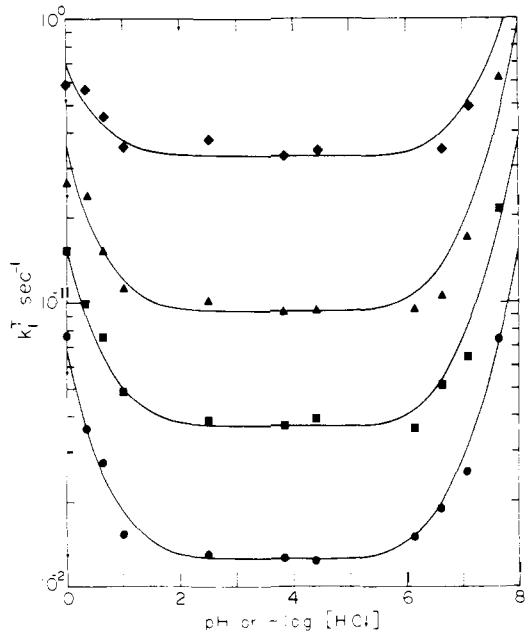


Figure 2. Derived rate constants for formation of hydrates of phenyl-substituted benzoylphosphonates. Substituents designated as in Figure 1. Curves drawn according to equations in text.

Table IV. Rate Constants for Decomposition of Conjugate Bases of Hydrate of Phenyl-Substituted Dimethyl Aroylphosphonates at 25 °C

substituent	$k_3 \times 10^{-3}$, s^{-1}	substituent	$k_3 \times 10^{-3}$, s^{-1}
3-bromo	5.83	4-methyl	14.2
none	12.0	4-methoxy	17.9

$$(\sigma^*_{CCl_3}/\sigma^*_{CCl_2}) = (\sigma^*_{CF_3}/\sigma^*_{CF_2}) \qquad (14)$$

This yields a value of 2.82 for that parameter. The pK_a of phenyl-substituted hydrates IH (pK_a^{XP}) can be obtained by adjustment of the pK_a of the correspondingly substituted trifluoroacetophenone (pK_a^{XT}):

$$pK_a^{XP} - pK_a^{XT} = 1.32(2.18 - 2.82) = -0.84 \qquad (15)$$

The pK_a values calculated by applying eq 15 to the data of Stewart and Van der Linden[11] follow: **1**, 10.35; **2**, 10.84; **3**, 10.99; **4**, 11.02.

The values of k_3 for **1–4** are related to observed rate constants k''_{obsd} and the equilibrium constant $K_1 = [IH]/[S]$. The data in Figure 1 and eq 10 were used to arrive at values of k_3. These are listed in Table IV.

Solvent Isotope Effect. It was found that absorbance of solutions of dimethyl benzoylphosphonate, after hydration equilibrium is attained, is identical in deuterium oxide and water. Therefore, K_1 has a solvent isotope effect of unity. The values obtained for solvent isotope effects on rate constants for dimethyl benzoylphosphonate, expressed as a ratio of the rate constant in water to the rate constant in deuterium oxide, are $k_1^U = 3.3$ and $k_3 = 1.0$. The method of Ballinger and Long[14] was used to estimate the isotope effect on K_a to be 4.

The solvent isotope effect on k_1^U is close to that observed for the hydration of aldehydes.[10] Calculations by Bunton and Shiner[15] based on a vibrational analysis procedure predict a value of 3.0 for this process. This is consistent with general acid or general base catalyzed hydration (by the solvent molecules). The lack of a solvent isotope effect on k_3 is consistent with the observation that the decomposition process is not subject to general acid or base catalysis.

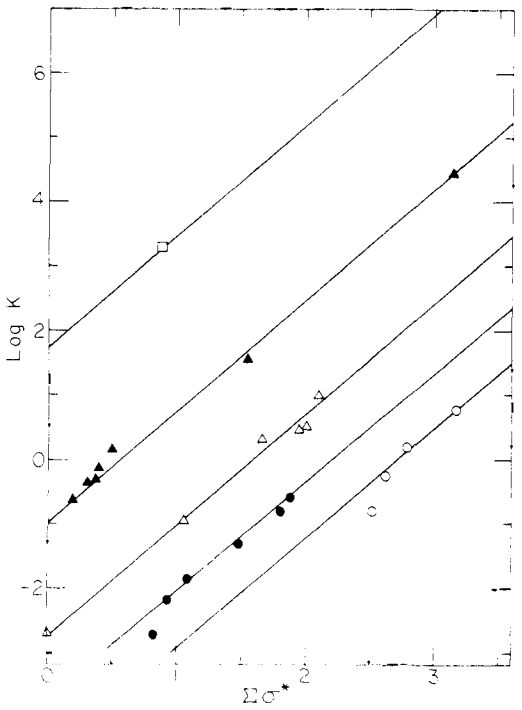


Figure 3. Equilibrium constant for hydrate formation from carbonyl compounds as a function of Taft parameters (see text) of both substituents (□ = formaldehyde, ▲ = substituted acetaldehydes, △ = substituted dialkyl ketones and dimethyl acetyl phosphonate, ● = substituted benzaldehydes, ○ = phenyl-substituted dimethyl benzoylphosphonates).

Table V. Observed and Derived Rate Constants (s^{-1}) for Hydrolysis of Dimethyl Benzoylphosphonate at Various Temperatures and Equilibrium Constants for Hydration at These Temperatures[a]

t, °C	K_1	k'_{obsd}	$10^4 k''_{obsd}$	$10^2 k_1^T$	$10^2 k_2^T$	$10^{-3} k_3$
15	2.17	0.105	2.41	7.2	3.3	3.45
25	1.53	0.154	7.46	9.3	6.1	12.0
35	1.03	0.231	20.5	11.7	11.4	39.4
45	0.680		26.4			112

[a] Ionic strength maintained at 1.0 with KCl.

Table VI. Activation Parameters for Hydrolysis of Dimethyl Benzoylphosphonate (Calculated at 25 °C)

	k_1^U	k_2^U	k_3
E_a, kcal/mol	4.4	11.3	21
$\Delta H^{\ddagger}$, kcal/mol	3.8	10.7	20.4
$\Delta S^{\ddagger}$, cal/(mol/deg)	−50	−28	28
$\Delta G^{\ddagger}$, kcal/mol	18.8	19.1	12.1

Activation Parameters. The effects of temperature on observed rate and equilibrium constants for reactions of dimethyl benzoylphosphonate were used to determine transition state activation parameters.[16] Values of rate constants at various temperatures are listed in Table V. Derived activation parameters are listed in Table VI. The large negative values for activation entropy associated with hydration and dehydration are consistent with catalysis by solvent molecules.[17] The larger negative value for hydration compared to dehydration is of course due to the different molecularity of the two processes compared to the respective starting materials. The positive value for k_3 is consistent with a unimolecular process leading to the transition state.[17] The enthalpy barriers for hydration and dehydration are relatively low. The reactions are retarded principally by the high entropy barriers at these temperatures.

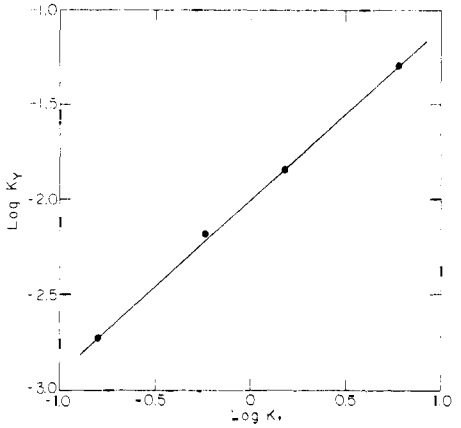


Figure 4. Equilibrium constant for hydration of phenyl-substituted dimethyl benzoylphosphonates (K_1) vs. equilibrium constant for hydration of substituted benzaldehydes (K_Y).

Free-Energy Relationships. The equilibrium constants for hydrate formation K_1 are expected to be related by a linear free energy equation to the Taft substituent parameters (σ^*) of the groups attached to the carbonyl group.[10] Since effects of phenyl substituents are defined by the Hammett parameter (σ), using the definition of σ^* and the value of σ^* for a benzene ring as a substituent (H) of 0.60,[12] the σ^* value for a benzene ring containing a substituent designated "X" is given by

$$\sigma_X^* = \sigma_X + \sigma_H^* = \sigma_X + 0.60 \qquad (16)$$

Figure 3 plots equilibrium constants for hydration of ketophosphonates, and other carbonyl compounds[10] whose hydration equilibria are known, as a function of substituent parameters, σ^*. Each class of compound defines a linear relationship with a common slope ($\rho^* = 1.7$)[10] giving a reasonable common fit. The greater stability of the aromatic carbonyl compounds is presumably a result of resonance stabilization of the carbonyl form.[10] The sets of aromatic derivatives show a deviation for the 4-methoxy substituted material. This suggests that further resonance interactions are important in this equilibrium; presumably the carbonyl form has additional stabilization.

The equilibrium constants for the two aromatic-substituted series give a good linear free energy plot with the *p*-methoxy compound on the line. The plot of the common points (Figure 4) has a slope slightly different from unity (with phosphonate on the X axis, the slope is 0.91), indicating that the common slope in Figure 3 is an approximation and not a phenomenon. The slope of 0.91 in Figure 4 implies that the ketophosphonate is slightly more sensitive to substituent effects than is the corresponding aldehyde.

Figure 5 shows the effect of substituents on the rate constant for addition of water to the carbonyl group of substrate molecules for the acid-catalyzed, base-catalyzed, and uncatalyzed reactions. Neither σ nor σ^+[18] gave a straight line, however. Since addition of water to a partially positive center is not analogous to the basis of either function alone but reflects an intermediate situation, the combined parameter approach appears justified. Thus, for each rate constant, log k_X (rate constant for compound with substituent "X") is plotted according to

$$\log\left(\frac{k_X}{k_H}\right) = \rho[\sigma + r(\sigma^+ - \sigma)]^{16} \qquad (17)$$

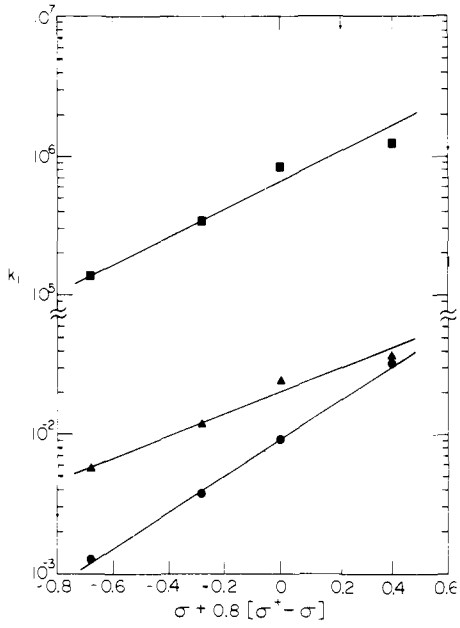


Figure 5. Yukawa-Tsuno plot of rate constants for addition of water to phenyl-substituted dimethyl benzoylphosphonates. (■ = hydroxide catalysis, $M^{-1}\,s^{-1}$; ▲ = acid catalysis, $M^{-1}\,s^{-1}$; ● = uncatalyzed, s^{-1}).

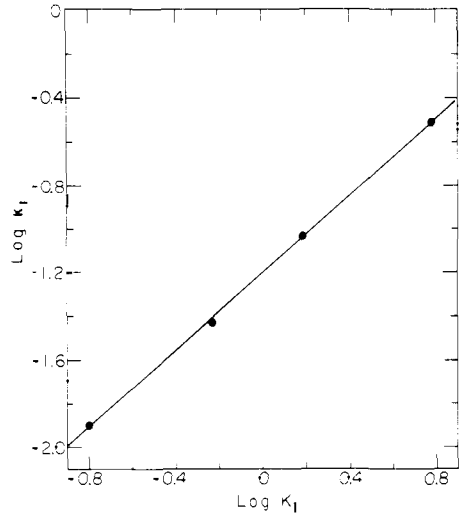


Figure 6. Equilibrium constant for formation of hydrates of phenyl-substituted dimethyl benzoylphosphonates vs. rate constants (s^{-1}) for formation of hydrate (uncatalyzed).

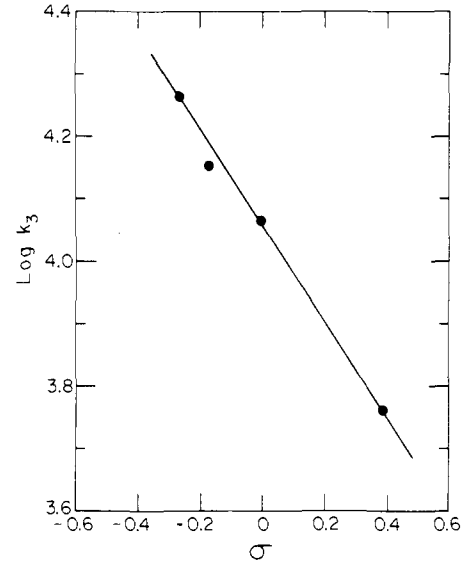


Figure 7. Hammett plot for k_3 (s^{-1}), the rate constant for conversion of conjugate base of hydrates of phenyl-substituted dimethyl benzoylphosphonates to substituted benzoates and dimethyl phosphite (25 °C).

A good fit, with a value for the parameter r of 0.8, is obtained for all three rate constants. The ρ value for the rate constant for hydroxide catalysis is 1.3; for acid catalysis ρ is 0.84; and for the uncatalyzed reaction ρ is 1.32.

Figure 6 is a plot of the rate constant for uncatalyzed hydration against the equilibrium constant for hydration for the compounds studied. The large slope of 0.87 suggests that the transition state closely resembles the hydrate, since it is anticipated that a limiting value for this slope would be 1.0.

The rate constants for the unimolecular decomposition of the conjugate bases of the carbonyl hydrates (listed in Table III) obey the Hammett equation (Figure 7). The reaction parameter ρ has a value of −0.75. This is consistent with a transition state in which developing positive charge at the hydrated carbon atom is stabilized by electron donation from the aromatic ring. To the extent that transition state stability is a function of the basicity of the leaving group, phosphonate expulsion serves as a model for the analogous reaction in ester hydrolysis where the leaving group, alkoxide ion, is approximately as basic as a phosphonate ion.[3] The incipient resonance-stabilized carboxylate product is the same in both cases, and the intermediates should be comparable in energy. The resonance stabilization of the ester makes the equilibrium between it and its hydrate much higher in energy than that between ketophosphonate and its hydrate.

Discussion

Mechanism of Hydrolysis of Dimethyl Benzoylphosphonates. The phosphonate diester group, as a substituent, promotes hydrate formation from the carbonyl derivative. It is strongly electron withdrawing but does not stabilize the carbonyl form by orbital overlap. The high barrier to hydration of esters and amides is largely the result of stabilization of their keto forms by overlap of lone pairs of their substituent groups.[1] We note that for the compounds **1–4** the ρ^* value of 1.7 for the equilibrium constant for hydration (Figure 3) is the same as that of other unstabilized carbonyl compounds.[10]

In Figure 5, the effect of substituents on the rate constants for acid-, base-, and water-catalyzed hydration reactions have been fitted to the Yukawa-Tsuno[18] equation. We know of no other case where rate constants for formation of neutral hydrates have been determined explicitly. Thus, these data provide a new set of information on a general process. The combined knowledge of equilibrium constants and rate constants for this series of compounds is important since these compounds also undergo further reaction to expel the phosphonate group. Therefore, a decomposition reaction proceeding from a tetrahedral intermediate is available for systematic analysis (Figure 7). The ρ value for decomposition is −0.75.

Uniqueness of the Kinetic Pattern. As we have noted, other cases of hydration-decomposition reactions have been studied.[2,19] However, this case is unique. The pH-k_{obsd} profile breaks owing to a change in rate-determining step from decomposition to hydration. Yet hydration can be observed separately when that step is not rate determining. Thus self-consistency between the rate constant for hydration observed directly and assumed by the pH dependence of decomposition has been demonstrated. This possibility arises because the hydration step leads to a favorable hydration equilibrium. The fact that hydration occurs to a large extent prevents steady-state conditions from applying in the pH region where decomposition becomes rate determining.

Application to Hydrolysis of Acetyl Phosphate Derivatives. The rate of C-O (acyl) cleavage of acetyl phosphate mono- and diesters (acetyl phenyl phosphate,[17] acetyl dimethyl phosphate[20]) is sensitive to the pK_a of the leaving group; the monoanion is cleaved much more slowly than is the neutral compound. Metal ions promote the hydrolysis of the monoanion[21,22] presumably by causing it to resemble the neutral compound. These effects could be due to the fact that a phosphate diester is a much weaker base than a phosphate monoester, and thus is a better leaving group.[22] Our results indicate that the rate-determining step in this reaction is probably addition of water to the carbonyl group to form the hydrated derivative, with expulsion of the leaving group being rapid. Thus, the rate differences are due to the differences in inductive (or resonance) effects of the substituents in the hydration step, and catalysis by metal ions is likely to be the result of promotion of hydration. If the hydrate is only a transition state, then, in effect, expulsion may also be assisted. These matters are under investigation.

Acknowledgments. We thank the National Research Council of Canada for an Operating Grant, David C. Pike for preliminary experiments, and Timothy Smyth for helpful discussions.

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Substrate Analogue Studies of the Specificity and Catalytic Mechanism of D-3-Hydroxybutyrate Dehydrogenase[1]

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Abstract: D-3-Hydroxybutyrate dehydrogenase from *Pseudomonas lemoignei* was analyzed by steady-state kinetics which indicate that an ordered binding mechanism (NADH and then acetoacetate on, hydroxybutyrate and then NAD off) applies. The specificity for binding and the specificity for catalysis of the enzyme to which NADH has bound were determined through the use of carboxylates, phosphonates, and a sulfonate that have structural features in common with acetoacetate. It was found that 3-keto carboxylates, with or without alkyl substituents at the 2 and 4 positions, and acetonylsulfonate are reduced enzymatically. The phosphonate monoanionic analogue of acetoacetate, methyl acetonylphosphonate, is unique in being a competitive inhibitor at pH 7 of reduction of acetoacetate (K_i = 1.65 mM). A variety of anions function as noncompetitive inhibitors. It was shown that V_{max} is independent of the substrate; therefore, it is unlikely that the catalytic step is rate determining. It is proposed that the active site's recognition of the substrate involves a cationic center to which the carboxyl group binds and a hydrogen-bond donor with which the carbonyl group of the substrate or competitive inhibitor can associate. The lack of rotational symmetry in the phosphonate monoester functional group leads to its being bound in a distorted manner compared to molecules that have this symmetry and are substrates. Reduction of the carbonyl group occurs only when a more critically limited spatial arrangement is attained, distinguishing an inhibitor from a substrate. The catalytic process in this system is suggested to involve a transition state for reduction which closely resembles the alcohol and NAD products.

Phosphonates and sulfonates which have structural and electronic features in common with carboxylates can be used to probe the specificity and catalytic mechanism of enzymes that normally catalyze reactions of carboxylates.[2,3] Differences in the inherent reactivity patterns of the various molecular types lead to diverse responses to the catalytic apparatus of an enzyme. In this paper, we report the results of a substrate analogue study of the highly specific bacterial enzyme, nicotinamide adenine dinucleotide-dependent D-3-hydroxybutyrate dehydrogenase (*Pseudomonas lemoignei*)[4] (eq 1). The enzyme is abbreviated HBDH; the cofactors are abbreviated NADH and NAD.

A major advantage of the structural analogue approach to the study of enzymic specificity and catalysis is that definitive information about the accommodations of an enzymic binding site can be obtained for an enzyme for which very little other information is yet available. Thus, once the basic, kinetic-reaction patterns of the enzyme are known, the effects of substrate analogues can be immediately evaluated. This approach is complementary to methods which analyze the protein

0002-7863/78/1500-7388$01.00/0