Hydration and Hydrolysis of Methyl Pyruvate. A Bifunctional Probe of Chemical and Enzymatic Catalysis

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Abstract: The hydration and hydrolysis reactions of methyl pyruvate were followed at 5.0 °C and an ionic strength of 1 M by using spectrophotometric methods. For the very slow hydrolysis reaction, an enzyme-coupled technique involving the use of lactate dehydrogenase was developed. The hydration and hydrolysis reactions were both shown to be susceptible to catalysis by general bases and by carbonic anhydrase from bovine erythrocytes (BCA). The series of bases chosen for this investigation range in values of pK_a from ca. 4.5 to 10 and represent those commonly employed as buffer components in enzyme assays. Of the general bases studied, diethylmalonate dianion showed a pronounced negative departure from Brønsted behavior, while the hydroxide ion showed a high positive deviation. Even higher positive deviations were observed for the BCA-catalyzed hydration (+3.1) and hydrolysis (+5.1) reactions of methyl pyruvate. The behavior of methyl pyruvate as a bifunctional substrate allows the dual manifestation of hydrase and esterase activities to be probed and, consequently, permits direct mechanistic comparisons to be made between these enzyme-catalyzed processes. The Brønsted relation is used as the basis for a comparison of the relative susceptibilities of the many substrates of BCA and also as an exploratory aid in the delineation of carbonic anhydrase catalyzed hydration and hydrolysis reactions.

Alkyl pyruvate esters undergo hydration[2] at the carbonyl group to yield I, the *gem*-diol, and hydrolysis[3] at the carbalkoxy group

$$\underset{\text{I}}{CH_3C(OH)_2CO_2R} \qquad CH_3COCO_2^- + \underset{\text{II}}{ROH} + H^+$$

to yield II, pyruvate, a proton (actually $B + H_3O^+ \rightleftharpoons BH^+ + H_2O$), and alcohol. It has been shown that both reactions are susceptible to catalysis by carbonic anhydrase[3,4] (carbonate hydro-lyase, EC 4.2.1.1.) from bovine erythrocytes (BCA).

As with most enzyme systems, the activity of BCA is highly pH dependent.[4-11] Since such kinetic studies are conducted in buffer solutions, a quantitative scrutiny of possible buffer catalysis is required for evaluating enzymatic rates and also for allowing comparisons to be made between the mechanisms of buffer and enzyme catalysis. The present work establishes that the hydration and hydrolysis of methyl pyruvate are, indeed, catalyzed by general bases.

The dual reactivity of methyl pyruvate allows direct comparisons to be made between its enzymatic hydration and hydrolysis and also between the relative sensitivities of these reactions toward general-base and enzymatic catalysis. BCA catalyzes many reactions that already proceed with significant rates in buffered solutions.[4-11] Although the mechanism of action may be somewhat different with regard to its various substrates, the active site of the enzyme exhibits certain common features that characteristically include juxtaposed general-base and nucleophilic subsites.[5-7,9] The advantage in using one and the same substrate to study hydration and hydrolysis reactions is that one variable parameter is removed from consideration.

This paper shows that an index of general-base and nucleophilic contributions to catalysis in the enzymatic mechanism may be obtained by comparing substrate sensitivity toward general bases with the deviation of bovine carbonic anhydrase from the Brønsted plot.

Experimental Section

The kinetic studies were carried out in deionized water at 5.0 °C in acetate, arsenate, phosphate, diethylmalonate, imidazole, *N*-methylimidazole, 1,2-dimethylimidazole, borate, and carbonate buffers. All buffer components with the exception of diethylmalonate were used in their commercially available reagent grade forms. The preparation of diethylmalonic acid was described in earlier work.[12] Methyl pyruvate was distilled prior to use through a Vigreux column under nitrogen gas; bp 43 °C (19 torr). The ionic strength of reaction solutions was adjusted by the addition of the appropriate quantities of sodium sulfate. Values of pH were determined before and after kinetic runs by means of a Beckman 101900 research pH meter. Stock solutions of NADH (Sigma, Grade III), the reduced form of nicotinamide adenine dinucleotide, were prepared in dilute buffers (ca. pH 8) immediately prior to use. The solutions were maintained at 0.0 °C during a given series of runs. Beef heart lactate dehydrogenase (Sigma, Type III) was obtained as an ammonium sulfate suspension. Stock enzyme solutions were prepared by dilution of small amounts of the suspension in 0.01 M NaCl immediately prior to the kinetic determinations. The stock enzyme solutions were kept ice-cold during the course of each series of kinetic runs. The methods of purification and standardization of lyophilized bovine carbonic anhydrase (Mann Research Laboratories) are described elsewhere.[13a,b]

The hydrolysis of methyl pyruvate was followed by using three independent methods. A direct spectrophotometric method using a Gilford high-speed recording spectrophotometer and one utilizing a Radiometer titrator, Type TT1c, were described in earlier work.[3] An enzyme-coupled system involving lactate dehydrogenase was also developed for use in the present investigation.

When the hydrolysis of methyl pyruvate occurs in the presence of NADH and lactate dehydrogenase, the enzymatic reduction of pyruvate anion and corresponding production of NAD result in a diminution of optical density at 340 nm (ϵ_{340}^{NADH} 6.2×10^3). The rate of the enzymatic oxidation of NADH is identical with the rate of ester hydrolysis when the hydrolysis step is rate limiting. A sufficiently rapid enzymatic oxidation of NADH to satisfy this requirement was attained under typical initial reaction conditions by using 0.0017 M methyl pyruvate, 5.0×10^{-5} M NADH, and ca. 7 units of LDH per 3-mL cuvette. It will be noted that the concentration of methyl pyruvate was far in excess of that of NADH and furthermore that the concentration of NADH is much larger

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Table I. Enzymatic and General-Base-Catalyzed Hydration and Hydrolysis of Methyl Pyruvate at 5.0 °C (Ionic Strength = 1.0 M)

		hydration			hydrolysis		
catalyst	pK_a' [a]	k_{cat}, M^{-1} s^{-1}	k_{calcd}, [b] M^{-1} s^{-1}	Brønsted [b] deviation, log units	k_{cat}, M^{-1} s^{-1}	k_{calcd}, [b] M^{-1} s^{-1}	Brønsted [b] deviation, log units
H_2O	−1.74	7.0×10^{-4} [d]	6.0×10^{-4}	+0.07	ca. 2×10^{-9} [e]	ca. 2.7×10^{-10}	ca. +0.87
$CH_3CO_2^-$	4.52	0.56	0.18	−0.06	8.69×10^{-6}	1.1×10^{-6}	−0.10
$HAsO_4^{2-}$	6.27	1.37	0.87	+0.20	1.10×10^{-5}	1.2×10^{-5}	−0.038
HPO_4^{2-}	6.54	1.24	1.1	+0.05	2.42×10^{-5}	1.7×10^{-5}	+0.15
$(C_2H_5)_2C(CO_2^-)_2$	6.70	0.256	1.3	−0.71	1.09×10^{-5}	2.1×10^{-5}	−0.28
BCA	7.0 [c]	2.2×10^3	1.7	+3.1	4.2	3.1×10^{-5}	+5.1
CH_3IM	7.51	2.17	2.7	−0.095	2.68×10^{-5}	6.1×10^{-5}	−0.36
IM	7.66	2.50	3.0	−0.079	7.57×10^{-5}	7.4×10^{-5}	+0.0099
OH^-	16.48	8.38×10^5	8.7×10^3	+2.0	3.89×10^2	9.5	+1.6

[a] As defined in text. [b] Values of k_{calcd} were determined from Brønsted plots (Figure 4) to correspond to the pK_a of each catalyst. The Brønsted deviations were calculated from the appropriate comparisons of k_{cat} and k_{calcd}. [c] Determined from inflection points of pH-rate profiles; see ref 3. [d] Determined from the data in Figure 3; $k_{H_2O} = k_0/55.5$ M. [e] Estimated value as described in text.

than its Michaelis constant with respect to lactate dehydrogenase.[14,15] As expected (provided that the ester hydrolysis is, indeed, rate limiting), production of NAD was observed to be a pseudo-zero-order process. Rates of hydrolysis were calculated from the slopes of linear plots of optical density vs. time:

$$v = k_{hydrolysis}[MP] = \text{slope}/\epsilon^{NADH}$$

The obvious advantage of this method of determining rates of hydrolysis is that only a minute fraction of ester hydrolysis results in a large spectrophotometric change due to the reduction of NADH. Accordingly, insignificant pH changes arise when the reaction is monitored, even in the presence of dilute buffers. The enzyme-coupled method was less reliable at low values of pH, due to decreased enzyme activity.[14] Thus, for reactions carried out below pH 5.5, one of the alternative methods of following the hydrolysis was employed. The LDH-coupled method was also avoided in the determination of carbonic anhydrase catalytic parameters since little is yet known about interactions between the two enzymes. Periodic checks were made in all kinetic series by using at least two of the methods to ascertain the validity of the data. All three methods gave results that were strictly in accord.

The much more rapid hydration of methyl pyruvate was followed directly by spectrophotometric measurements as described earlier.[2] The pseudo-first-order rate constants obtained were multiplied by the final fraction of hydration, $\chi = 0.84$ (5.0 °C),[2] to give the forward rate constant (eq 1).

$$k_{hydration} = k_{obsd} = 0.84 \times k_{obsd} \quad (1)$$

The hydration[2-4] and hydrolysis[3] of methyl pyruvate are catalyzed both by general bases and by BCA. The forward rate constant for these reactions is equal to a sum of kinetic terms:

$$k_f = k_0 + k_{OH^-}[OH^-] + k_B[B] + k_{BCA}[BCA] \quad (2)$$

In the absence of enzyme, the catalytic rate coefficient for a general base was determined from a series of kinetic runs in the buffer pair B,BH^+ at constant buffer ratio while the concentrations of base and conjugate acid were varied simultaneously. Plots of k_f vs. [B] have a slope $S = k_B$ and an intercept $I = k_0 + k_{OH^-}[OH^-]$. Values of k_0 and k_{OH^-} were determined from such data obtained at the various pH's of the different buffers employed in this work. Activity coefficients, $f_\pm$ were calculated from

$$\log f_\pm = \frac{-0.49Z^2I^{0.5}}{1 + 1.5I^{0.5}} \quad (3)$$

where I and Z stand for the ionic strength of the solution and the charge of the ion under consideration, respectively.

For both the hydration and hydrolysis of methyl pyruvate, the catalytic component associated with bovine carbonic anhydrase was calculated from Lineweaver–Burk plots in which $k_{BCA} = k_2/K_m$ at substrate concentrations much smaller than K_m.[16]

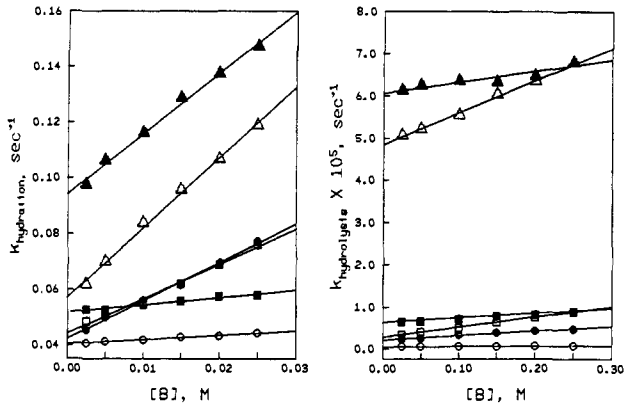

Figure 1. Plots of the general-base-catalyzed hydration (left) and hydrolysis (right) of methyl pyruvate at 5.0°C, μ = 1 M: (○) acetate buffers; (●) arsenate buffers; (□) phosphate buffers; (■) diethylmalonate buffers; (△) imidazole buffers; (▲) *N*-methylimidazole buffers.

The method of half-neutralization was used to obtain pK_a' values of the conjugate acids of the various bases used in the present work. The mean pH value for each set of buffers with a buffer ratio of unity was taken to be pK_a'.

Results and Discussion

While the hydrolyses of phenolic esters[6,8,17] and lactones[18] are catalyzed by general bases and/or nucleophiles, the susceptibility of the hydrolysis of methyl pyruvate to catalysis by general bases represents an unusual phenomenon for an alkyl ester. According to Figure 1, the general-base-catalyzed nature of the hydration and hydrolysis of methyl pyruvate is described by an equation that includes kinetic terms for each basic catalyst present in solution:

$$k_f = \sum k_{cat}[cat] = k_0 + k_{OH^-}[OH^-] + k_B[B] \quad (4)$$

where it is assumed that $k_0 = k_{H_2O}[H_2O]$. Both reactions of methyl pyruvate are rather insensitive toward acid catalysis,[2] as one would expect with a substrate strongly activated by the electron-withdrawing inductive effect caused by the carbmethoxy group (for hydration) or by the carbonyl group (for hydrolysis). In a similar way, general-acid catalysis is not observed for the hydrolysis of alkyl esters of 2,2-dichloro- or 2,2-difluoroacetic acid.[19] The linearity of the plots in Figure 1 precludes any significant catalytic contribution by a third-order kinetic term

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(16) The constants k_2 and K_m refer to the turnover number and Michaelis constant, respectively, in the simplified mechanism

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

(17) Kirsch, J. F.; Jencks, W. P. *J. Am. Chem. Soc.* **1964**, *86*, 843.
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(19) (a) Jencks, W. P.; Carriuolo, J. *J. Am. Chem. Soc.* **1960**, *82*, 1778. (b) *Ibid.* **1961**, *83*, 1743. (c) It might be noted that for the hydrolysis of ethyl dichloroacetate,[17] it has been proposed that bases stronger than ammonia behave as nucleophilic catalysts, while those weaker than ammonia behave as general-base catalysts. This hypothesis is based on a break in the Brønsted plot, showing a lower slope in what is presumed to be the general-base-catalyzed region and a higher slope in the nucleophilically catalyzed region.[17]

Table II. BCA and Chemical Catalysis of Hydration and Hydrolysis Reactions

compound	reaction	T, °C	β	k_{H_2O}, $M^{-1}s^{-1}$	k_{OH^-}, $M^{-1}s^{-1}$	pK_{BCA}[a]	K_m^{BCA}, M	k_{BCA}^{max},[b] $M^{-1}s^{-1}$	k_{calcd}[c,d]	Brønsted deviation BCA, log units
carbon dioxide	hydration	25.0		4.7×10^{-4}	6.0×10^3	6.85	0.015	5.5×10^7	0.3	+8.3
acetaldehyde[e]	hydration	0.0	0.47	2.8×10^{-5}	7.9×10^3	7.00	0.65	1.4×10^3	0.60	+3.4
propionaldehyde[f]	hydration	0.0	0.45	1.7×10^{-5}	2.3×10^3	6.6	0.20	1.2×10^3	0.31	+3.6
isobutyraldehyde[f]	hydration	0.0	0.46	9.3×10^6	1.8×10^3	5.6	0.15	2.1×10^2	0.20	+3.0
p-nitrophenyl acetate	hydrolysis	25.0	0.89	1.5×10^{-8} [h]	9.5	7.5	0.0067[i,j]	4.7×10^2 [i]	0.053	+3.9
methyl pyruvate	hydration	5.0	0.39	7.0×10^{-4}	8.4×10^5	7[k]	0.39[k]	2.2×10^3 [k]	1.7	+3.1
methyl pyruvate[g]	hydrolysis	5.0	0.58	1.9×10^{-9}	3.9×10^2	7	0.20	4.2	3.1×10^{-5}	+5.1

[a] Determined from inflection points of pH rate profiles. [b] $k_{BCA}^{max} = k_2/K_m$ at pH_{max}. For the hydrolysis of methyl pyruvate, $k_{BCA}^{max} = 2k_2/K_m$ at the inflection pH. [c] With the exception of carbon dioxide, β values of k_{calcd} were determined for each substrate from the respective Brønsted plots to correspond to the pK_a listed in column 7 of this table. For carbon dioxide, the value given here as k_{calcd} is actually $k_{HPO_4^{2-}}$ since its pK_a is almost identical with that for BCA. [d] References 9 and 27. [e] References 5, 12, and 22. [f] References 7 and 21. [g] Reference 20. [h] Reference 29. [i] Reference 6. [j] K_m varies with pH. The value chosen here is that at the inflection. [k] Reference 3.

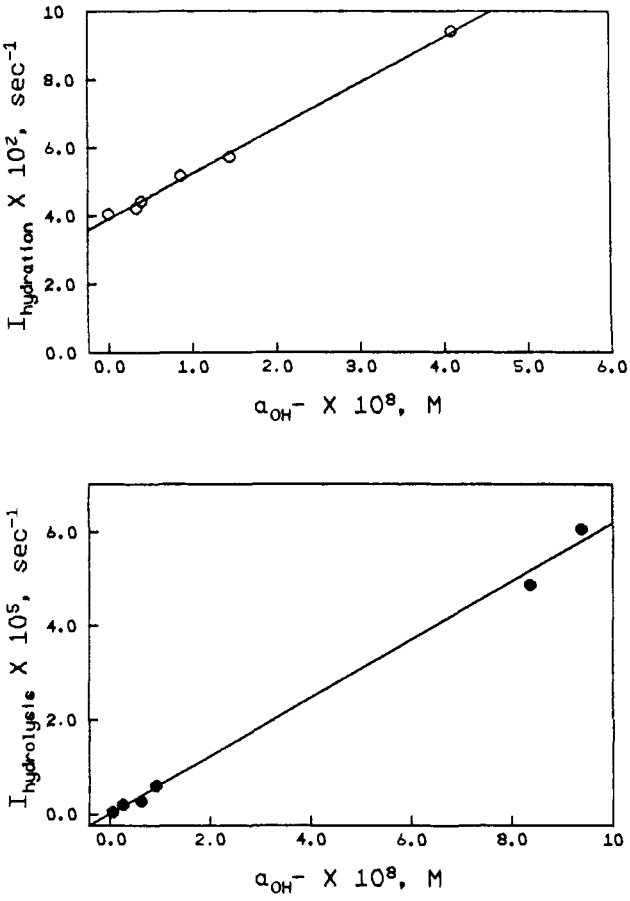

Figure 2. Plots of the spontaneous and hydroxide-catalyzed hydration (○) and hydrolysis (●) of methyl pyruvate. For the hydration and hydrolysis reactions, $I = k_0 + k_{OH^-}[OH^-]$.

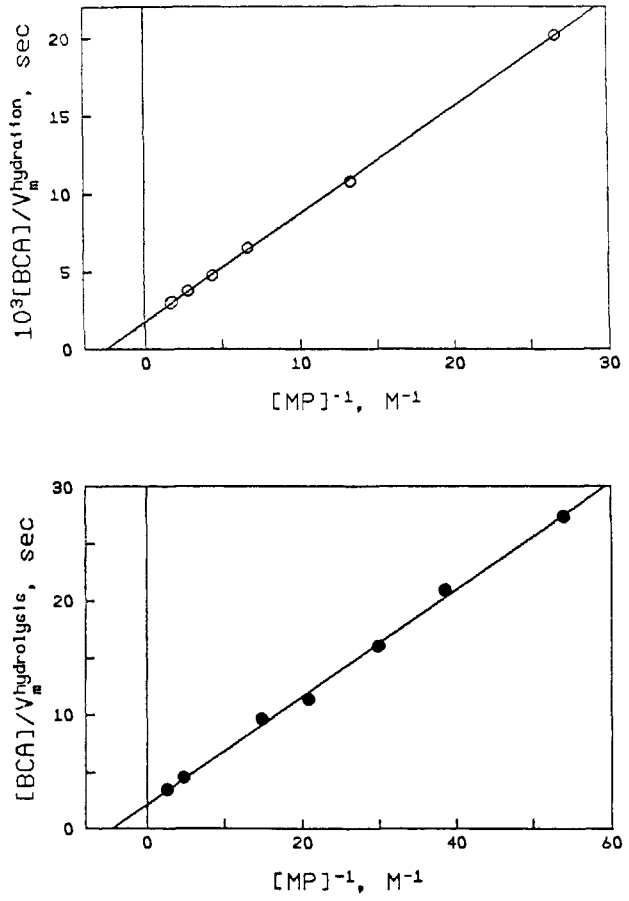

Figure 3. Lineweaver–Burk plots for the BCA-catalyzed hydration (○) and hydrolysis (●) of methyl pyruvate.

involving substrate, general base, and conjugate acid.

By use of the intercept values, $I_{hydration}$ and $I_{hydrolysis}$, from Figure 1, the catalytic coefficients for hydroxide ion were evaluated: k_{OH^-} = slope $\times f_{\pm}$ (Figure 2). The spontaneous rate constant, k_0, for the hydrolysis of methyl pyruvate was so small that it was necessary to estimate its value from data obtained in acetate buffers, where the catalytic contribution by hydroxide ion is minimal. A summary of the catalytic coefficients, k_{cat}, determined for the bases studied is given in Table I.

Inasmuch as bovine carbonic anhydrase possesses both hydrase[5,7,9,10] and esterase activities,[3,6,8,11] alkyl pyruvate esters provide unique substrates for the investigation of enzyme kinetics. Since such esters undergo both hydration[2] and hydrolysis,[3] a single compound may be used to analyze and compare both hydrase and esterase activities. As illustrated in Figure 3, the enzymatically catalyzed hydration and hydrolysis of methyl pyruvate both obey the Michaelis–Menten mechanism. Interestingly, the values of K_m for the two reactions *of the same substrate* differ[20] (Table II), perhaps suggesting a difference in the positioning of methyl pyruvate in the active cavity of the enzyme for the two reactions.

Brønsted plots were constructed for the catalysis of the hydration and hydrolysis of methyl pyruvate. It was assumed that

(20) The actual value of K_m for the BCA-catalyzed *hydrolysis* is even smaller than that obtained from Figure 3, since the experimental K_m is based on *total* ester concentration, $[CH_3COCO_2CH_3] + [CH_3C(OH)_2CO_2CH_3]$. See ref 3 for further discussion.

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(22) Bell, R. P.; Rand, M. H.; Wynne-Jones, K. M. A. *Trans. Faraday Soc.* **1956**, *52*, 1093.

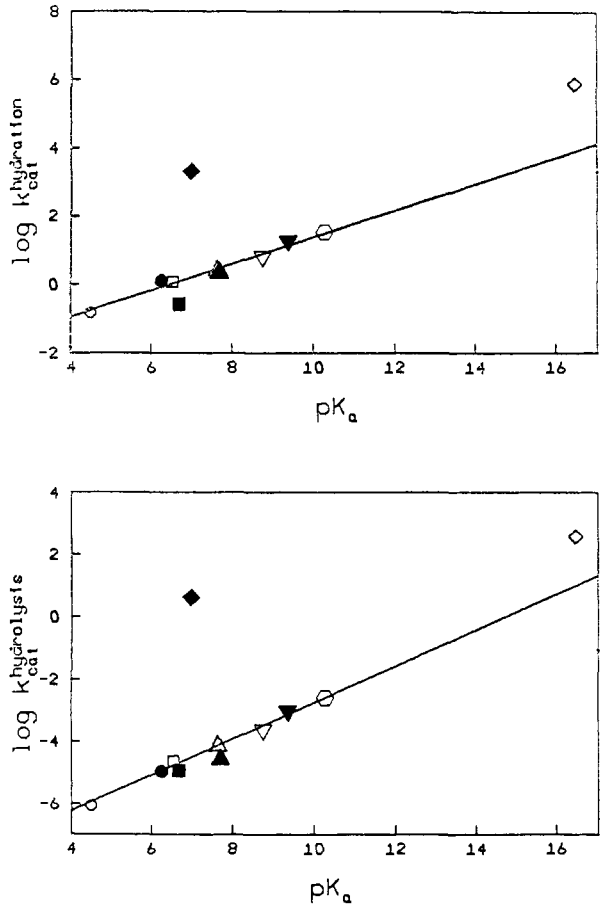


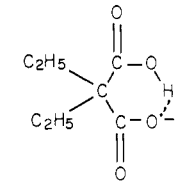
Figure 4. Brønsted plots for the hydration (top) and hydrolysis (bottom) of methyl pyruvate: (○) acetate; (●) arsenate; (□) phosphate; (■) diethylmalonate; (△) imidazole; (▲) *N*-methylimidazole; (▽) 1,2-dimethylimidazole; (▼) borate; (⊙) HCO_3^-/CO_3^{2-}; (◆) BCA; (◇) hydroxide. Statistical corrections were not made.

the slope of the plot was dictated by the catalytic coefficients associated with the series of general bases indicated in the legend (Figure 4). A comparison of Brønsted coefficients for related reactions is given in Table II. It will be noted that the value of β for hydration of methyl pyruvate is lower than the corresponding values for the hydration of aliphatic aldehydes. The lower β value is in accord with the greater reactivity of the substrate brought about by the electron-withdrawing inductive effect of the carbomethoxy group.

For the methyl pyruvate substrate, it was determined that the Brønsted coefficient is considerably larger for hydrolysis than for hydration. This observation is in accord with the finding that the hydrolysis of many other esters[17-19] exhibits higher β values than the hydrations of aliphatic aldehydes.[7] It should be noted that the slope of the Brønsted catalysis plot is considerably higher for the hydrolysis of *p*-nitrophenyl acetate than for the hydrolysis of methyl pyruvate. In this regard, it is known that Brønsted plots associated with nucleophilic catalysis have higher slopes than those for general-base catalysis.[17,19,23,24] For example, because of the favorable pK_a of the leaving group in *p*-nitrophenyl acetate, its hydrolysis is subject to nucleophilic catalysis by bases as weak as imidazole.[17] Furthermore, is has been observed for this reaction that phosphate and arsenate dianions exhibit large negative deviations to the Brønsted plot and appear to behave as general bases. On the other hand, it would be expected that the hydrolysis of methyl pyruvate, a substrate activated in the acyl group but possessing a poor leaving group, would be subject to general-base catalysis. This is, indeed, indicated in Figure 4 and quantitatively in Table I by the fact that imidazole, *N*-methylimidazole, phosphate, and arsenate all show relatively small Brønsted deviations.

Diethylmalonate dianion exhibits a negative Brønsted deviation in regard to both the hydration and hydrolysis of methyl pyruvate. In general, it has been observed for other reactions subject to general-base catalysis that the monohydrogen phosphate dianion is considerably more catalytic than diethylmalonate dianion despite the fact that the latter is a slightly stronger base.[12,23] There, in fact, is a distinct advantage associated with the relatively low catalytic effectiveness of the components of diethylmalonate, namely, that such buffers can be employed to regulate pH around physiological levels. Indeed, their use in reactions that are sensitive to general-acid and general-base catalysis facilitates the precise isolation of the enzymatic component from the overall rate.[5-7,11b]

The higher pK_a of the conjugate acid of diethylmalonate dianion relative to that of aliphatic monocarboxylate ions corresponds to enhanced stability of diethylmalonate monoanion through intramolecular hydrogen bonding (III) and, to a lesser extent, the



III

statistical advantage associated with four rather than two oxygen atoms available for the acceptance of a proton. However, when diethylmalonate dianion functions as a general-base catalyst, only partial stabilization would be gained by hydrogen bonding in the developing carboxylic acid group or the transition state. Thus, the relative catalytic effectiveness of diethylmalonate dianion in general-base-catalyzed reactions would be expected to vary depending on the mechanism and the extent of proton transfer in the transition state. Normal catalytic behavior, then, would be expected to range from that corresponding to aliphatic monocarboxylate ions to that associated with a general base with a pK_a value around neutrality.

It will be noted for both the hydration and hydrolysis of methyl pyruvate that catalysis by hydroxide ion exhibits a very high positive deviation, in comparison to the general bases investigated (Figure 4 and Table II). We suggest that the hydroxide ion behaves as a nucleophilic catalyst and, further, that the high positive deviation indicates at least the proclivity toward such catalysis by acyl-activated esters with a poor leaving group.

The Brønsted equation and other linear free-energy relationships allow only approximate predictions of catalytic behavior, and deviations from ideality may be explained in terms of many factors.[25,26] However, the various kinetic determinations taken together in such a correlation allow one to establish a norm to which catalysis of reactions by enzymes can be compared. Carbonic anhydrase is one of the few enzymes that catalyzes reactions which show already significant catalytic contributions from buffer components.[4-11] The erythrocyte enzyme is unusually versatile.[3-11] It exhibits both hydrase and esterase activities and is reactive toward many different substrates.[3-11] Accordingly, a valid point of comparison of enzymatic effectiveness among substrates of the same reaction and even between different reactions is the magnitude of the positive deviation from the corresponding Brønsted plot. From the data in Table II, it is clear that the enzymatic hydration of carbon dioxide stands alone at the height of catalytic efficiency.[27,28] It is also instructive to note

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(24) (a) Pocker, Y.; Ronald, B. P. *J. Am. Chem. Soc.* **1978**, *100*, 3122. (b) Pocker, Y.; Ronald, B. P.; Ferrin, L. *Ibid.* **1980**, *102*, 7725.
(25) Kovach, I. M.; Elrod, J. P.; Schowen, R. L. *Ibid.* **1980**, *102*, 7530.
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that the positive Brønsted deviations for the BCA-catalyzed hydration and hydrolysis of methyl pyruvate are even more pronounced than those for the corresponding hydroxide ion catalyzed processes. Interestingly, for the hydration of methyl pyruvate, the enzymatic rate coefficient, k_{BCA}^{max}, is similar to that for the hydration of aliphatic aldehydes.[21] Indeed, for the reversible hydration reactions included in Table II, the positive Brønsted deviations are of comparable magnitude. It will be noted that for the hydrolysis of methyl pyruvate, the positive deviation of k_{BCA}^{max} from Brønsted behavior is second only to that for the hydration of CO_2.

The hydrolysis reaction exhibits a greater sensitivity toward general-base catalysis but a smaller relative deviation of k_{OH^-} from the extrapolated Brønsted slope. The hydration reaction, on the other hand, exhibits a smaller sensitivity toward general-base catalysis but a larger relative deviation of k_{OH^-} from the extrapolated Brønsted slope. The deviation of the enzymatic coefficient, k_2/K_m, is much larger for hydrolysis than for hydration. The above observations imply that there are general-base components in the enzymatic mechanism of carbonic anhydrase. This is in accord with the fact that the vast majority of carbonic anhydrase catalyzed reactions either require proton transfer as part of the stoichiometry of the reaction (e.g., hydrolysis) or include proton-transfer steps in their most reasonable mechanistic portrayals (e.g., hydration).[28,30] Since the concentrations of hydronium or hydroxide ions are too low at neutral pH values to permit sufficiently high rates of turnover, general catalysis is almost certainly operative in many, if not all, of these reactions.

Indeed, due to the presence of zinc-bound water (or OH^-) and of a proton-transfer group (imidazole), the catalytic site of carbonic anhydrase would be expected to show, inter alia, the characteristics of a general-base and a nucleophilic catalyst. Through catalytic versatility studies,[3-12] we note that the best substrates of the enzyme prove to be compounds, the reactions of which are catalyzed by general bases and which also have at least a tendency toward nucleophilic catalysis.

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Registry No. Methyl pyruvate, 600-22-6.

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Reaction of a Benzvalene with Sulfur Dioxide. A Chemical Cascade[1]

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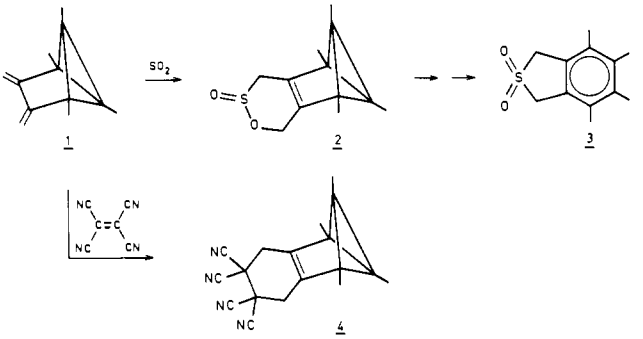
Abstract: The reaction between benzvalene derivative **4** and sulfur dioxide has been investigated in the temperature range −95 to +80 °C and found to follow a cascade-type pathway. The first step in the reaction leading to dipolar ion **5** is thought to be a charge-controlled attack of SO_2 at the central bond of the bicyclobutane moiety. Rearrangement and ring closure subsequently lead to isomeric sulfones **7** and **8** and sultine **9**. The latter compound readily extrudes sulfur monoxide, affording cyclopentadiene **10**. Both the facile sulfone–sultine rearrangement and the loss of sulfur monoxide occur at exceptionally low temperatures.

Sulfur dioxide has been known as a reagent in organic chemistry for almost a century,[2] and in recent years an avalanche of research about its chemical properties has appeared in literature.[3] Most studies of organic reactions of sulfur dioxide concern its behavior toward (di)enes. Sulfur dioxide has been reported to give π complexes with alkenes,[4] and quite recently has been found to induce the "ene" reaction.[5] The cheletropic reaction of sulfur dioxide with dienes, a $(2 + 4)(n + \pi\pi)$ type of reaction, has been known for a long time,[6] and recently[7] the $(2 + 4)(\pi + \pi\pi)$ mode of addition has also been reported. In the reaction of diene **1** with sulfur dioxide, a strong interaction between the bicyclobutane moiety and the unsaturated sites in the remaining part of the skeleton probably plays a role. This interaction induces some interesting facts. For example, diene **1** reacts in Diels–Alder reactions with a rate comparable to that of cyclopentadiene.[8] On

Scheme I



(1) With chemical cascade we imply a reaction involving a series of observable consecutively formed isomeric products. See also ref 9.

(2) As an example: the reaction of sulfur dioxide with alkenes was reported in 1888 by W. Solonina. In 1935 Staudinger and Ritzenthaler proved the product to be a polymer. See: Staudinger, H.; Ritzenthaler, B. *Ber.* **1935**, *68*, 455 and references cited therein.

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