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$$k_{CO_3^{2-}} = k_{HCO_3^-}\left(\frac{[\text{pteridine}]}{[\text{pteridine}\cdot H^+]}\right)\left(\frac{[HCO_3^-]}{[CO_3^{2-}]}\right) = k_{HCO_3^-}\left(\frac{K_1}{[H^+]}\right)\left(\frac{[H^+]}{K_{HCO_3^-}}\right)$$

$$= \frac{(k_{HCO_3^-})(K_1)}{K_{HCO_3^-}} = \frac{(1.5\ 1\ \text{mol}^{-1}\text{min}^{-1})(10^2)}{(60\ \text{sec/min})10^{-10}}$$

$$= 2.5 \times 10^{10}\ 1\ \text{mol}^{-1}\ \text{sec}^{-1}$$

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The Alcohol–Bicarbonate–Water System. Structure–Reactivity Studies on the Equilibria for Formation of Alkyl Monocarbonates and on the Rates of Their Decomposition in Aqueous Alkali[1]

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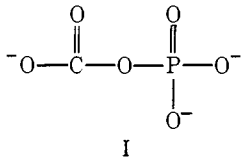
Abstract: The decomposition rates for the alkyl monocarbonates in aqueous alkaline solutions have been measured, and it has been shown that these reactions are characterized by a Bronsted β value for the leaving group of -1.1. This correlation provides a method for estimating the pK_a of weakly acidic alcohols. The equilibria for the reaction, $ROH + HCO_3^- \rightleftharpoons ROCO_2^- + H_2O$, have been measured for a smaller series of alcohols. The data thus obtained were used to calculate a Bronsted β value of 1.4 for the reaction, $RO^- + CO_2 \rightleftharpoons ROCO_2^-$, which shows that the carboxylate group is significantly more electron withdrawing than hydrogen in this reaction. The β value for nucleophilic attack of alkoxide ion on carbon dioxide was found to be 0.3. These results may be used to predict that carbonyl phosphate can exist only in very small amounts in aqueous solutions of bicarbonate and hydrogen phosphate dianion and that its rate of decomposition would be on the order of 10 sec^{-1}. It is suggested that "activated CO_2" represents low-entropy CO_2 fixed at the active site of carboxylating enzymes and made available by the ATP-mediated dehydration of bicarbonate or the decarboxylation of carboxybiotin in situ.

The available evidence derived from studies of a number of enzymic carboxylation reactions has led investigators to propose that enzyme-bound carbonyl phosphate I is an intermediate in these reactions. The existence of this interme-

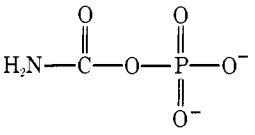


I

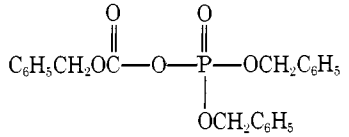
diate has been postulated in enzymic carboxylations requiring biotin[2,3] as well as in carbamyl phosphate synthetases from ureotelic vertebrates[4] and *E. coli*.[5] All of these enzymes require the utilization of a molecule of ATP that is cleaved to ADP and inorganic phosphate during the course of the reaction.

Support for the intermediacy of I is provided by the existence of bicarbonate-dependent ATPase activity for these enzymes,[4,5] a bicarbonate-dependent exchange of labeled ADP into ATP,[5e] and oxygen-18 labeling experiments, which demonstrate that the inorganic phosphate produced contains an oxygen originally present in the bicarbonate.[3,4b] Carbamyl phosphate II is a structural analog of carbonyl phosphate and is active as a phosphoryl donor toward ADP to give a rapid synthesis of ATP in the presence of the biotin carboxylase component of acetyl CoA carboxylase.[5f] On the other hand, kinetic studies with carbamyl phosphate synthetase from liver indicate that ammonia reacts before ADP is released; i.e., any active carbonyl phosphate–enzyme intermediate that is formed must also contain ADP.[6]

Derivatives of carbonyl phosphate such as carbamyl phosphate II[7] and dibenzylcarbobenzoxy phosphate III,[8a] and other alkyl esters of I[8b,8c] are known compounds. Attempts to observe metal ion promoted addition of phosphate to carbon dioxide with the formation of the carbonyl phosphate trianion I were unsuccessful,[8a] and so far this compound has not been isolated.



II



III

In view of the stability of compounds II and III and because alkyl monocarbonates have been extensively studied by others,[9-11] it seemed possible that the equilibrium governing the formation and breakdown of carbonyl phosphate might be in an accessible range. We reasoned that a structure–reactivity study of a series of related compounds would supply information which could be used to predict the behavior of I in nonenzymic and possibly in enzymic reactions. Thus, we selected for study the following equilibrium reaction:

$$\mathrm{ROH} + \mathrm{HCO_3^-} \overset{K_{eq}}{\rightleftharpoons} \underset{\mathrm{IV}}{\mathrm{ROCO_2^-}} + \mathrm{H_2O} \qquad (1)$$

in which ROH represents a series of aliphatic alcohols of varying pK_a. In addition to the position of equilibrium for this reaction, we have studied the rate of breakdown of the alkyl monocarbonates IV, as a function of the pK_a of the precursor alcohols.

Earlier workers have already established some facts about this system.[9,10] It has been shown that the breakdown of ethyl monocarbonate is pH independent at high pH where the alkyl monocarbonate would be completely in the ionized form. Since the reaction stoichiometry requires the loss of a mole of hydroxide ion from the solution, it has been concluded that the reaction must proceed in a stepwise fashion as shown in Scheme I.[9-11]

Scheme I

$$\mathrm{ROCO^-} \rightleftharpoons \mathrm{RO^-} + \mathrm{CO_2} \qquad (2)$$

$$\mathrm{CO_2} + \mathrm{OH^-} \rightleftharpoons \mathrm{HCO_3^-} \qquad (3)$$

$$\mathrm{RO^-} + \mathrm{H_2O} \rightleftharpoons \mathrm{ROH} + \mathrm{OH^-} \qquad (4)$$

$$\mathrm{HCO_3^-} + \mathrm{OH^-} \rightarrow \mathrm{CO_3^{2-}} + \mathrm{H_2O} \qquad (5)$$

overall $$\mathrm{ROCO^-} + \mathrm{OH^-} \rightarrow \mathrm{ROH} + \mathrm{CO_3^{2-}} \qquad (6)$$

In a series of investigations extending over the years 1927–1958, Faurholt and his coworkers studied the bicarbonate–alkyl monocarbonate equilibrium in a variety of aqueous alcohol solutions at 0°.[9] In addition these investigators studied the rate of alkyl monocarbonate formation in strongly basic (0.1 *M* NaOH) aqueous alcohol solutions which were shaken with gaseous carbon dioxide. From the knowledge of the equilibrium constants as well as the relative rates of attack of alkoxide and hydroxide on carbon dioxide, the rates of breakdown of the alkyl monocarbonates were calculated.

The high pH at which the rate of attack of alkoxide on carbon dioxide was studied produces some uncertainty in the calculated rate constants. Thus Faurholt has defined:

$$\frac{k_{CO_2}\cdot\mathrm{RO^-}}{K_{\mathrm{RO^-}}} = k' = \frac{(\%\ \mathrm{ROCO^-})(k_{CO_2}\cdot\mathrm{OH^-})}{(\%\ \mathrm{CO_3^{2-}})[\mathrm{ROH}]_{\mathrm{total}}}$$

where $K_{RO^-} = K_w/K_a$ for ROH and $(k_{CO_2}\cdot RO^-)$ and $(k_{CO_2}\cdot OH^-)$ are the rate constants for attack of alkoxide and hydroxide ions on carbon dioxide. It may be shown that at pH 13 this equation does not hold for alcohols of pK lower than 15. The analyses were carried out using barium chloride solution to precipitate excess carbonate at 0°; a second precipitation after heating precipitated the carbonate from alkyl monocarbonate. Some decomposition of the alkyl monocarbonates could take place during the analytical procedures and thereby affect the results. In the present work, we have sought to change the experimental design.

Experimental Section

Materials. Commercial ethyl chloroformate, methyl chloroformate, 2,2,2-trichloroethyl chloroformate, isobutyl chloroformate, butyl chloroformate, hexyl chloroformate, propyl chloroformate, isopropyl chloroformate, 3-chloropropyl chloroformate, 2-chloroethyl chloroformate and *tert*-butyl azidoformate were redistilled and were stored in desiccators over Drierite in the freezer or were used immediately after distillation. 2-Methoxyethyl chloroformate, propargyl chloroformate, and 2,2-dichloroethyl chloroformate were prepared from the reaction of phosgene (12.5% solution in benzene) with the appropriate redistilled alcohol.[12] Freshly distilled dimethylaniline was used as a reagent for the latter two preparations. Great caution was exercised for the reactions of phosgene. They were run in the hood and flasks containing morpholine in ether were used to trap excess phosgene evolving from the reaction mixture during the reactions and after the acylations were completed. 2-Methoxyethyl chloroformate and 2,2-dichloroethyl chloroformate were purified by distillation under reduced pressure; NMR and ir analyses confirmed their purity. Propargyl chloroformate boiled so close to the reaction solvent that purification was carried out by preparative gas chromatography on a 6-ft 20% SE-30 column at 100°, injection port temperature of 140°. Thus prepared, the propargyl chloroformate was contaminated with ca. 20% propargyl chloride. The latter compound did not appear to interfere with the kinetic runs. Reagent grade methanol and absolute ethanol were used to prepare the alcoholic bicarbonate solutions for the equilibrium measurements; all other alcohols were redistilled before preparation of the solutions. Reagent grade inorganic salts were used without purification; solutions prepared from boiled glass-distilled water for the kinetic runs were deaerated with an aspirator prior to use. Tropaeolin O, pK_a = 11.06 (K & K Lab-

Table I. Experimental Conditions for the Measurement of the Rate of Decomposition of Alkyl Monocarbonates at 25.0° in Water at Ionic Strength 1.0 (KCl) Containing 5% Acetonitrile

Alcohol	[Monocarbonate][a], M	pH[b]	Indicator	ΔA[c]	No. runs
CH_3OH	1.3×10^{-3}	11.82–11.02	Tropaeolin O 495 nm	0.150	3
CH_3OH	6×10^{-3}				
	1.2×10^{-2}	11.5	*d*	*d*	5
C_2H_5OH	1.6×10^{-3}	11.72–11.20	Tropaeolin O 495 nm	0.200	3
C_2H_5OH	3×10^{-3}				
	6×10^{-3}	11.5	*d*	*d*	3
$CH_3OCH_2CH_2OH$	1.3×10^{-3}	11.75–11.20	Tropaeolin O 495 nm	0.250	6
$CH_3OCH_2CH_2OH$	2×10^{-3}				
	4×10^{-3}	11.5	*d*	*d*	4
$ClCH_2CH_2OH$	9.1×10^{-4}	11.58–11.00	Alizarin 568 nm	0.180	5
$ClCH_2CH_2CH_2OH$	1.59×10^{-3}	11.7–11.3	Tropaeolin O 485 nm	0.130	8
$HC{\equiv}CCH_2OH$	5.3×10^{-4}	11.7–11.5	Tropaeolin O 495 nm	0.150	3
Cl_2CHCH_2OH	7.8×10^{-4}	11.6–11.0	Tropaeolin O 495 nm	0.180	5
Cl_3CCH_2OH	5.0×10^{-4}	11.95–11.7	Tropaeolin O 495 nm	0.150	2
		11.7–11.4			3
		11.7–11.1			4
i-C_4H_9OH	1.52×10^{-3}	11.7–11.1	Tropaeolin O 485 nm	0.150	3
n-C_3H_7OH	1.63×10^{-3}	11.7–11.1	Tropaeolin O 485 nm	0.190	
i-C_3H_7OH	2.04×10^{-3}	11.7–11.0	Tropaeolin O 485 nm	0.200	5
n-C_4H_9OH	1.58×10^{-3}	11.7–11.0	Tropaeolin O 485 nm	0.200	4
n-$C_6H_{13}OH$	1.52×10^{-3}	11.7–11.3	Tropaeolin O 485 nm	0.350	8
t-C_4H_9OH	1.50×10^{-3}	11.7–11.0	Tropaeolin O 485 nm	0.250	2

[a] Calculated initial concentration of alkylchloroformate esters injected in dry acetonitrile (5% v/v). [b] The pH ranges shown include the pH change associated with attack of hydroxide ion on the chloroformate as well as the decomposition of alkyl monocarbonate. [c] Absorbance change for the monocarbonate decomposition. Total absorbance change included chloroformate hydrolysis and was about three times the figures shown. [d] pH-stat runs.

oratories, Plainview, N.Y.) and Alizarin, pK_a = 11.40 (Eastman Kodak, Rochester, N.Y.) were used without further purification. Reagent grade acetonitrile for the kinetic runs was stirred with calcium hydride, then distilled from the hydride, and stored over molecular sieves (Davison type 4A grade 514, 8–12 mesh) in bottles equipped with serum caps. Solutions of the chloroformates in acetonitrile were made up on the day of use.

Kinetic Measurements. All runs were carried out at 25.0 ± 0.1° and at ionic strength of 1.0 unless otherwise noted. Kinetic runs were carried out using either Gilford 2000 or Gilford 240 recording spectrophotometers and a rapid mixing apparatus[13] or conventional spectroscopic techniques. A few kinetic and equilibrium determinations were carried out on a Radiometer Copenhagen pH-stat type TTTlc equipped with a type B glass electrode. pH measurements were made on Radiometer Copenhagen pH meter Model 26.

A summary of the reaction conditions for the kinetic runs is given in Table I. The alkyl chloroformates were added rapidly or injected into aqueous solutions of either Alizarin or Tropaeolin O adjusted to ca. pH 11.7. The decomposition of the alkyl monocarbonates thus formed was followed by monitoring the absorbance at an appropriate wavelength.

Equilibrium Measurements. Solutions of alcohols (1.5–4 M) and potassium or cesium bicarbonate (2–6.9 M) were permitted to stand for at least 24 hr. Proton magnetic resonance spectra of the solutions prepared from ethanol, methanol, and propargyl alcohol contained peaks with the appropriate splitting patterns which were assigned to the hydrogen atoms attached to the carbon bearing the $-OCO_2^-$ group of the alkyl monocarbonates. Equilibrium constants for the reactions were calculated from the distribution of the peak areas for the alcohol and the alkyl monocarbonate together with the known initial concentrations of the alcohol, bicarbonate ion, and water. (The volume of water needed to make a standard volume of the original solutions was measured by pipet.) The spectra of methoxyethanol–bicarbonate solutions were too complex to be analyzed by this method.

The spectrum of chloroethanol in D_2O containing bicarbonate from a solution which was allowed to stand for 1 week showed the formation of a new singlet at 0.11 ppm upfield from the overlapping triplets from the original alcohol.[14]

The spectrum of trifluoroethanol in H_2O or D_2O was found to be identical in the presence and absence of bicarbonate. We estimate that 2% of the adduct should have been observable; this gives an upper limit of 0.16 for the equilibrium constant, K_{eq}, in this system.

A carbon-13 Fourier transform NMR spectrum was measured for the methanol–deuterium oxide–cesium bicarbonate system. When the initial concentrations were $[CH_3OH] = 2.47\ M$, $[D_2O] = 43.75\ M$, and $[CsHCO_3] = 5.23\ M$, a peak for the adduct was observed which was 24.6% of the total methyl signal. No attempt was made to determine whether the methyl signals from the alcohol and the adduct were representative of the molar concentrations of these species. Examination of the spectrum for trifluoroethanol in D_2O containing cesium bicarbonate (5.20 M) revealed no additional peaks which could be ascribed to the adduct after 13,614 pulses.

Measurement of the proton NMR spectra for isopropyl alcohol and isopropyl alcohol-d_6 (prepared by the lithium aluminum hydride reduction of acetone-d_6) in 3.07 M cesium bicarbonate did not reveal any peaks which could be attributed to the adduct.

The equilibrium constant for the methoxyethanol system was measured from the total amount of hydroxide solution needed to maintain constant pH on the pH stat during the kinetic runs made by this method.

Solubility problems prohibited the measurement of the equilibrium constants for *n*-propyl alcohol, the butyl, and higher alcohols.

Table II. Experimental Conditions for Measurements of the Equilibrium $HCO_3^- + ROH \rightleftharpoons ROC(=O)O^- + H_2O$

Alcohol	Initial molarities					Chemical shift, ppm[a]	% adduct at equil.
	ROH	$CsHCO_3$	$KHCO_3$	H_2O	D_2O		
CH_3OH	2.46		1.80	50.5		0.19	9.9
	2.46		1.80		45.6	0.19	8.7
	2.46	6.20		51.1		0.19	25.8
C_2H_5OH	1.72		1.80	51.1		0.27	5.6
	1.72		1.80		45.5	0.28	4.5
$HC{\equiv}CCH_2OH$	1.73	6.20		52.2		0.27	5.5
	1.73		1.80		45.5	0.27	2.0
F_3CCH_2OH	1.00	6.20		51.0		Adduct was not observed[b]	

[a] Chemical shift for the $-CH_2-OCO_2^-$ downfield from the resonance of corresponding hydrogen atoms in the alcohol. [b] If it is assumed that 2% of the adduct would have been observed, an upper limit of 0.16 can be assigned to the equilibrium constant.

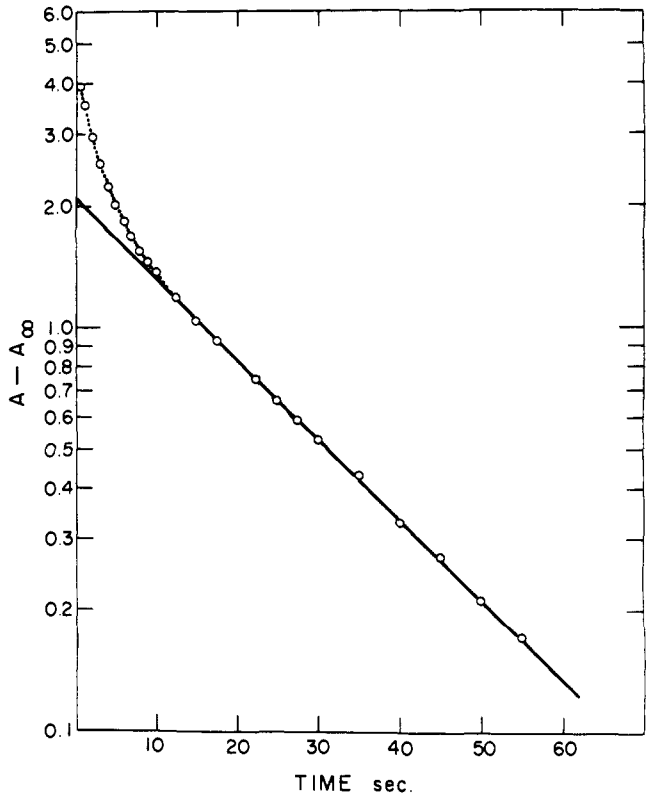


Figure 1. Decomposition of 2-chloroethyl chloroformate at pH 11.58-11.00 followed by observing the absorbance change of alizarin at 568 nm.

The experimental conditions for the equilibrium measurements are summarized in Table II.

Results

The alkyl monocarbonates IV were formed in situ by the rapid addition of the corresponding chloroformate esters or *tert*-butyl azidoformate to aqueous solutions at pH 11.4-11.9. At these levels of hydroxide ion concentration, attack of hydroxide ion on the carbonyl carbon of the chloroformate or *tert*-butyl azidoformate was extremely rapid. During this initial rapid uptake of hydroxide ion, the substrate was entirely converted to the monocarbonate. The disappearance of the latter compound could then be followed. The method depends on the rapid quenching of the carbon dioxide by reaction with hydroxide ion. At 25°, the second-order rate constant for this reaction is 8500 M^{-1} sec^{-1},[15] which gives a pseudo-first-order rate constant of 42.5 sec^{-1} for carbon dioxide disappearance at pH 11.3. This is sufficiently faster than the decomposition rate being measured so that hydration of carbon dioxide is not the rate-determining step for most of the reactions studied. However, this step could be important in the decomposition of trichloroethyl monocarbonate at pH 11.

Since the decomposition of the alkyl carbonate is not accompanied by a readily measurable change in the uv spectrum of the reaction mixtures, an indirect indicator method was devised for the kinetic runs. According to eq 6, 1 mol of hydroxide ion is removed from the reaction mixture as 1 mol of alkylmonocarbonate decomposes. This stoichiometric relationship holds as long as the pH is high enough so that the carbonate (pK_a = 9.77 at μ = 1.0) is almost completely converted to the dianion and the pH is low enough so that the ionization equilibrium for the alcohol lies on the un-ionized side. These conditions are met in the pH range 11.0-11.6, where the kinetic runs were made. (For kinetic runs made at constant pH on the pH stat, it was not necessary to maintain the pH high enough to convert all bicarbonate to carbonate since as long as the $[HCO_3^-]/[CO_3^{2-}]$ ratio is constant, the rate of hydroxide ion disappearance is directly proportional to the rate of decomposition of alkylmonocarbonate ion.)

The disappearance of hydroxide ion was measured indirectly by measuring the change in absorbance of the indicators Tropaeolin O or Alizarin which were present in concentrations of 1-2.5 × 10^{-4} M. Titrations of the reaction mixtures (without the chloroformates) with standard hydrochloric acid showed that absorbance of the indicator solutions is a linear function of the hydroxide ion concentration over the small pH ranges covered by the reactions. Absolute absorbances at the beginning of the runs were ca. 1.400; at the end of a run, they had dropped to ca. 0.800.

A typical logarithmic plot of the kinetic runs is shown in Figure 1. An initial steep curved slope which characterized the rapid attack of hydroxide on the chloroformate to yield the monocarbonate is followed by a less steep slope which is linear for 3-4 half-lives. That the second slope does indeed represent first-order alkyl monocarbonate decomposition was confirmed by comparison of three of the rate constants obtained by using the chloroformate-indicator method with the corresponding rate constants for the alkyl monocarbonate decomposition measured with the pH stat. In the latter experiments, an alcohol (2-8 M) was dissolved in an aqueous bicarbonate solution (0.4 M), and the mixture was permitted to stand for 24 hr until the alkyl monocarbonate equilibrium had been established. Aliquots of the reaction mixtures were then injected into sodium hydroxide solutions at pH 11 to 11.3 in the pH-stat apparatus set at 11 to 11.3. The rate of consumption of 0.5 M NaOH required to maintain a constant pH in the reaction mixture is a measure of the rate of alkyl monocarbonate breakdown.

The values for the equilibrium constant, K_{eq}, for eq 1 and the rate constants for alkyl monocarbonate decomposition

Table III. Summary of Rate and Equilibrium Constants for the Reactions of Carbon Dioxide with Alcohols at 25°

Compd	pK_a[a]	k_{decomp},[b] sec^{-1}	K_{eq}[b]	K_{eq}',[b] M	k_{attack},[b] M^{-1} sec^{-1}
CH_3CH_2OH	16	$8.62 (\pm 0.20) \times 10^{-4}$	1.80	1.47×10^{8}	1.27×10^{5}
CH_3CH_2OH[c]	16	$1.1 (\pm 0.4) \times 10^{-3}$			
H_2O	15.7	1.9×10^{-4}	1.00	4.5×10^{7}	8.50×10^{3}
CH_3OH	15.54	$1.65 (\pm 0.07) \times 10^{-3}$	3.57	1.02×10^{8}	1.68×10^{5}
CH_3OH[c]	15.54	$1.63 (\pm 0.06) \times 10^{-3}$			
$CH_3OCH_2CH_2OH$	14.82	$6.52 (\pm 0.20) \times 10^{-3}$	1.7	9.22×10^{6}	6.01×10^{4}
$CH_3OCH_2CH_2OH$[c]	14.82	$5.0 (\pm 0.8) \times 10^{-3}$			
$ClCH_2CH_2CH_2OH$		$2.17 (\pm 0.26) \times 10^{-3}$			
$ClCH_2CH_2OH$	14.31	$4.67 (\pm 0.17) \times 10^{-2}$			
$HC{\equiv}CCH_2OH$	13.55	$3.00 (\pm 0.33) \times 10^{-1}$	0.50	1.45×10^{5}	4.35×10^{4}
Cl_2CHCH_2OH	12.89	1.15 ± 0.08			
Cl_3CCH_2OH	12.24	2–5			
F_3CCH_2OH	12.43		0.16[d]	3.52×10^{3}[d]	
i-C_4H_9OH		$4.58 (\pm 0.05) \times 10^{-4}$			
n-C_4H_9OH		$5.42 (\pm 0.58) \times 10^{-4}$			
i-C_3H_7OH	15.91[e]	$3.35 (\pm 0.11) \times 10^{-4}$			
n-C_3H_7OH	15.87[e]	$4.70 (\pm 0.07) \times 10^{-4}$			
n-$C_6H_{13}OH$		$3.80 (\pm 0.43) \times 10^{-4}$			
t-C_4H_9OH	16.04[e]	$2.99 (\pm 0.03) \times 10^{-4}$			

[a] pK_a values are taken from P. Ballinger and F. A. Long, *J. Am. Chem. Soc.*, 82, 795 (1960), except as otherwise noted. [b] Rate constants k_{attack} and k_{decomp} refer to the reverse and forward steps of eq 2. Equilibrium constants K_{eq} and K_{eq}' refer to eq 1 and the reverse of eq 2, respectively. The actual measured molarity of water was used in determination of K_{eq}. [c] pH-stat runs. [d] Upper limit (Table II). [e] Values taken from S. Takahashi, L. A. Cohen, H. K. Miller, and E. G. Peake, *J. Org. Chem.*, 36, 1205 (1971).

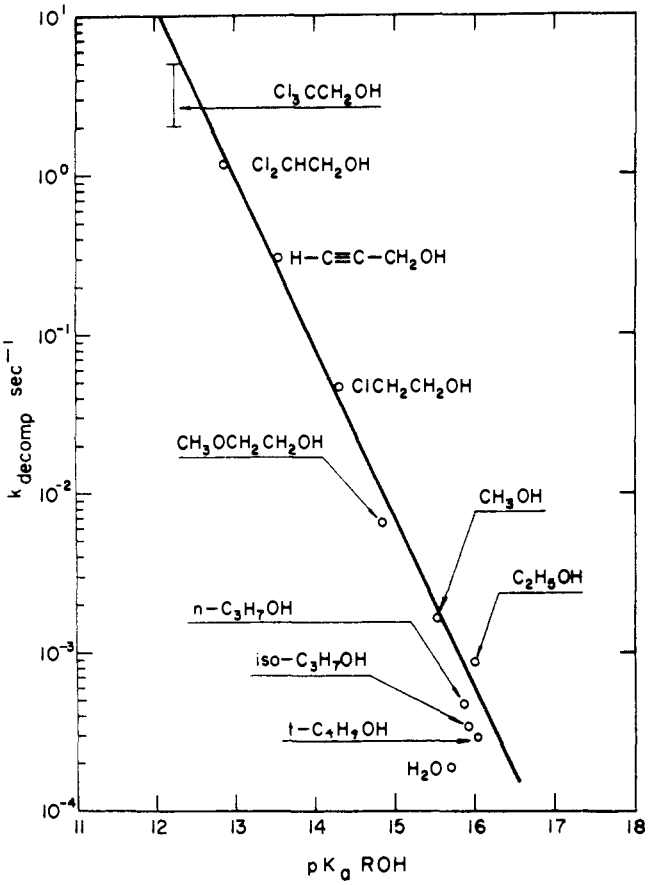


Figure 2. Bronsted plot for the rate of decomposition of alkyl monocarbonate anions in water at ionic strength 1.0 (KCl) at 25°. The value of $-\beta_{lg}$ is 1.1 ± 0.1.

are given in Table III. The actual molarity of water was used in the determination of values for K_{eq}. Values of the rate constants for the attack of RO^- on CO_2 may be calculated in the following way. Equation 2 in the reverse direction is the sum of eq 1, 3, and the water ionization reaction minus the alcohol ionization reaction. Therefore

$$k_{attack} = k_{decomp} \frac{K_{eq} K_3 K_a(H_2O)}{K_a(ROH)}$$

The value for the equilibrium constant for eq 3, $K_3 = 4.5 \times 10^7\ M^{-1}$, was obtained from the values of the rate constants for the forward and reverse steps ($k_f = 8500\ M^{-1}\ sec^{-1}$ and $k_r = 1.9 \times 10^{-4}\ sec^{-1}$).[15] The values for the equilibrium constant, K_{eq}' for eq 2 in reverse were calculated from the equation

$$K_{eq}' = \frac{[ROCO_2^-]}{[RO^-][CO_2]} = \frac{k_{attack}}{k_{decomp}}$$

and are shown in Table III.

Miller and Case measured the decomposition of ethyl monocarbonate by two different methods at 25.1° and found values for k_{decomp} of 9.06 (±0.1) × 10^{-4} sec^{-1} and 8.30 (±0.1) × 10^{-4} sec^{-1}.[10] These values are in fair agreement with the value of 8.62×10^{-4} sec^{-1} obtained by the decomposition of ethyl chloroformate.

The data of Faurholt and coworkers can be used to calculate K_{eq}. These calculations yield values of 4.51 and 1.91 at 0° for methanol and ethanol, respectively.[9] These values are in fair agreement with the values at 25° obtained in this work (see Table III) and suggest that the equilibrium constants are not very dependent on temperature. The decomposition rate constants measured by Faurholt et al. at 0° are approximately 20-fold smaller than those obtained in the present work at 25° and show the same order of reactivity for the alcohols that were examined in both series.

Discussion

The rates of decomposition of the alkyl monocarbonates are sensitive to electronic effects. A plot of log k vs. pK_a of the corresponding alcohols (Figure 2) has a slope, β, for the leaving group of −1.1 ± 0.1.

This Bronsted plot exhibits a good fit to the data for alcohols of known pK_a and provides a method for the estimation of the pK_a of weakly acidic alcohols that may be simpler and less subject to error than potentiometric titration. The solid line in Figure 2 is drawn from eq 7:

$$\log k_{decomp} = -1.1\ pK_a + 14.3 \qquad (7)$$

Based on this equation and the observed rate constants, the following pK_a values may be estimated: 3-chloro-1-propanol, 15.5; ethyl alcohol, 15.8; n-propyl alcohol, 16.0; n-butyl

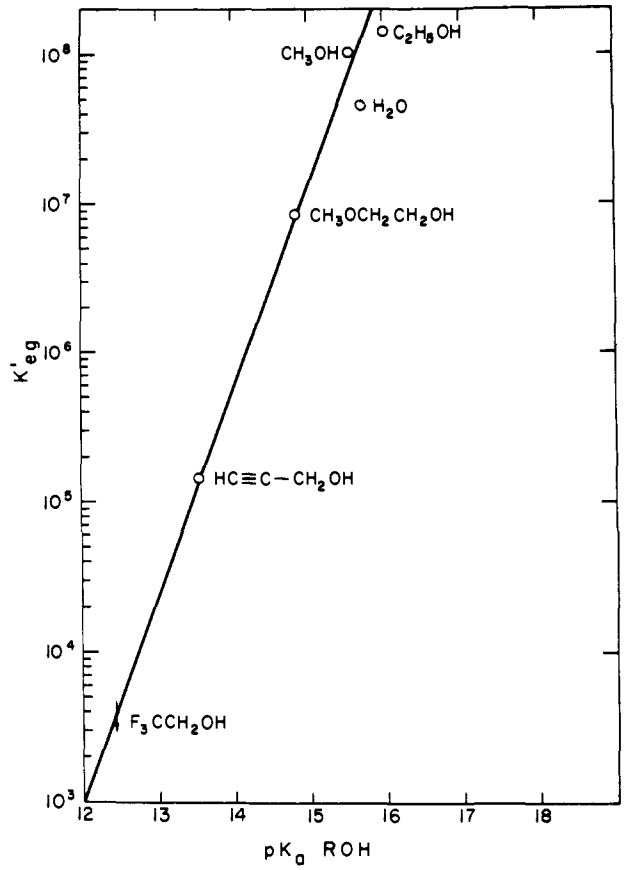


Figure 3. Bronsted plot for the equilibrium constant for formation of the alkyl monocarbonates from alkoxide and carbon dioxide. The value of β is 1.4 ± 0.1.

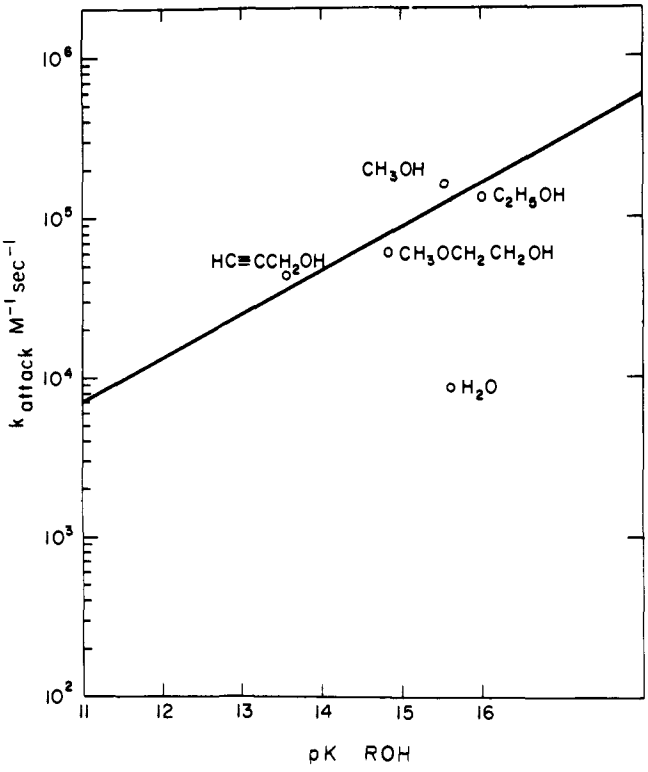


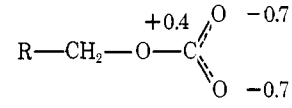
Figure 4. Bronsted plot for the rate of attack of alkoxide ions on carbon dioxide. The line is drawn with a slope, β_{nuc}, of 0.3.

alcohol, 15.9; isobutyl alcohol, 15.95; isopropyl alcohol, 16.2; *tert*-butyl alcohol, 16.2; and *n*-hexyl alcohol, 16.05. The calculated value of 16.5 for water is anomalous; however, it is similar to a value of 16.4 based on the rate constants for reactions of acetate esters and acetic acid.[16] These pK_a values will, of course, reflect any differences in solvation free energy for the formation of the transition state compared with formation of alcoholate ion in the ionization reaction.

A plot of log K_{eq}' vs. pK_a of the alcohol for eq 2 is shown in Figure 3. The value of β_{eq} for carbon dioxide transfer to alkoxide ion is 1.4 ± 0.1. The Bronsted plot for the attack of alkoxide ions on carbon dioxide (Figure 4) is consistent with the expected β_{nuc} value for attack of alkoxide attack on carbon dioxide of 1.4 − 1.1 = 0.3 ± 0.1. Based on a pK_a of 15.74, the point for water shows a small negative deviation from the line for K_{eq}' and a larger deviation from the line for k_{attack}.

The transition state for alkyl monocarbonate decomposition must occur rather late along the reaction coordinate and, conversely, the attack of alkoxide ions on CO_2 must occur early on the reaction coordinate to give the β_{nuc} value of 0.3. A similar small sensitivity to the basicity of the nucleophilic reagent is found in the attack of alcoholate ions on *p*-nitrophenyl acetate and the attack of amines on cyanic acid.[17] In the attack step of alkoxide on carbon dioxide, no leaving group is involved. The hybridization change from the linear carbon dioxide molecule to the trigonal alkyl monocarbonate molecule involves a greater change in bond angles (180–120°) than the corresponding change when an acyl carbon proceeds to a tetrahedral intermediate and/or transition state (120–109°). The results suggest that the highest point on the reaction coordinate in either direction is reached when the carbon dioxide molecule is distorted only slightly from its linear configuration.

The β value for K_{eq}' is larger (1.4) than the formal change in charge on the alcohol oxygen atom (1.0) for the entire reaction. Since the β value for the loss of one negative charge on an alcoholate ion upon protonation is 1.0, by definition, the β value of 1.4 for K_{eq}' means that electron-donating substituents stabilize the alkyl monocarbonates relative to the free alcohols, ROH, by the amount that would be expected if there were an effective positive charge of +0.4 on the alcohol oxygen atom of the ester and a charge of −0.7 on each of the other oxygen atoms. This effect of substituents shows that, in spite of the negative charge on the carboxylate group, there is sufficient electron delocalization from the alcohol oxygen atom into the carboxylate group to make this group rather strongly electron withdrawing relative to hydrogen. The Hammett σ values for $-CO_2'$ located in the meta and para positions are −0.10 and 0.00, respectively. The negative value for the meta position results from the electron-donating charge effect, and the value for the para position indicates that a compensating electron-withdrawing resonance effect is in operation.[18] The downfield chemical shifts of the methylene hydrogen atoms of 0.19–0.27 for the alkyl monocarbonates and 0.42 for ethyl acetate, compared with the corresponding alcohols, indicate a larger deshielding for the latter compound and an approximate parallelism of the chemical shifts with the "effective charges" on the alcohol oxygen atoms of +0.4 for the alkyl monocarbonates and +0.7 for alkyl acetates.[19,20]



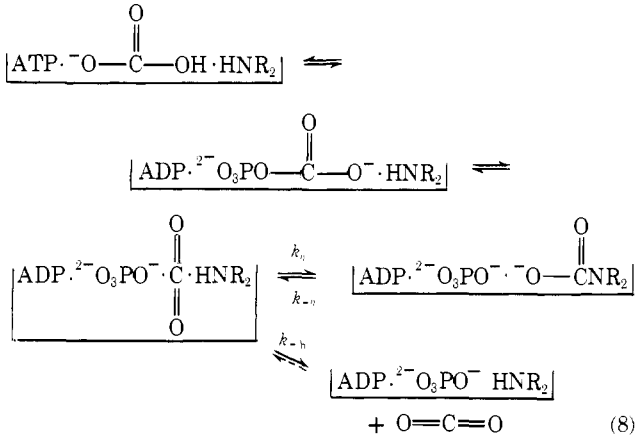
It is of interest to compare the β_{lg} value of −1.1 obtained in this study with the value of −1.15 obtained in the study of alkyl and aryl *N*-phenylcarbamates.[21] It is apparent that

the development of charge on the leaving alkoxide or aryloxide group is independent of whether the elimination product is carbon dioxide or phenyl isocyanate. It is also worth noting that, when ketene is the elimination product as is the case in the Ecb1 hydrolyses of aryl acetoacetates, the value of β_{lg} is -1.29.[22] The alkyl acetoacetates do not hydrolyze by an elimination mechanism and this is apparent from the β_{lg} value of -0.05 for these compounds.

Some previously known facts can be rationalized in terms of these data. Faurholt found that the adduct of sodium phenoxide treated with carbon dioxide in anhydrous toluene was "instantaneously" decomposed in water.[9] Based on the present work, the predicted rate of breakdown in water at 25° is approximately 2×10^3 sec^{-1} for phenyl monocarbonate anion.

The data permit calculation of the expected rate and equilibrium constants for carbonyl phosphate. Based on the pK_a for hydrogen phosphate dianion of 12.0, the rate of decomposition of this species should be about 10 sec^{-1}. The equilibrium constant K_{eq}' will be about 10^3 M^{-1} and, from this, the equilibrium constant K_{eq} may be calculated to be about 0.13. Electrostatic repulsion of the negative charges will have an additional destabilizing effect so that the direct observation of carbonyl phosphate in solution will be difficult.

The nonenzymic rate of decomposition of carbonyl phosphate, $k_{decomp} \geq 10$ sec^{-1}, appears to be adequate to account for the observed bicarbonate-dependent ATPase activity of carbamyl phosphate synthetase ($k \geq 0.3$ sec^{-1})[6] with relatively little or no assistance from catalysis by the enzyme if carbonyl phosphate is the immediate product of the reaction of ATP and bicarbonate. In eq 8, the irreversible step of the hydrolysis reaction is shown as the dissociation of carbon dioxide from the enzyme (k_h); alternatively, carbonyl phosphate may itself dissociate from the enzyme and undergo decomposition in solution.[23] According to this



(8)

mechanism, the bound carbon dioxide can alternatively react with a bound amine acceptor, such as ammonia, glutamine, or biotin, to give the carbamate product of the reaction (k_n). In the case of carbamyl phosphate synthetase, carbamate is presumably phosphorylated by a second mole of ATP to give carbamyl phosphate.[4,5]

It has not been clear why bicarbonate ion and carboxybiotin, which are unreactive toward nucleophilic attack, are utilized in biological carbon dioxide "activation" in preference to the chemically much more reactive carbon dioxide molecule itself. The mechanism of eq 8 provides a possible answer to this problem. The small carbon dioxide molecule has little binding energy available for its fixation at the active site in a favorable concentration and position, with the corresponding loss of translational and rotational entropy, for rapid reaction.[24] According to the mechanism of eq 8, the role of the ATP-mediated phosphorylation of bicarbonate is to *deliver* a reactive molecule of carbon dioxide with a low entropy and hence a high Gibbs free energy, as a consequence of its localization in the correct position at the active site to react with the amine acceptor. The high local concentration of this molecule of carbon dioxide provides an effective driving force for its reaction with the bound acceptor so long as it reacts with the acceptor more rapidly than it dissociates into solution. A molecule may have a high Gibbs free energy that makes it effectively an "energy-rich" compound as a consequence of its fixation and decreased entropy, as well as chemical activation, and the Gibbs energy requirement for the reversible formation of such a molecule may be supplied by coupling its formation to the hydrolysis of ATP. It is known that the binding of inorganic phosphate adjacent to ADP on myosin and next to a carboxylate group of sodium–potassium ATPase makes possible the reversible synthesis of bound ATP and an acyl phosphate, respectively.[25]

The hypothesis that bound carbon dioxide represents the active intermediate in biotin-dependent reactions is consistent with several experimental observations. Carboxybiotin generally undergoes more rapid decarboxylation when bound to the active site of an enzyme than in solution, by a factor of up to 10^6 in the case of biotin-mediated decarboxylation reactions.[2,26] If the rate-determining step of this reaction is the dissociation of bound carbon dioxide, the addition of acceptor molecules that decrease the steady-state concentration of carbon dioxide at the active site would decrease the observed rate of decarboxylation. This is consistent with the observed inhibition of carboxybiotin breakdown by inorganic phosphate,[27] which can react with the bound carbon dioxide to give carbonyl phosphate (eq 8). Enzymes in this class also catalyze the biotin-independent decarboxylation of malonyl CoA and other carboxylation products.[28] The inhibition of this decarboxylation by biotin can be accounted for similarly by a trapping of bound carbon dioxide to give carboxybiotin. The long lifetime of carboxybiotin in solution means that this molecule is well fitted to serve as a coenzyme carrier of activated carbon dioxide from one active site to another.[2,26,29] The biotin group provides a large binding energy to bring the carboxylate group into the active site where it is activated for decarboxylation, perhaps by being forced into a poor ion-solvating environment,[30] to deliver carbon dioxide at a high local concentration and in the proper position for reaction. If the enzyme activated the carboxylate group toward nucleophilic attack by interaction with an electrophilic group, such as a metal ion, it might be expected that this interaction would decrease the driving force for decarboxylation by stabilizing the negative charge on the carboxylate group and thereby cause a decrease, rather than an increase, in the rate of decarboxylation.[29,31]

If this hypothesis is correct and bound carbon dioxide can dissociate from the enzyme before reacting with water, there should be a detectable formation of carbon dioxide that is not equilibrated with labeled oxygen in the solvent from the ATPase, substrate decarboxylation, or biotin decarboxylation activity of these enzymes. If these enzymic reactions represent activation of the carboxylate group toward nucleophilic attack by water, rather than the normal acceptor molecule, oxygen from the solvent will be incorporated into the product. Conversely, since the dissociation of carbon dioxide should be reversible, it is possible that a sufficiently high concentration of labeled carbon dioxide in the medium could give incorporation into carbamyl phosphate or other reaction products of this class of enzymes without passing through bicarbonate or requiring the cleavage of ATP.[32]

The extrapolated equilibrium constant K_{eq}' of $\sim 10^3\ M^{-1}$ means that the equilibrium constant for the dissociation of bound carbonyl phosphate to carbon dioxide and phosphate trianion will be small. If the phosphate becomes protonated to the dianion, the apparent equilibrium constant at pH 8.0 is $\sim 0.1\ M^{-1}$ so that a considerable fraction of the bound carbon dioxide will exist as carbonyl phosphate, depending on the "effective molarity" of bound carbon dioxide and phosphate relative to each other at the active site. The value of K_{eq} ~ 0.13 (based on the actual molarity of water and ROH = $^{2-}O_3POH$, eq 1) gives a value of $\Delta G°$ for the hydrolysis of carbonyl phosphate of -3.6 kcal/mol (for the reaction of eq 1 in reverse and based on the usual convention that the activity of liquid water = 1.0). Thus, even after allowance for an additional electrostatic effect that will favor hydrolysis, it does not appear that carbonyl phosphate is a "high energy" compound at pH 8. This will not significantly affect the overall activation process so long as any carbonyl phosphate that is formed at the active site is not free to dissociate into the solution, i.e., as long as it is an intermediate in a coupled process taking place at the active site. However, the Gibbs free energy of hydrolysis (ΔG) will become more negative if the reactants and products are in dilute solution and will also become more negative at lower pH, as the reaction is pulled toward hydrolysis by protonation of phosphate dianion and bicarbonate.

Thus, two roles are apparent for the coupled hydrolysis of ATP to ADP and phosphate in carbon dioxide activation: (1) a kinetic role in supplying low-entropy, activated carbon dioxide through the dehydration of bicarbonate at the active site; and (2) a thermodynamic role in forcing the overall carboxylation reaction toward product formation.

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References and Notes

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