

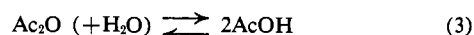
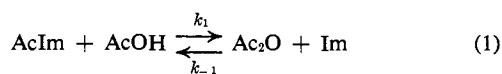
The Free Energy of Hydrolysis of Acetic Anhydride¹

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Abstract: The equilibrium constant for the formation of acetic anhydride and imidazole from acetylimidazole and acetic acid was found to be 3.2×10^{-5} in aqueous solution at 25°, from the ratio of the rate constants of the forward and back reactions. Comparison with the known free energy of hydrolysis of acetylimidazole gives a value of $-15,700$ cal/mole for the free energy of hydrolysis of acetic anhydride. The apparent equilibrium constant for the reaction of acetylimidazole with acetic acid in acetic acid solution is 0.031; this value increases with increasing imidazole concentration. The difference between the equilibrium constants in water and in acetic acid is attributed to the formation of the imidazolium acetate ion pair in acetic acid. Acetate ion is a general base catalyst for the hydrolysis of both acetylimidazole and acetylimidazolium ion.

As a part of a series of investigations on the free energies of hydrolysis of activated acyl compounds in water,² it was of interest to determine the free energy of hydrolysis of acetic anhydride. It is known that this reaction is strongly exothermic^{3,4} and that acetic anhydride will extract water from concentrated sulfuric acid and dilute oleum.⁵ The free energy of hydrolysis of acetic anhydride (eq 3) has been obtained from the equilibrium constant for the reaction of acetic acid with acetylimidazole (eq 1) and the free energy of hydrolysis of acetylimidazole^{2,6} (eq 2).



Experimental Section

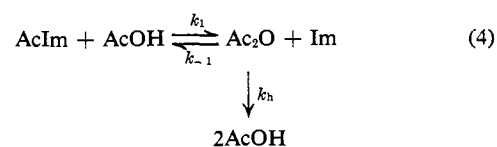
Acetylimidazole was prepared by the method of Boyer.⁷ Other compounds were reagent grade commercial materials. Organic compounds were generally recrystallized or redistilled. Water was glass distilled.

Kinetic measurements were carried out as described previously.⁸ Acetylimidazole disappearance or formation was followed spectrophotometrically at 245 or 260 m μ . The extinction coefficient of acetylimidazole in acetic acid at 260 m μ was found to be 782 M⁻¹ cm⁻¹ by dissolving a known amount of acetylimidazole in acetic acid and extrapolating the measured absorbance to zero time. Measured absorbances of acetylimidazole were corrected for the absorbance of acetic anhydride and imidazole if necessary. The ionic strength in aqueous solution was maintained by the addition of potassium chloride.

Results

The equilibrium constant for the reaction of acetylimidazole with acetic acid to form acetic anhydride (eq 1) cannot be measured directly in aqueous solution

because of the rapid hydrolysis of acetylimidazole. However, the equilibrium constant may be obtained from the ratio of the rate constants, $K_{\text{eq}} = k_1/k_{-1}$. The over-all rate constants for the reactions of acetylimidazole with acetic acid and of acetic anhydride with imidazole have been determined in water,⁹⁻¹⁰ but in order to calculate the equilibrium constant for the reaction it is necessary to know what fraction of each observed over-all rate constant represents nucleophilic reaction and what fraction represents general acid-base catalysis of hydrolysis. Acetic anhydride is known to react with imidazole in a nucleophilic reaction,^{9,10} so that the observed rate constant for this reaction,¹⁰ 8820 M⁻¹ min⁻¹, may be taken as k_{-1} . That this is indeed a nucleophilic reaction, and that general base catalysis of acetic anhydride hydrolysis by imidazole is not significant, was confirmed by measuring the yield of acetylimidazole formed upon the addition of acetic anhydride to 0.05 M imidazole by conversion to the hydroxamic acid,¹⁰ the yield of acetylimidazole was found to be 97-98%. The fraction of the over-all reaction of acetylimidazole with acetic acid that gives acetic anhydride as product was determined by measuring the amount that the catalysis by acetate buffers of the hydrolysis of acetylimidazole is inhibited by imidazole. As shown in eq 4, catalysis of acetylimidazole hydrolysis by a nucleophilic pathway involves the intermediate formation of acetic anhydride, which then undergoes hydrolysis. Now, if imidazole is added to the reaction mixture in sufficient concentration to react



with the acetic anhydride ($k_{-1}[\text{Im}]$) more rapidly than does water (k_h), all of the acetic anhydride that is formed will be converted back into acetylimidazole and no catalyzed hydrolysis will be observed. General base catalysis by acetate buffers is not subject to such inhibition and will not be affected by the addition of imidazole.

The results are illustrated in Figure 1. The acetate-catalyzed hydrolysis of acetylimidazole at pH 4.94 is

(9) D. M. Brouwer, M. J. van der Vlugt, and E. Havinga, *Proc. Koninkl. Ned. Akad. Wetenschap.*, **B60**, 275 (1957).

(10) J. F. Kirsch and W. P. Jencks, *J. Am. Chem. Soc.*, **86**, 837 (1964).

(1) Supported by grants from the National Science Foundation (GB-1648) and the National Institute of Child Health and Human Development of the National Institutes of Health (HD-01247). F. B. and R. B. were supported by NSF Predoctoral Fellowships.

(2) J. Gerstein and W. P. Jencks, *J. Am. Chem. Soc.*, **86**, 4655 (1964), and references therein.

(3) J. B. Conn, G. B. Kistiakowsky, R. M. Roberts, and E. A. Smith, *ibid.*, **64**, 1747 (1942).

(4) I. Wadsö, *Acta Chem. Scand.*, **16**, 471, 479 (1962).

(5) J. A. Leiston, *J. Chem. Soc.*, 298 (1955).

(6) E. R. Stadtman in "The Mechanism of Enzyme Action," W. D. McElroy and B. Glass, Ed., Johns Hopkins Press, Baltimore, Md., 1954, p 581.

(7) J. H. Boyer, *J. Am. Chem. Soc.*, **74**, 6274 (1952).

(8) W. P. Jencks and J. Carriuolo, *J. Biol. Chem.*, **234**, 1272, 1280 (1959).

inhibited by low concentrations of imidazole hydrochloride, with half-maximal inhibition at approximately 0.011 *M*. The dashed lines in the figure show the rate of hydrolysis of acetylimidazole in the presence and absence of 0.8 *M* acetate buffer, so that the distance between these lines represents the amount of acetate-catalyzed hydrolysis of acetylimidazole. If the catalyzed hydrolysis were all caused by nucleophilic reaction to give acetic anhydride, imidazole would cause complete inhibition of the catalyzed reaction; the observed maximal inhibition is 19% of the maximal possible inhibition which means that 19% of the reaction of acetylimidazole with acetate under these conditions gives acetic anhydride. The remaining 81% represents general acid-base catalysis of acetylimidazole hydrolysis by acetate buffer. The results of a series of similar experiments at different pH values and buffer ratios are summarized in Table I. Similar results were obtained at all except the highest pH values, with an average maximal inhibition by imidazole of 22% of the acetate-catalyzed reaction.

Table I. Inhibition of the Acetate-Catalyzed Hydrolysis of Acetylimidazole by the Back-Reaction with Imidazole at 25°^a

Fraction of acetic acid	$k_{\text{obsd}}, \text{min}^{-1}$			Fraction of inhibition ^c
	Acetate buffer		0.8 <i>M</i> + Im ^b	
	0.8 <i>M</i>	0.032 <i>M</i>		
0.75	1.19	0.83	1.11	0.24
0.50	0.77	0.39	0.68	0.22
0.35	0.56	0.24	0.50	0.19
0.25	0.44	0.166	0.37	0.25
0.10	0.173	0.058	0.154	0.18

^a Ionic strength maintained at 1.4 *M* with potassium chloride. The acetylimidazole concentration was 2×10^{-4} *M*. ^b Maximum inhibition by imidazole hydrochloride. ^c Maximum fractional decrease in acetate-catalyzed hydrolysis of acetylimidazolium ion. Corrected for a small contribution of the acetate-acetylimidazole reaction at the highest pH values.

The rate law for the hydrolysis of acetylimidazole catalyzed by acetate buffers is given in eq 5. The value of k_A was found to be 1.3 $M^{-1} \text{min}^{-1}$, in agree-

$$\text{rate} = k_A[\text{AcIm}][\text{AcOH}] + k_B[\text{AcIm}][\text{AcO}^-] \quad (5)$$

ment with a previous determination.⁸ (This term actually represents acetate catalysis of the hydrolysis of acetylimidazolium ion,⁸ but the kinetic distinction is not significant for the purposes of this treatment.) The value of k_B , which represents catalysis of the hydrolysis of free acetylimidazole by acetate ion, was found to be 0.017 $M^{-1} \text{min}^{-1}$ from the increase in the rate of acetylimidazole hydrolysis at pH 7.1 caused by the addition of increasing concentrations of acetate ion up to 1.0 *M*. The latter reaction shows no inhibition by imidazole and represents, therefore, general base catalysis of acetylimidazole hydrolysis by acetate ion, analogous to the previously reported general base catalysis of acetylimidazole hydrolysis by imidazole.⁸ This term is of very little significance in the pH range of the experiments shown in Table I, but does account for a decrease in the inhibition by imidazole which was observed at high pH values.

The steady-state rate expression for the hydrolysis of acetylimidazole with the intermediate formation of

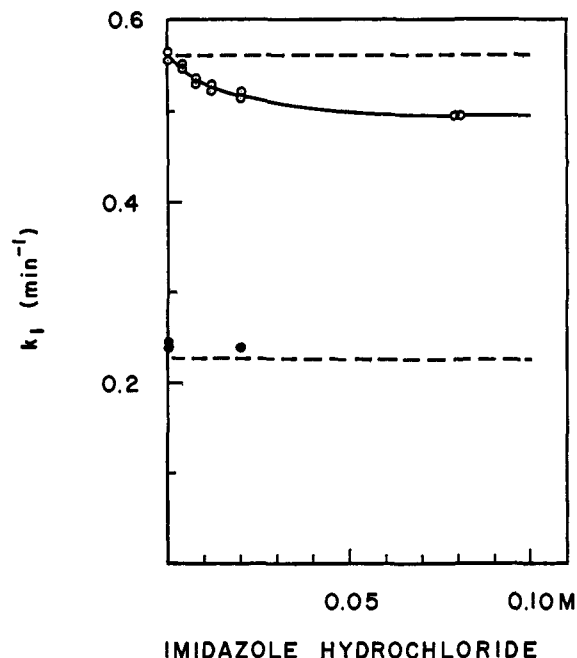


Figure 1. Inhibition of the acetate-catalyzed hydrolysis of acetylimidazole by the back-reaction with imidazole at pH 4.95 ± 0.01, ionic strength 1.4 *M*, and 25°: O, 0.8 *M* acetate buffer, 35% acetic acid; ●, 0.032 *M* acetate buffer. The dashed lines show the rate of acetylimidazole hydrolysis in the presence and absence of 0.8 *M* acetate buffer.

acetic anhydride (eq 4) is given in eq 6. The inhibition

$$\frac{d[\text{AcIm}]}{dt} = \frac{k_1 k_h [\text{AcOH}][\text{AcIm}]}{k_{-1}[\text{Im}] + k_h} \quad (6)$$

of the catalyzed hydrolysis by imidazole becomes significant when the $k_{-1}[\text{Im}]$ term becomes significant compared to k_h , and when the hydrolysis is 50% inhibited these two terms should be equal. Since the value of k_h in the presence of acetate is equal¹⁰⁻¹² to $0.17 + 0.24[\text{CH}_3\text{COO}^-]$ (min^{-1}), a rough value of k_{-1} may be calculated from the inhibition data. At pH 4.64, half-maximal inhibition is observed in the presence of 0.014 *M* imidazole hydrochloride or 3.8×10^{-5} *M* imidazole free base, which gives a value of k_{-1} of 7000 $M^{-1} \text{min}^{-1}$. This is in reasonable agreement with the directly measured value¹⁰ of k_{-1} of 8820 $M^{-1} \text{min}^{-1}$ and serves to confirm the treatment.

Since the rate constant for the nucleophilic reaction of acetic acid with acetylimidazole is $0.22 \times 1.3 = 0.286 M^{-1} \text{min}^{-1}$, the over-all equilibrium constant for the reaction of acetic acid with acetylimidazole to form acetic anhydride (eq 1) is $0.286/8820 = 3.2 \times 10^{-5}$, which corresponds to a standard free energy of 6200 cal/mole. The free energy of hydrolysis of acetylimidazole to (uncharged) acetic acid and imidazole² is -9490 cal/mole (based on an activity of pure water of 1.0) so that the free energy of hydrolysis of acetic anhydride is -15,700 cal/mole. At pH 7.0 the free energy of hydrolysis of acetic anhydride to two molecules of acetate is -21,800 cal/mole.

In acetic acid solutions containing 5.5 *M* water and in methanol the solvolysis of acetylimidazole was found to be too rapid to permit direct measurement of the

- (11) M. Kilpatrick, Jr., *J. Am. Chem. Soc.*, **50**, 2891 (1928).
 (12) A. R. Butler and V. Gold, *J. Chem. Soc.*, 2305 (1961).

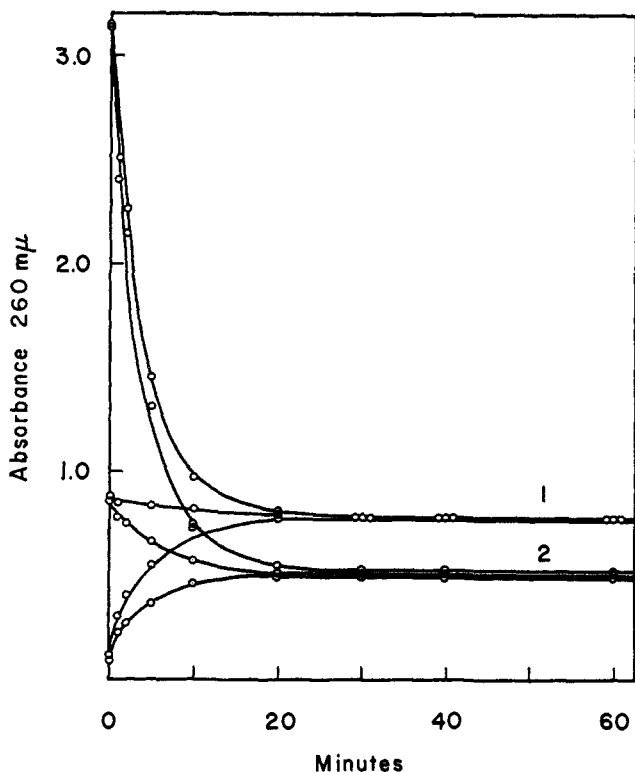


Figure 2. The approach to equilibrium from both directions in the reaction of acetic anhydride with imidazole in acetic acid containing 2% acetonitrile at 25° (total $[Ac_2O + AcIm] = 4.1 \times 10^{-3} M$): upper curves, 0.196 M imidazole, $K = 0.042$; lower curves, 0.097 M imidazole, $K = 0.036$.

equilibrium constant. However, the equilibrium for acetic anhydride formation could be measured directly in acetic acid as solvent. The results of one series of experiments in which the formation and disappearance of acetylimidazole was followed spectrophotometrically and equilibrium was approached from both directions are shown in Figure 2. At a given concentration of imidazole, the same equilibrium position is reached from both directions, but the apparent equilibrium constant varies with the concentration of imidazole. As shown in Figure 3, both imidazole and N-methylimidazole increase the apparent equilibrium constant for acetic anhydride formation. The value of the apparent equilibrium constant in acetic acid, extrapolated to zero imidazole concentration, is 0.031.

The equilibrium constant was found to be unaffected by varying the concentration of sodium acetate in acetic acid from 0.006 to 0.10 M in the presence of 0.03–0.067 M imidazole. The equilibrium constant was also found to be unaffected by the addition of 1% water.

Discussion

The value of $-15,700$ cal/mole for the free energy of hydrolysis of acetic anhydride may be compared with -9250 cal/mole for succinic anhydride, calculated from the data of Higuchi, *et al.*¹³ The difference between these values for equilibria may in turn be compared with the differences in the rates of intermolecular and intramolecular reactions which are thought to go through transition states which resemble succinic anhydride. The difference between the free energies of the transi-

(13) T. Higuchi, T. Miki, A. C. Shah, and A. K. Herd, *J. Am. Chem. Soc.*, **85**, 3655 (1963).

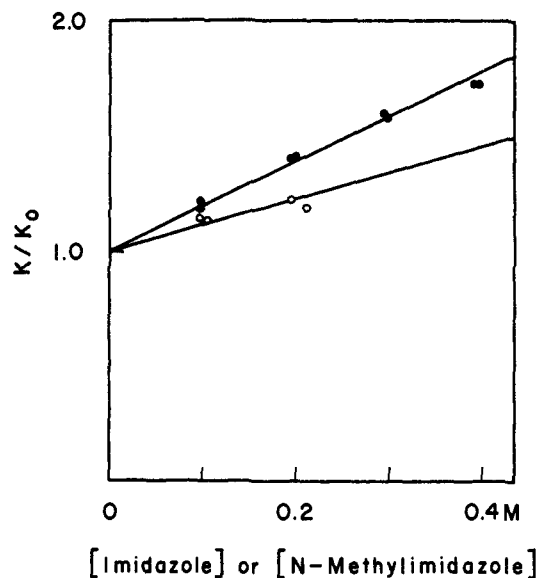


Figure 3. The effect of imidazole (●) and N-methylimidazole (○) concentration on the apparent equilibrium constant for the reaction of acetic anhydride with imidazole in acetic acid at 25°.

tion states for the intramolecular solvolysis of phenyl γ -(N,N-dimethylamino)butyrates and the intermolecular reactions of 1 M trimethylamine with phenyl acetates is approximately 5000 cal/mole,¹⁴ and a similar difference is observed for monophenyl succinate, compared with the intermolecular reaction of phenyl acetate with acetate.¹⁵ The heat of hydrolysis of acetic anhydride is $-14,000$ cal/mole, while that for succinic anhydride is $-11,200$ cal/mole, a difference of 2800 cal/mole.^{3,4} The much larger difference of 6450 cal/mole in the free energies of hydrolysis of these anhydrides is, therefore, largely an entropy effect. This is consistent with the fact that the stabilization of the transition states in the intramolecular reactions is almost entirely an entropy effect, albeit a somewhat larger entropy effect than might be expected.^{14,15} The reason for the difference in the enthalpies of hydrolysis of acetic and succinic anhydrides is not evident.

The fact that the free energy ($-15,700$ cal/mole) is larger than the heat ($-14,000$ cal/mole) of hydrolysis of acetic anhydride is a consequence of the convention that the activity of pure water is taken as 1.0 for the free energy of hydrolysis. If the activity of water is taken as equal to its molar concentration, the free energy of hydrolysis is $-13,300$ cal/mole.

It is of interest that the greater part of the reaction of acetic acid with acetylimidazole is general acid-base catalysis of hydrolysis, rather than a nucleophilic reaction. This reaction presumably actually represents general base catalysis by acetate ion of the hydrolysis of acetylimidazolium ion and is analogous to the general base catalysis of the hydrolysis of acetylimidazolium and N-methylacetylimidazolium ions by imidazole and N-methylimidazole, respectively, to the catalysis of *p*-nitrophenyl acetate and acetic anhydride hydrolysis by acetate ion, and to general base catalysis of ester

(14) T. C. Bruice and S. J. Benkovic, *ibid.*, **85**, 1 (1963).

(15) Calculated from the data of E. Gaetjens and H. Morawetz, *ibid.*, **82**, 5328 (1960), and of M. L. Bender and M. C. Neveu, *ibid.*, **80**, 5388 (1958), extrapolated to 25°. The difference is probably somewhat greater than this, because the intermolecular reaction of acetate with phenyl acetate probably does not represent a nucleophilic reaction.¹⁶

hydrolysis.^{8,11,12,16-19} The relatively weak general base catalysis of the hydrolysis of acetylimidazole by acetate ion is analogous to general base catalysis by imidazole of acetylimidazole hydrolysis;⁸ the catalytic constants for these two catalysts give a (rather uncertain) Brønsted slope of 0.4.

The point for acetic anhydride falls more than two orders of magnitude below the line relating the logarithms of equilibrium constants for ester formation to the pK of the alcohols, to which there is a remarkably good fit of the points for a series of esters, including phenyl acetates.² Evidently, there is either some special instability of acetic anhydride, compared to other acetates, or acetic acid has an unexpectedly low acidity, compared to phenols and other alcohols. A possible explanation is that cross-conjugation causes a specific loss of resonance stabilization in acetic anhydride, compared to acetic acid, and that this loss is more important than the loss of resonance stabilization upon the esterification of other alcohols, such as phenols.

The apparent equilibrium constant for the reaction of acetylimidazole with acetic acid is three orders of magnitude more favorable in acetic acid than in aqueous solution. By analogy with the behavior of pyridine in acetic acid, imidazole would be expected to abstract a proton from acetic acid to form an undissociated imidazolium-acetate ion pair in this solvent.^{20,21}

(16) A. R. Butler and V. Gold, *J. Chem. Soc.*, 1334 (1962).

(17) T. H. Fife, *J. Am. Chem. Soc.*, **87**, 4597 (1965).

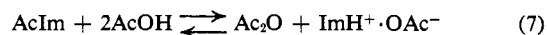
(18) R. Wolfenden and W. P. Jencks, *ibid.*, **83**, 4390 (1961).

(19) W. P. Jencks and J. Carriuolo, *ibid.*, **83**, 1743 (1961).

(20) S. Bruckenstein and I. M. Koltoff, *ibid.*, **78**, 10 (1956).

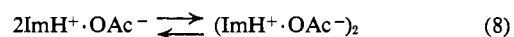
(21) This conclusion is supported by the qualitative observation that the solution of imidazole in acetic acid is strongly exothermic, whereas

Consequently, the equilibrium expression for the reaction of acetylimidazole with acetic acid as solvent should be written according to eq 7 for which the



equilibrium constant is $0.031/17.4 = 1.78 \times 10^{-3} M^{-1}$.

The increase in the apparent equilibrium constant for acetic anhydride formation with increasing concentrations of imidazole or N-methylimidazole suggests the occurrence of complex formation of these compounds with one of the products of the reaction. One possible type of complex formation is the formation of quadruple ions²⁰ of imidazolium acetate (eq 8), although evidently



no such reaction takes place with sodium acetate. If this formulation is correct, the effect of added imidazole on the apparent equilibrium constant is given by eq 9,

$$K_{\text{obsd}}/K_0 = K_c[\text{Im}] + 1 \quad (9)$$

in which K_c is the equilibrium constant of eq 8. The data shown in Figure 3 are fitted by values of K_c of 2.0 and 1.1 M^{-1} for imidazole and N-methylimidazole, respectively. However, other types of complex formation may also be envisaged.²²

the solution of imidazole in water is endothermic.⁴ Furthermore, S. Kushner and D. Epstein have shown (unpublished experiments) that the infrared difference spectrum of 0.5 M imidazole in acetic acid shows peaks at 1530 and 1585 cm^{-1} which are similar to the peaks in the difference spectra in acetic acid of 0.5 M sodium acetate (1530 cm^{-1}) and 0.5 M imidazolium sulfate (1585 cm^{-1}). These spectra were taken with calcium fluoride cells with 0.05-mm path length.

(22) See, for example, K. A. Connors and J. A. Mollica, Jr., *J. Am. Chem. Soc.*, **87**, 123 (1965).