[CONTRIBUTION NO. 308 FROM THE GRADUATE DEPARTMENT OF BIOCHEMISTRY, BRANDEIS UNIVERSITY, WALTHAM, MASSACHUSETTS 02154]

Equilibria and Rates for Acetyl Transfer among Substituted Phenyl Acetates, Acetylimidazole, O-Acylhydroxamic Acids, and Thiol Esters[1]

BY JOSEPH GERSTEIN AND WILLIAM P. JENCKS

RECEIVED JULY 6, 1964

The equilibrium and rate constants for acetyl transfer from acetylimidazole to a series of substituted phenols have been determined. The effects of polar substituents on the transition state of the reaction are intermediate between those on the reactants and on the products for the equilibrium $AcImH^+ + {}^-OPhX \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} AcOPhX + Im$. Determination of the equilibrium constants for acetyl transfer to N-methylacetohydroxamic acid and N-acetyl-β-mercaptoethylamine has made possible the calculation of the free energies of hydrolysis of these compounds, the phenyl acetates, acetylimidazole, and other "energy-rich" compounds for which the equilibrium constants for reactions with these compounds are known.

In order to consider in detail the effect of structure on reactivity and the mechanism of acyl transfer reactions, it is desirable to know the rate constants in both directions and the equilibrium constants for a series of such reactions. The results of such determinations for a series of phenyl acetates and imidazole are reported here. In addition, some rate and equilibrium constants for acetyl transfers involving acetylimidazole, the ester group of N,O-diacetyl-N-methylhydroxylamine, and the thiol ester group of N,S-diacetyl-β-mercaptoethylamine have been determined. By relating these values to the free energy of hydrolysis of thiol esters,[2-3] the results permit the calculation of the free energies of hydrolysis of these and related "energy-rich" compounds.

Experimental

Materials.—Commercially available phenols and imidazole were redistilled or recrystallized. Substituted phenyl acetates and N,O-diacetyl-N-methylhydroxylamine were kindly supplied by Dr. Jack Kirsch. N-Acetylimidazole, m.p. 101–103°, was prepared by the method of Boyer[4] and showed λ_{max} 245 mμ, ε 2920; reported[5] λ_{max} 245 mμ, ε 3000; the latter value was used for calculation of acetylimidazole concentration in these experiments.

N,S-Diacetyl-β-mercaptoethylamine[6] (N-2-mercaptoethylacetamide acetate) was prepared by the dropwise addition with vigorous stirring of 0.3 mole of acetic anhydride to 0.1 mole of mercaptoethylamine hydrochloride in 30 ml. of water at 0–3°. The solution was maintained at pH 8.0 by the simultaneous addition of 8 *M* potassium hydroxide. After standing for 20 min. at room temperature, the mixture was extracted with ether and the ether extract was dried with sodium sulfate. After evaporation of solvent the product was distilled; b.p. 140–142° (0.85 mm.), $n^{24}D$ 1.5068, *d* 1.11. An aqueous solution of N-acetyl-β-mercaptoethylamine (N-2-mercaptoethylacetamide) was prepared by the reaction of 0.7 mmole of N,S-diacetyl-β-mercaptoethylamine with 1.8 mmoles of potassium hydroxide in 3.2 ml. of water at 25° for 40 min. followed by neutralization with a small excess of hydrochloric acid. Measurement of thiol appearance by the nitroprusside reaction[7] showed that the reaction was complete in 15 min. under these conditions and ultraviolet spectroscopy indicated that the solution did not contain detectable amounts of thiol ester or thiazoline. N-Methylacetohydroxamic acid was prepared in a similar manner by the saponification of N,O-diacetyl-N-methylhydroxylamine. In a typical preparation 1.7 mmoles of N,O-diacetyl-N-methylhydroxylamine was added to 4.0 mmoles of potassium hydroxide in 6 ml. of water at 25° and the mixture was allowed to stand for 1 hr. before neutralization with 0.75 ml. of 2 *M* hydrochloric acid. A control experiment in which the release of hydroxamic acid was followed by the addition of aliquots to ferric chloride solution[8] showed that the reaction was complete in 28 min. under these conditions.

Determination of pK_a' Values.—The pK_a' of N-methylacetohydroxamic acid was found by titration to be 8.79 ± 0.02 at 25° and ionic strength 1.0. Measurement of the pH of diluted samples gave a pK_a' value of 8.85 at ionic strengh 0.01. These values were confirmed by measurements of the absorbance of the N-methylacetohydroxamate anion at 260 mμ. In a series of tris(hydroxymethyl)aminomethane (Tris) buffers N-methylacetohydroxamate anion exhibits an ultraviolet absorption maximum at 227 mμ, ε 1.96×10^4, in 0.01 *M* NaOH, and has an extinction coefficient of 1575 at 260 mμ. The corresponding acid shows only end absorption at 227 mμ (ε 8.9×10^3 in 0.01 *M* HCl) and has negligible absorption at 260 mμ (ε *ca.* 7). Acetohydroxamate anion exhibits a weaker absorption maximum at 215 mμ (ε 6.6×10^3). The spectrophotometric method gave a pK_a' value for N-methylacetohydroxamic acid at 25° of 8.78 ± 0.03 at ionic strength 1.0 and 8.84 ± 0.02 at ionic strength 0.01. In 6.2% acetonitrile at 25° and ionic strength 1.0 the pK_a' was found to be 8.97 ± 0.03 by the same method.

The pK_a' of the thiol group of N-acetyl-β-mercaptoethylamine was found to be 9.38 ± 0.04 at 25° and ionic strength 1.0 by measurement of the absorption of the thiol anion at 250 mμ (ϵ_{250} 3120) at a series of pH values between 9.2 and 9.7 and in 0.01 *M* NaOH. A slow oxidation of the thiol in the alkaline solutions necessitated a (small) extrapolation of the absorbance readings to zero time. The pK_a' was found to be 9.47 ± 0.05 at ionic strength 0.01. The pK_a' of *p*-chorophenol at 25° and ionic strength 1.0 was found to be 9.20 ± 0.03 in water and 9.29 ± 0.04 in 6.2% acetonitrile by measurements of the absorption of the *p*-chlorophenolate anion at 298 mμ; the absorption of *p*-chlorophenol is negligible at this wave length. The pK_a' of *p*-nitrophenol at 25° and ionic strength 1.0 in 2% acetonitrile was found to be 7.02 ± 0.02 in a similar manner, utilizing the absorption of the *p*-nitrophenolate anon at 401 mμ. The pK_a' values of other phenols[9] and of acetylimidazolium ion[10] were taken from the literature.

Rate Measurements.—The rates of reactions of phenols with acetylimidazole were generally followed by observing the decrease in acetylimidazole absorption at 245 mμ (248 mμ in the case of *p*-chlorophenol and 260 mμ in the case of N-methylacetohydroxamic acid). When the absorption of the phenol at this wave length was large, the measurements were made against a blank cuvette containing all the constituents of the reaction mixture except acetylimidazole. Spectrophotometric measure-

(1) Supported by research grants from the National Science Foundation and from the National Cancer Institute of the National Institutes of Health (CA-03975) and a training grant from the National Institute of Neurological Diseases and Blindness (5-T1-NB-5241).

(2) W. P. Jencks, S. Cordes, and J. Carriuolo, *J. Biol. Chem.*, **235**, 3608 (1960).

(3) W. P. Jencks and M. Gilchrist, *J. Am. Chem. Soc.*, **86**, 4651 (1964).

(4) J. H. Boyer, *Biochem. Prepn.*, **4**, 54 (1955).

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

(6) T. Wieland and E. Bokelmann, *Ann.*, **576**, 20 (1952).

(7) E. R. Stadtman, "Methods in Enzymology," Vol. III, S. P. Colowick and N. O. Kaplan, Ed., Academic Press, Inc., New York, N. Y., 1957, p. 939.

(8) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **159**, 21 (1945).

(9) M. M. Fickling, A. Fischer, B. R. Mann, J. Packer, and J. Vaughan, *J. Am. Chem. Soc.*, **81**, 4226 (1959).

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

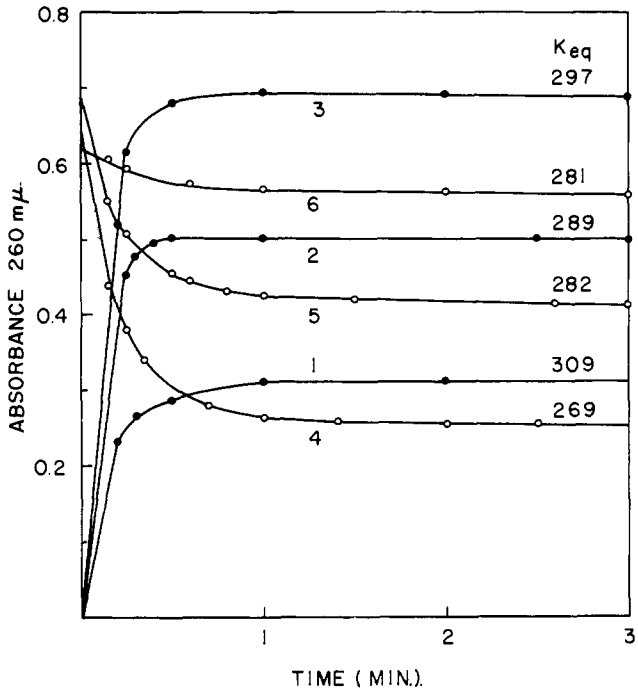


Fig. 1.—Determination of the equilibrium constant for acetyl transfer between N-methylacetohydroxamic acid and imidazole by measurement of acetylimidazole absorption at 260 mμ. Initial concentrations for curves 1-3: 9.0×10^{-3} *M* hydroxamic acid, 0.238 *M* imidazole as the free base, 1.94, 3.24, and 4.53×10^{-3} *M* N,O-diacetyl-N-methylhydroxylamine, pH 7.2; curves 4-6: 4.5×10^{-3} *M* hydroxamic acid, 0.192 *M* imidazole as the free base, 3.4-3.8 $\times 10^{-4}$ *M* acetylimidazole, 0.65, 1.30, and 1.94×10^{-3} *M* N,O-diacetyl-N-methylhydroxylamine, pH 7.0. Ionic strength maintained at 1.0 with potassium chloride. The calculated values of the equilibrium constant are shown above the curves.

ments were made with a Zeiss PMQ II spectrophotometer equipped with a brass cuvette holder through which water at 25 ± 0.1° was circulated. Reactions were carried out with a large excess of phenol so that the observed rates followed pseudo-first-order kinetics and were much faster than the rates of acetylimidazole hydrolysis. For those reactions in which the absorption of the phenol was too high for readings in a 1 cm. path length cuvette, a quartz insert was inserted and the reaction was followed with a 0.2- or 0.05-cm. path length. The reaction of *m*-nitrophenol with acetylimidazole was followed with acetylimidazole present in excess by measuring the disappearance of the absorption of the phenol at 338 mμ. First- and second-order rate constants were obtained as described previously.[10] The observed pseudo-first-order rate constants were corrected for the rate of acetylimidazole hydrolysis, measured under the same experimental conditions; this correction was small in all cases.

Direct Measurement of Equilibrium Constants.—The experimental conditions used for the direct determination of equilibrium constants are given in Table II. The measurement of the equilibrium constant for acetyl transfer between imidazole and the hydroxyl group of N-methylacetohydroxamic acid (eq. 1) is described here in detail as an example of the methods used. The

$$\mathrm{AcIm} + \mathrm{CH_3C(=O)N(CH_3)OH} \rightleftharpoons \mathrm{Im} + \mathrm{CH_3C(=O)N(CH_3)OAc} \quad (1)$$

reaction was followed by measuring the change in absorption at 260 mμ attributed to acetylimidazole (Fig. 1). The initial concentrations of reactants were varied over the ranges shown in Table II. A solution of acetylimidazole in dilute imidazole buffer, pH 7.0, was prepared before each experiment and was kept at 0°. The concentration of acetylimidazole in this solution was determined by measurement of the absorbance at 245 mμ of diluted aliquots. Solutions of N-O-diacetyl-N-methylhydroxylamine were prepared before each experiment and were shown to be free of hydroxamic acid by the absence of color development upon addition of aliquots to acidic ferric chloride solution.[8] The rate of hydrolysis of this compound was shown to be negligible at the pH values at which equilibrium measurements were made. The reactants were brought to 25° and the reaction was initiated by the addition of acetylimidazole in imidazole buffer to the other reactants in a 4-ml. cuvette. The contents was mixed by inversion and the absorption at 260 mμ was followed until the readings were stable over a period of several min. (Fig. 1). The pH of the mixture was then determined. The rate of acetylimidazole hydrolysis under the same conditions of temperature, imidazole concentration, pH, and ionic strength was determined separately. The amount of acetylimidazole which had hydrolyzed during the time required to reach equilibrium was estimated by multiplying the average concentraion of acetylimidazole by the hydrolysis rate constant and the time. This correction was less than 1% of the acetylimidazole concentration in this experiment and was less than 7% in all experiments. The measured absorbance at 260 mμ was corrected for the absorption of N-methylacetohydroxamate anion, determined from the initial concentration of N-methylacetohydroxamic acid, the pH, and the amount of this compound which was formed or utilized during the approach to equilibrium. This correction was about 5% of the observed absorbance in this experiment. The corresponding correction for the reaction with phenol was also 5%, but it was as much as 30% in the reactions with *p*-methoxyphenol, which necessitated the use of successive approximations to make an accurate correction in the latter reaction. The equilibrium concentration of acetylimidazole was then calculated, based on ϵ_{260} 1810. The equilibrium concentrations of the other reactants were determined from the initial concentrations and the amount of reaction which had taken place.

The measurements of equilibria involving phenyl acetates were carried out in a similar manner, except that quartz inserts were used to reduce the path length of 1.0-cm. cuvettes to 0.2 or 0.05 cm. The path lengths were calibrated with solutions of known absorbance. The determination of the equilibrium constant for acetyl transfer between *p*-chlorophenol and N-methylhydroxamic acid presented technical difficulties because of the low solubility of *p*-chlorophenyl acetate in water and because it was not possible to carry out the reaction under conditions in which the pH remained constant during the reaction. Consequently, experiments in which *p*-chlorophenyl acetate was added initially were carried out in 6.2% acetonitrile; the results in this solvent showed moderately good agreement with those obtained in water in the absence of added *p*-chlorophenyl acetate (Table II). The pH values of the reaction mixtures used for the spectrophotometric measurements were obtained by following the pH of identical reaction mixtures as a function of time. The rate of hydrolysis of N,O-diacetyl-N-methylhydroxylamine was determined by following the appearance of N-methylacetohydroxamate at 272 mμ under the conditions of the equilibrium experiments and was found to proceed with a rate constant of 0.018 min.$^{-1}$ at pH 8.53 and 0.013 min.$^{-1}$ at pH 8.49 in 6.2% acetonitrile. The value of ϵ_{298} for *p*-chlorophenoxide was found to be 2420 ± 20 for two samples of recrystallized and one of resublimed *p*-chlorophenol; Spencer and Williams[11] report ϵ_{298} 2600.

The reaction of acetylimidazole with *p*-nitrophenol was followed by measurement of the absorption of the *p*-nitrophenolate ion at 401 mμ. The concentration of *p*-nitrophenol was calculated from the measured pH and the pK_a' of *p*-nitrophenol, which was measured separately under the same experimental conditions.

Reactions of N-acetyl-β-mercaptoethylamine were carried out in 0.05 *M* Tris buffer which contained 10^{-4} *M* ethylenediaminetetraacetic acid. It was shown in control experiments that the thiol did not undergo significant oxidation and the thiol ester and N,O-diacetyl-N-methylhydroxylamine did not undergo hydrolysis during the time interval required for the attainment of equilibrium.

The reaction of N,S-diacetyl-β-mercaptoethylamine with N-methylacetohydroxamic acid was followed by measurement of the concentration of free thiol by the nitroprusside reaction.[7] The assay mixture contained 2.0 ml. of saturated sodium chloride, 0.4 ml. of 1.6 *M* potassium carbonate to which had been added 0.8 *M* hydrochloric acid, 0.4 ml. of 2.7% sodium nitroprusside, and 0.10 ml. of sample. All reagents were made up in 10^{-4} *M* ethylenediaminetetraacetic acid. Readings were taken 30 sec. after the addition of sample.

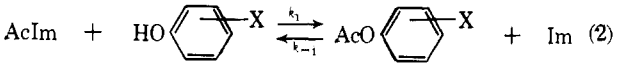
(11) B. Spencer and R. T. Williams, *Biochem. J.*, **48**, 537 (1951).

The concentrations of reactants and products at equilibrium were calculated from absorbance measurements after the initial rapid change in absorbance had leveled off. In reaction mixtures in which the final readings were not perfectly stable, because of hydrolysis, the equilibrium position was determined by the graphical method of Stadtman[5] as well as by measurements at several time intervals after the initial rapid change in absorption; no significant differences were found between the results of these procedures.

Results

Equilibrium constants were obtained from measurements of the rate of the reaction in both directions, from the relationship $K_{eq} = k_1/k_{-1}$, or by direct measurement of the equilibrium concentrations of the reactants and products. In a few instances the results of the two methods were compared and internal consistency was demonstrated by showing that the same equilibrium constant was obtained by comparing two different pairs of reactions. All measurements were made at 25° and at an ionic strength maintained at 1.0 with potassium chloride.

Rate Measurements.—The rate constants, k_{-1}, for the reaction of imidazole with a series of substituted phenyl acetates (eq. 2) were taken from a recently reported series of measurements which had been carried



out under the same experimental conditions.[12] The rate constants of the reverse reaction, k_1, were determined by measuring the rate of acetylimidazole disappearance with the phenol present in large excess, except in the case of *m*-nitrophenol, for which reaction acetylimidazole was present in excess and the disappearance of phenol was measured spectrophotometrically. Pseudo-first-order kinetics were followed for at least two half-times in each reaction. The results obtained for the reaction of *p*-chlorophenol with acetylimidazole are shown in Fig. 2, as an example. The rate constant for acetylimidazole hydrolysis was determined under the same conditions and was subtracted from the observed rate constants if necessary, but in most instances this correction was negligible. The reactions were carried out in dilute imidazole buffer to avoid side reactions. Doubling the concentration of imidazole buffer did not affect the rate constant or extent of reaction. The same rate constant was observed if the pH was increased from 7.04 to 7.25. This demonstrates that the rate of the reaction is independent of pH in this pH region and follows the rate law[10]

$$\text{rate} = k_1[\text{AcIm}][\text{ROH}] \qquad (2a)$$

Since the mechanism of the reaction actually involves the attack of phenolate anion on the conjugate acid of acetylimidazole,[10] the rate may be described more appropriately, for mechanistic purposes, by the kinetically indistinguishable rate law

$$\text{rate} = k_2[\text{AcImH}^+][\text{RO}^-] \qquad (2b)$$

in which $k_2 = k_1K_{\text{AcImH}^+}/K_{\text{ROH}}$; K_{ROH} and K_{AcImH^+} are the acid dissociation constants for the phenol and the conjugate acid of acetylimidazole, respectively.

The rate constants, k_1 and k_2, for the reactions of acetylimidazole with a series of phenols and N-methylacetohydroxamic acid are summarized in Table I. The value of k_1 of 20.1 M^{-1} min.$^{-1}$ for the reaction with phenol at ionic strength 1.0 is in satisfactory agreement with the corresponding values of 16–19 M^{-1} min.$^{-1}$ measured previously in the absence of added salt.[10] For each reaction with a substituted phenol it was shown that the rate constant is not altered by doubling the concentration of imidazole buffer, which demonstrates that the reactions proceed to completion and are not subject to significant catalysis by imidazole under the experimental conditions which were used. For the reactions with phenol, *p*-chlorophenol, *m*-nitrophenol, and N-methylacetohydroxamic acid it was shown that the rate constants are independent of pH over a range of 0.2–0.3 pH unit, which demonstrates that the rate law of eq. 2a and 2b holds for these reactions. Since it was not possible to measure the rate constant for the reaction of acetylimidazole with *p*-nitrophenol directly, for technical reasons, this rate constant was calculated from the rate constant for the reverse reaction and the directly measured equilibrium constant (see below).

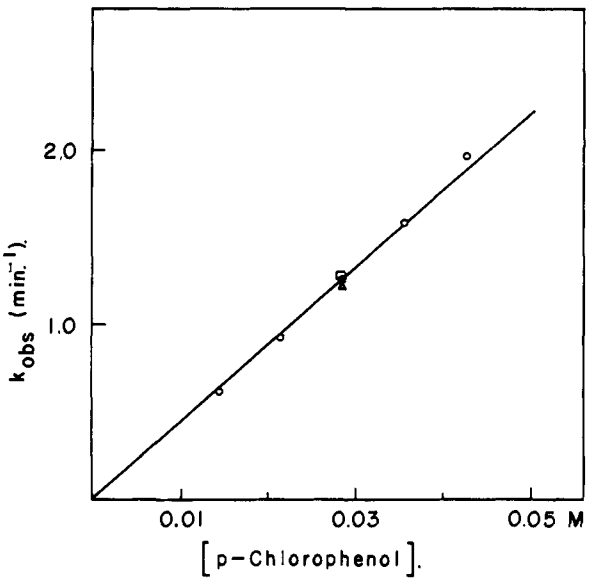


Fig. 2.—Rate of disappearance of acetylimidazole as a function of *p*-chlorophenol concentration at 25° and ionic strength 1.0: O, pH 7.04, 0.007 *M* imidazole; □, pH 7.25, 0.01 *M* imidazole; △, pH 7.05, 0.014 *M* imidazole.

In contrast to the reactions with phenols, the observed rate constants for the reaction of N-methylacetohydroxamic acid with acetylimidazole increase with increasing imidazole buffer concentration at constant pH (Fig. 3, upper curve). The second-order rate constant of 510 M^{-1} min.$^{-1}$ for the reaction (Table I) was obtained by dividing the observed pseudo-first-order rate constant for acetylimidazole disappearance, extrapolated to zero imidazole concentration, by the concentration of N-methylacetohydroxamic acid. The dependence of the rate upon imidazole concentration was shown to be caused partly by the fact that the reaction does not proceed to completion at the higher imidazole concentrations and partly by catalysis of the reaction by imidazole. Since the hydroxamic acid and imidazole are present in large excess, the observed reaction is pseudo first order in both directions and, in any given experiment, $k_{obsd} = k_f + k_r$, where k_f and k_r are the pseudo-first-order rate

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

TABLE I

RATES OF REACTIONS WITH ACETYLIMIDAZOLE AT 25° AND IONIC STRENGTH 1.0

ROH	Init. concn. of acetylimidazole, M	Concn. of imidazole buffer (as free base), M	Concn. of ROH, M	No. of detmn.	pH	k_1,[a] M^{-1} min.$^{-1}$	k_2[b] $\times 10^{-6}$, M^{-1} min.$^{-1}$	Wave length, mμ	pK_a' ROH
p-Methoxyphenol	2.8×10^{-3}	0.015	0.02-0.10	5	7.2	25.3[c]	96.0	245[d]	10.20[e]
p-Methylphenol	2.0×10^{-3}	.015	.023-0.07	7	7.2	17.7[c]	69.0	245[d]	10.19[e]
Phenol	2.7×10^{-3}	.015	.034- .10	6	7.3[f]	20.1[c,h]	48.2	245[d]	9.99[g]
p-Chlorophenol	7.8×10^{-4}	.007	.015- .043	7	7.0[f]	43.3[c]	24.8	248[i]	9.38[e]
m-Nitrophenol	0.016-0.062	.004-0.02	.0013	7	6.7[f]	66.5[c,j]	3.6	338[i]	8.35[e]
p-Nitrophenol	...	...	...	..	7.1	38.3	0.13	[k]	7.14[g]
N-Methylacetohydroxamic acid	5.0×10^{-4}	.03	.0025-0.0095	11	6.9[f]	510[l]	87.5	260	8.85[m]

[a] For rate = k_1[acetylimidazole][ROH]. [b] For rate = k_2[AcImH$^+$][RO$^-$]. [c] Doubling of the imidazole concentration resulted in no significant change in rate. [d] Path length of 0.5 mm. [e] Ref. 13. [f] Rates found to change by less than 7% upon increasing the pH by 0.2 unit. [g] Ref. 9. [h] $k_1 = 17.9\ M^{-1}$ min.$^{-1}$ at pH 7.3 and $\mu = 0.25$. [i] Path length of 2 mm. achieved by use of quartz insert. [j] Reaction shown to have gone to completion by production of same final absorbance at 338 mμ (owing to *m*-nitrophenyl acetate) at all concentrations of acetylimidazole. [k] Calculated from the equilibrium constant (Table II) and the rate constant of the reverse reaction (ref. 12.). [l] The catalytic constant for imidazole in this reaction ($k_{cat.Im}$) is 800 l.^{2} M^{-2} min.$^{-1}$ (see Results). [m] This work.

constants for the forward and reverse reactions, respectively, and $K_{eq}' = k_f/k_r$, where K_{eq}' is the equilibrium ratio of N,O-diacetyl-N-methylhydroxylamine to acetylimidazole under the conditions of the experiment.[14] The value of K_{eq}' was evaluated for each

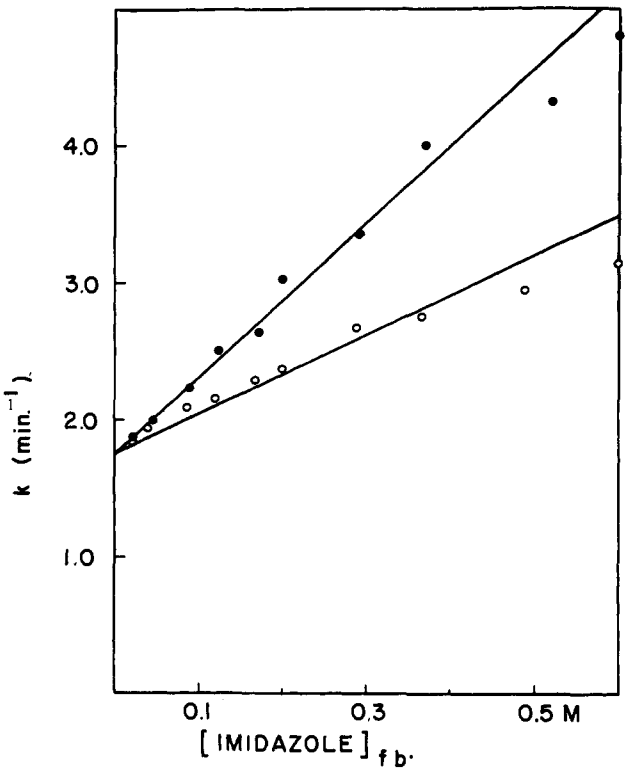


Fig. 3.—Effect of imidazole concentration on the rate of the reaction of 5×10^{-4} M acetylimidazole with 3.5×10^{-3} M N-methylacetohydroxamic acid at 25° and ionic strength 1.0: ●, k_{obsd}; ○, $k_f = k_{obsd}([AcIm]_I - [AcIm]_{eq})/[AcIm]_I$, where $[AcIm]_I$ and $[AcIm]_{eq}$ are the initial and equilibrium concentrations of acetylimidazole, respectively (see text).

experiment from the measured absorbance at zero time, the absorbance after the initial reaction had proceeded to equilibrium, and the absorbance after the acetylimidazole and N,O-diacetyl-N-methylhydroxylamine had undergone complete hydrolysis. The values of k_f calculated in this manner are shown as the lower curve in Fig. 3. The values of k_f increase with increasing imidazole concentration to an extent which is much larger than the experimental error in the determination of k_f. This indicates that the reaction is subject to general base catalysis by imidazole. The value of the catalytic constant of the reaction for the rate law

$$\text{rate} = k_1[\text{AcIm}][CH_3\overset{O}{\overset{\|}{C}}\text{—}\overset{CH_3}{\overset{|}{N}}\text{OH}] + k_c[\text{AcIm}][CH_3\overset{O}{\overset{\|}{C}}\text{—}\overset{CH_3}{\overset{|}{N}}\text{OH}][\text{Im}] \quad (3)$$

is 800 M^{-2} min.$^{-1}$. Based on the apparent second-order rate constant for the reverse reaction of 4.27 in the presence of 1 M imidazole[12] and on the fact that the ratio of the catalyzed and uncatalyzed reactions must be the same in both directions, the rate constant for the uncatalyzed reaction of imidazole with N,O-diacetyl-N-methylhydroxylamine is 1.7 M^{-1} min.$^{-1}$ and the equilibrium constant, from the ratio of the rate constants in the two directions, is 510/1.7 = 300.

Equilibrium Measurements.—In a number of instances it was possible to measure the equilibrium position of the reactions directly, because the transacylation reactions occur much faster than hydrolysis at readily attainable concentrations of reactants. The concentration of acetylimidazole or phenol was measured directly in the reaction mixture by spectrophotometry, with the use of quartz inserts to obtain short path length in 1-cm. cuvettes. The concentration of free thiol in the reaction of N,O-diacetyl-N-methylhydroxylamine with N-acetylmercaptoethylamine (eq. 4) was determined by the nitroprusside reaction in

$$CH_3\overset{O}{\overset{\|}{C}}\text{—}\overset{CH_3O}{\overset{|}{N}}\text{—}\overset{O}{\overset{\|}{C}}CH_3 + HSCH_2CH_2\overset{H}{\overset{|}{N}}\text{—}\overset{O}{\overset{\|}{C}}CH_3 \rightleftharpoons HO\overset{H_3C}{\overset{|}{N}}\text{—}\overset{O}{\overset{\|}{C}}CH_3 + CH_3\overset{O}{\overset{\|}{C}}SCH_2CH_2\overset{H}{\overset{|}{N}}\text{—}\overset{O}{\overset{\|}{C}}CH_3 \quad (4)$$

buffered solutions. A typical experiment for the measurement of the acetyl-transfer reaction between imidazole and the hydroxyl group of N-methylacetohydroxamic acid (eq. 1) is described in detail in the Experimental section and is illustrated in Fig. 1. The experimental conditions and the results are summarized in Table II. In each case equilibrium was approached

(13) A. I. Biggs, *Trans. Faraday Soc.*, **52**, 35 (1956).

(14) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," John Wiley and Sons, Inc., New York, N. Y., 1956, p. 172.

TABLE II

CONDITIONS FOR DIRECT DETERMINATIONS OF EQUILIBRIUM CONSTANTS AT 25° AND IONIC STRENGTH 1.0[a]

Acetylimidazole, $M \times 10^2$	Compound	Init. concn. ROH, $M \times 10^2$	Init. concn. AcOR, $M \times 10^3$	Imidazole,[b] M	Wave length, mμ	No. of detn.	pH	K_I[c] Range	K_I[c] Av.
		Acetylimidazole + ROH ⇌ AcOR + imidazole							
20–80	*p*-Nitrophenol	1.98[d]	0.74	0.014	401[e]	5	7.12	0.9–1.1	1.0
20–60	*p*-Nitrophenol	1.98[d]	0.74	0.014	401[e]	5	7.2	1.0–1.4	1.2
30–50	*p*-Nitrophenol	1.83[d]	0.94	0.014	401[e]	3	7.12	1.0–1.1	1.1
								Mean	1.1
0–1	*p*-Methoxyphenol	18	7.5	0.2–0.35	245	6	7.6	132–139	136
0–2.0	Phenol	30	10	0.234	245	7	7.5	45.6–48.1	47
0.8–3.0	Phenol	30	10	0.234	245	5	7.5	45.3–51.5	49[f]
0	N-Methylacetohydroxamic acid	0.9–1.4	1.9–4.5	0.24	260	4	7.2	289–309	298
0.36		0.45	0.6–2.0	0.19	260	3	7.0	269–282	278
								Mean	287

p-ClPhOAc + AcN(Me)OH ⇌ *p*-ClPhOH + AcN(Me)OAc

AcN(Me)OAc, $M \times 10^2$	ClPhOH, $M \times 10^3$	PCPA, $M \times 10^3$	AcN(Me)OH, $M \times 10^3$	Wave length, mμ	No. of detn.	pH	K_I Range	K_I Av.
11–66	3.9	...	19–48	298	5	8.5[g]	9.3–13.3	11.6
10	1.5	3.5	4.3–9.5	298	2	8.5[g]	8.3–10.5	9.4[h]
							Mean	11.2

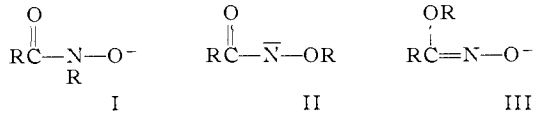
AcN(Me)OAc + HSEtNHAc ⇌ AcN(Me)OH + AcSEtNHAc

AcN(Me)OAc, $M \times 10^2$	HSEtNHAc, $M \times 10^2$	AcN(Me)OH, M	AcSEtNHAc, $M \times 10^2$	Wave length	No. of detn.	pH	K_I Range	K_I Av.
0–1.5	0–1.6	0.1–0.2	1.1–1.9	[i]	4	8.23[j]	19.2–21.0	20.1

[a] Maintained with KCl. [b] As the free base. [c] For eq. 2, with all reactants in the uncharged form. [d] Total *p*-nitrophenol concentration. [e] In 2% acetonitrile. [f] Ionic strength 0.25. [g] 0.17 M Tris buffer. [h] In 6.2% acetonitrile. [i] Concentration of RSH measured by the nitroprusside reaction (ref. 7). [j] 0.05 M Tris buffer, 10^{-4} M ethylenediaminetetraacetate.

from both directions and was attained from a range of different concentrations of starting materials. The equilibrium constants, K_I, refer to the concentrations of nonionized reactants and products at ionic strength 1.0 and 25°.

Structure of the Anion of Acetohydroxamic Acid.— Possible structures for the anion of unsubstituted hydroxamic acids are I, II, and III (R = H). It has recently been suggested, from the results of ultraviolet and infrared spectroscopic studies, that the anion



I II III

of benzohydroxamic acid exists partly or entirely in forms II or III (R = H), in which a proton has been removed from the nitrogen atom.[15-16] The anion of N-methylacetohydroxamic acid can exist only in form I (R = CH_3). The difference in ultraviolet spectrum between the anions of acetohydroxamic acid (λ_{max} 215 mμ, ε 6.6 × 10^3) and N-methylacetohydroxamic acid (λ_{max} 227 mμ, ε 1.96 × 10^4) is consistent with a difference in structure between these two anions. On the other hand, the fact that N-methylacetohydroxamic acid (pK_a 8.8) is a *stronger* acid than acetohydroxamic acid (pK_a 9.4)[17a] means that dissociation of a hydroxamic acid to structure I occurs readily. Acetohydroxamic acid must, therefore, dissociate largely or entirely to structure I and the possibility should be considered that the spectral differences represent only an effect of methyl substitution on the spectrum of I.[17b] Since the effect of methyl substitution on the dissociation constant is in the opposite direction from that expected from the inductive effect of the methyl group, it is probable that specific solvation of the N–H of acetohydroxamic acid provides some stabilization of this molecule; such solvation differences may also be expected to affect the ultraviolet spectra.

(15) R. E. Plapinger, *J. Org. Chem.*, **24**, 802 (1959).

(16) O. Exner and B. Kakáč, *Collection Czech. Chem. Commun.*, **28**, 1656 (1963); O. Exner, *ibid.*, **29**, 1337 (1964).

(17) (a) W. M. Wise and W. W. Brandt, *J. Am. Chem. Soc.*, **77**, 1058 (1955); (b) Steinberg and Swidler (personal communication) have determined the dissociation constants of N-methyl- and O-methylbenzohydroxamic acid and of ethylbenzohydroximic acid and conclude that the anion of benzohydroxamic acid exists in forms I and II in approximately equimolar concentration.

Discussion

The equilibrium constants and free energies for acetyl-transfer reactions which involve acetylimidazole, substituted phenyl acetates, a thiol ester, and the acetyl ester group of N,O-diacetyl-N-methylhydroxylamine are summarized in Table III according to two conventions. The values of K_I refer to the nonionized species of reactants and products. The values of K_{II} refer to the ionic species of the reactants which actually undergo reaction and are related to K_I by the ionization constants of the compounds. For nucleophilic reagents the reactive species is the anion and for acetylimidazole it is the acetylimidazolium cation.[10]

Where comparison is possible, there is moderately good agreement between the values of K_I determined from the ratio of the rate constants in two directions and from direct measurement of equilibrium concentrations. For the reaction of acetylimidazole with *p*-methoxyphenol, for example, the two methods give values of 129 and 136, respectively. After correction for catalysis of the reaction by imidazole, the rate measurements give an equilibrium constant of 300 for the reaction of acetylimidazole with N-methylacetohydroxamic acid, compared to the directly measured value of 287. The value of 37 from the rate measurements for the reaction of acetylimidazole with phenol is probably more accurate than the value of 48 from direct measurements, because of the high blanks attributed to phenol absorption in the latter measure-

ments. A further internal check of the consistency of the data can be made for the reactions involving acetylimidazole, *p*-chlorophenyl acetate, and N,O-diacetyl-N-methylhydroxylamine. The equilibrium constant for acetyl transfer from acetylimidazole to *p*-chlorophenol is 27 and that for acetyl transfer from *p*-chlorophenyl acetate to N-methylacetohydroxamic acid is 11.2. These values give a calculated equilibrium constant of 302 for acetyl transfer from acetylimidazole to N-methylacetohydroxamic acid, which is in good agreement with the directly measured value of 287. There is also close agreement between our results and those obtained by Stadtman for acetyl transfer from acetylimidazole to a thiol.[5] The equilibrium constants for the reactions of acetylimidazole with N-methylacetohydroxamic acid and of N,O-diacetyl-N-methylhydroxylamine with N-acetyl-β-mercaptoethylamine are 287 and 210, respectively. This gives an equilibrium constant of 5770 for the reaction of acetylimidazole with N-acetylmercaptoethylamine, which may be compared with Stadtman's value of 5640 for the reaction of acetylimidazole with the thiol group of glutathione.

TABLE III

SUMMARY OF EQUILIBRIUM CONSTANTS AND FREE-ENERGY DIFFERENCES FOR ACETYL TRANSFER[a]

X–	K_I $\frac{[IM][X\text{-}Ac]}{[AcIm][X\text{-}H]}$	$-\Delta F_I°$, cal./mole	K_{II} ($\times 10^{-3}$) $\frac{[Im][X\text{-}Ac]}{[AcImH^+][X^-]}$[b]	$-\Delta F_{II}°$, cal./mole
p-MeOPhO–[b]	129	2900	49,200	11,800
p-MeOPhO–	84	2600	32,500	11,600
PhO–	37	2100	8,750	10,600
p-ClPhO–	27	1900	1,550	9,700
m-O_2NPhO–	5	940	15	6,900
p-O_2NPhO–	1.1	60	0.37	4,750
AcN(Me)O–	287	3270	4,020	10,200
AcHNEtS–[c]	5770	5030	420,000	12,800
	$\frac{[p\text{-ClPhOH}][XAc]}{[p\text{-ClPhOAc}][XH]}$		$\frac{[p\text{-ClPhO}^-][XAc]}{[p\text{-ClPhOAc}][X^-]}$	
AcN(Me)O–	11.2	1400	4.4×10^{-4}	850
	$\frac{[AcHNEtSAc][XH]}{[AcHNEtSH][XAc]}$		$\frac{[AcHNEtSAc][X^-]}{[AcHNEtS^-][XAc]}$	
AcN(Me)O–	20.1	1730	7.7×10^{-5}	2,500

[a] Ionic strength 1.0, 25°. The values for the reaction of acetylimidazole with phenol and *p*-methoxyphenol are based on rate measurements which are thought to be more accurate than the direct equilibrium constant determinations for these reactions. [b] Based on pK_a' of 7.2 for imidazoleH$^+$ at ionic strength 1.0 (ref. 12). [c] Calculated from the equilibrium constants for AcIm + AcN(Me)OH ⇄ Im + AcN(Me)OAc and AcN(Me)OAc + AcHNEtSH ⇄ AcHNEtSAc + AcN(Me)OH.

Structure–Reactivity Correlations.—The equilibrium constants for ester formation from acetic acid and a series of alcohols and thiol alcohols, $K = [RCOXR]\cdot[H_2O]/[RCOOH][HXR]$, calculated from the equilibrium constants for acetyl transfer and the known equilibrium constant for N,S-diacetyl-β-mercaptoethylamine formation,[3] are plotted logarithmically against the pK_a of the alcohol in Fig. 4. The hydrolysis of oxygen esters is favored by electron-withdrawing substituents on the alcohol moiety, and a line drawn through the values for a series of aliphatic alcohols and phenols is linear. The sensitivity of the equilibrium constant to the nature of the leaving group is large. The slope of the line of Fig. 4 is 0.70, which is close to the corresponding value of approximately 0.8 for the sensitivity of the rate of attack of a series of related nucleophilic reagents on an activated ester or amide, such as *p*-nitrophenyl acetate or acetylimidazolium cation.[18,19] The corresponding correlation of log K with Hammett's σ-values for the series of substituted phenyl acetates is linear with a slope of −1.40, based upon a σ-value of 1.27 for the *p*-NO_2 group.[20] The equilibrium constants for acetyl transfer between 4-pyridinealdoxime methiodide acetate and several thiol esters have been determined,[21] and comparison of these values with the equilibrium constants for thiol ester formation[3] permits the calculation of the equilibrium constant for the formation of the ester group of this oxime acetate. The equilibrium constant for the formation of acetyl hypochlorite is 0.0025, based on the molar concentration of water,[22] and 4.5×10^{-5}, based on the convention that the activity of pure water is 1.0. This value is not exactly comparable to the other equilibrium constants, because it was measured in acetic acid containing traces of water, but it is improbable that the solvent effect would change the result by more than an order of magnitude.

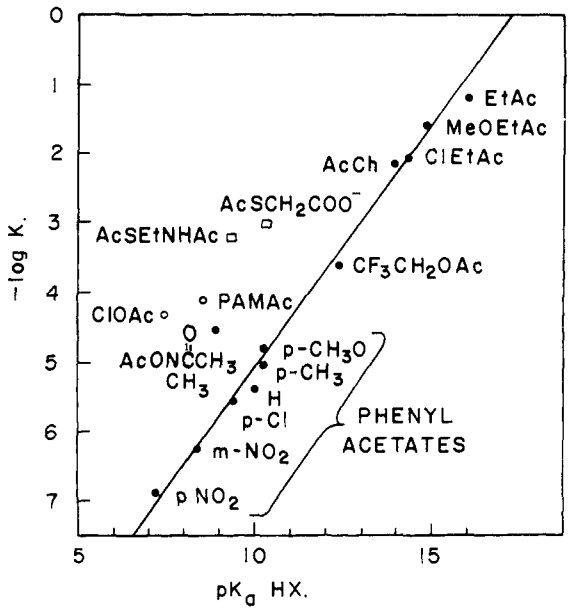


Fig. 4.—Comparison of the equilibrium constants for the formation of acetate esters from acetic acid and a series of alcohols with the pK_a of the alcohol: O, oxygen esters. Solid circles are this work and ref. 3. ClOAc is acetyl hypochlorite[22] (in acetic acid solution) and PAMAc is 4-pyridinealdoxime acetate[21] (at 37°); □, thiol esters. The equilibrium constants are based on an activity of water of 1.0.

The oxygen atoms of hydroxylamine and its derivatives and of hypochlorite are unusually effective nucleophilic reagents toward the acyl group of *p*-nitrophenyl acetate.[19] The oxygen atoms of hydroxylamine[19] and of N-methylacetohydroxamic acid (Table I) are also unusually effective nucleophilic reagents toward acetylimidazolium cation; the latter compound is about 15-fold more reactive than phenolate ions of comparable basicity. This high reactivity of atoms adjacent to an electronegative atom with a free electron

(18) T. C. Bruice and R. Lapinski, *J. Am. Chem. Soc.*, **80**, 2265 (1958).
(19) W. P. Jencks and J. Carriuolo, *ibid.*, **82**, 1778 (1960).
(20) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940.
(21) J. J. O'Neill, H. Kohl, and J. Epstein, *Biochem. Pharm.*, **8**, 399 (1961).
(22) (a) P. B. D. De la Mare, I. C. Hilton, and C. A. Vernon, *J. Chem. Soc.*, 4039 (1960); (b) J. O. Edwards and R. G. Pearson, *J. Am. Chem. Soc.*, **84**, 16 (1962).

pair has been called the *α-effect*.[22b] It is of interest to compare this kinetic effect with the thermodynamic stability of the products of these reactions. It is apparent that the products of these reactions are more stable than might be expected from the acidity of the alcohol (Fig. 4). The positive deviation for the equilibrium constant of N,O-diacetyl-N-methylhydroxylamine is very similar to the positive deviation of the rate constant for the reaction of N-methylacetohydroxamate with acetylimidazolium. It may be concluded that whatever factor results in a stabilization of the transition state for nucleophilic reactions of these compounds also results in a stabilization of the products, relative to the phenyl acetates. There are other examples of favorable equilibria, as well as rates, in additions of this class of compound to the carbonyl group.[19] It may be noted that it is difficult to account for such equilibria by ordinary resonance effects, which have been invoked to explain the unusual reactivities of these compounds.[22]

For a given pK_a of the leaving group, thiol esters are some two orders of magnitude more stable than the corresponding oxygen esters. This result is not unexpected in view of the relative bond strengths of oxygen and sulfur to carbon and hydrogen, the large size of the sulfur atom which is reflected in the relatively high acidity of thiols, the decreased electronegativity of sulfur compared to oxygen, and the larger resonance stabilization of oxygen than of thiol esters.

It is of interest to compare the equilibrium constants with the rate constants in both directions for the reversible reactions of imidazole with phenyl acetates. The rate constants for the reactions of imidazole with substituted phenyl acetates under the same experimental conditions that were used for the equilibrium constant determinations have been reported previously.[12] The rate constants for the reactions of substituted phenols with acetylimidazole display little sensitivity to the nature of the phenol when they are expressed in terms of the uncharged reactants according to the rate law of eq. 2a. However, it is more appropriate for a discussion of mechanism to use the rate law of eq. 2b and the equilibrium constant

$$K = [\mathrm{Im}][\mathrm{AcOR}]/[\mathrm{AcImH^+}][^-\mathrm{OR}]$$

based on the over-all reaction

$$\mathrm{AcImH^+} + {}^-\mathrm{OC_6H_5X} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{Im} + \mathrm{AcOC_6H_5X} \qquad (5)$$

in which the compounds are expressed in the form of the ionic species which undergo reaction. Logarithmic plots of the rate constants in the two directions against the equilibrium constant for this reaction show negative deviations for the rate constants for the *p*-nitro substituent (Fig. 5). The slope of the line drawn through the points for the less electron-withdrawing substituents is 0.39 for log k_2 and −0.60 for log k_{-2} against log K_{eq}. It may be concluded that for these substituents, electronic effects on the transition state are approximately midway between those on the reactants and products. The same comparison may be made in terms of the ρ–σ correlation. The value of ρ for K_{eq} is −3.37, while that for k_2 is −1.54 and that for k_{-2} is 1.83. It was concluded previously that in the reaction of imidazole with a series of acetates,

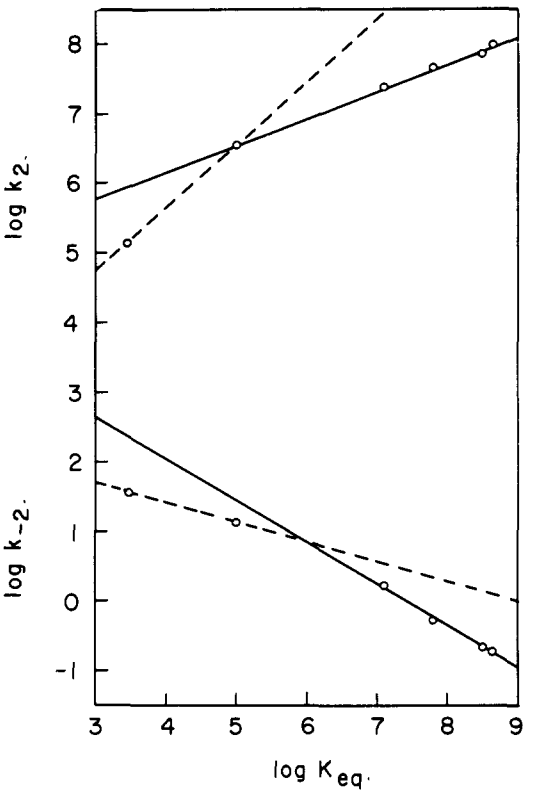
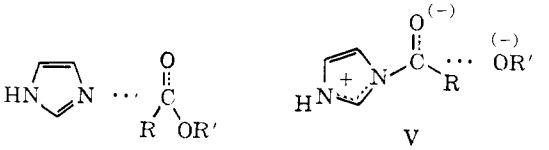


Fig. 5.—Logarithmic plot of the equilibrium constants against the rate constants in the two directions for the reaction of acetylimidazolium and phenolate ions (eq. 5) at 25°.

the substituted phenyl acetates fall in the region intermediate between those compounds with a good leaving group, in which the transition state reflects principally the attack of imidazole (IV), and those compounds with a poor leaving group, in which the transition state reflects principally the expulsion of the leaving group (*e.g.*, V).[12] Thus, it is not unexpected that substituent effects on the transition state should



be intermediate between those on the starting materials and the products for these compounds. In the case of *p*-nitrophenol, the transition state should reflect the attack of imidazole (IV) to a greater extent than in the case of the other phenols. For *p*-nitrophenol the sensitivity of the rate of imidazole attack on the phenyl acetate to substituents should, therefore, be diminished and the sensitivity of the rate of phenolate attack on acetylimidazolium ion should be increased, because the negative charge of the phenolate ion should be almost completely lost in the transition state. These expectations are borne out by the results, as shown by the dashed lines in Fig. 5. The same situation is evident in ρ–σ correlations for k_2 and k_{-2}, in which the rate constants for the *p*-nitro substituent fall below the lines for both correlations. These relationships are shown schematically in the transition-state diagrams of Fig. 6. It should be kept in mind that such results may also be interpreted in terms of a skewed transition

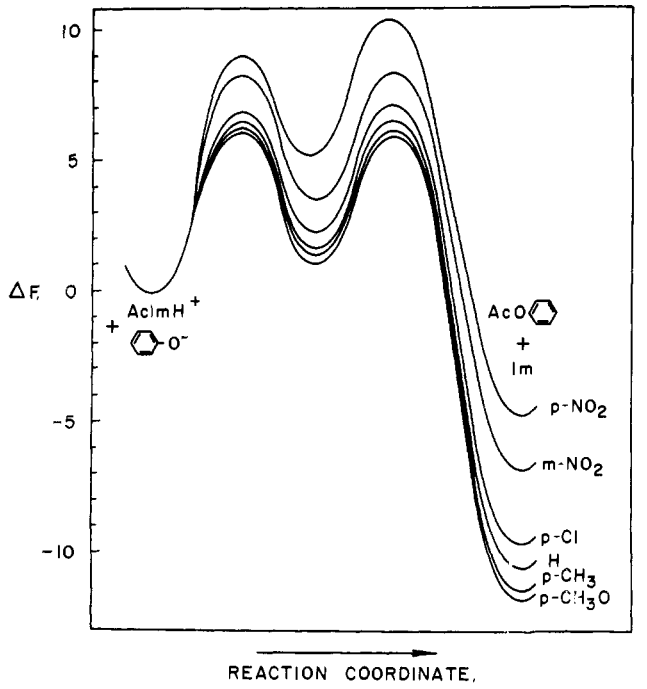


Fig. 6.—Schematic, semiquantitative transition-state diagram for the reversible reactions of imidazole with substituted phenyl acetates (see text). The depth of the valley corresponding to the hypothetical tetrahedral addition intermediate is arbitrary.

state and do not necessarily require the existence of a metastable tetrahedral addition intermediate.

The sensitivities to substituent effects of the addition of an acyl group (eq. 5) and of a proton (eq. 6) to substituted phenolate anions may be compared by a

$$H^+ + {}^-OC_6H_4X \rightleftharpoons HOC_6H_4X \qquad (6)$$

logarithmic plot of the equilibrium constants for eq. 6 against the pK_a of the phenol. The slope of such a plot is 1.72. This value and the large ρ-value of -3.37 for the equilibrium constants of eq. 5 show that the equilibrium addition of the electron-withdrawing acyl group to the phenolate ion is considerably more sensitive to polar effects than is the addition of a proton. Substituent effects on the ionization of phenols[23a] and on the rates of intramolecular displacement of phenols from monophenyl succinates and glutarates[23b] are reflected principally in the entropy rather than the enthalpy of the reactions; it will be of interest to determine to what extent this may hold true for acyl-transfer equilibria.

A comparison of the reactivity of a number of nucleophilic reagents of varying structure toward the acetylimidazolium cation has been presented previously.[19] In a plot of log k against the pK_a of the attacking nucleophilic reagent, the points for a given class of compound fall near a slope of 0.8, in a manner similar to that observed for similar correlations with p-nitrophenyl acetate.[18,19] The rate constants for substituted phenolate anions and for the anion of N-methylacetohydroxamic acid may now be added to this correlation. If the reactivities of compounds which carry a negative charge on the attacking atom toward acetylimidazolium cation and p-nitrophenyl acetate are compared with those of uncharged molecules, it is apparent that rates of the attack of anions on acetylimidazolium cation are displaced upward by about 1.3 to 1.8 logarithmic units, corresponding to a decrease in the free energy of activation of approximately 2000 cal./mole which may be ascribed to electrostatic effects. The positive deviation of the N-methylacetohydroxamate anion in this correlation has been discussed above.

(23) (a) P. Fernandez and L. G. Hepler, *J. Am. Chem. Soc.*, **81**, 1783 (1959); (b) E. Gaetjens and H. Morawetz, *ibid.*, **82**, 5328 (1960).

TABLE IV

FREE ENERGIES OF HYDROLYSIS OF DERIVATIVES OF ACETIC ACID AND RELATED COMPOUNDS AT 25°

Compound	$-\Delta F°$[a]	$-\Delta F_{pH\ 7}°'$[b]	Ref.
Acetylimidazole[c]	9490	12,970	[d]
p-Nitrophenyl acetate[e]	9430	13,010	[d]
m-Nitrophenyl acetate[e]	8550	11,610	[d]
p-Chlorophenyl acetate[e]	7590	10,650	[d]
Phenyl acetate[e]	7390	10,450	[d]
p-Methylphenyl acetate[e]	6890	9,950	[d]
p-Methoxyphenyl acetate[e]	6590	9,650	[d]
Acetyl hypochlorite	5950	9,214	[i]
Acetyl phosphate	6690[f]	10,300	5[g]
N,O-Diacetyl-N-methylhydroxylamine[c,g]	6190	9,250	[d]
4-Pyridinealdoxime acetate[i]	5670	8,730	21(37°)
Amino acid esters	1970	8,400	2(39°)
Acetate thiol esters[h]	4460	7,520	3
Adenosine triphosphate (→ADP, Pi)		7,300[k]	24
Adenosine triphosphate (→AMP, PP)		7,700[k]	2, 25
Trifluoroethyl acetate	4970	8,030	3
Acetylcholine	2940	6,000	3
Chloroethyl acetate	2840	5,900	3
Methoxyethyl acetate	2180	5,240	3
Ethyl acetate	1660	4,720	3
Glutamine		3,400	21
Propionamide		2,100	26
Peptides		500	27
Acetohydroxamic acid		+200	28

[a] Standard free energy of hydrolysis based on a standard state of 1 M concentrations of the *uncharged* reactants and products and an activity of pure water of 1.0 (convention I of ref. 2). [b] Standard free energy of hydrolysis at pH 7.0 based on a standard state of 1 M total stoichiometric concentration of reactants and products, except hydrogen ion, and on an activity of pure water of 1.0 (convention III of ref. 2). Values for derivatives of acetic acid are based on a thermodynamic pK_a of 4.76 for acetic acid and a ΔF for ionization of acetic acid at pH 7.0 of 3060 cal./mole. Values for $\Delta F_{pH\ 7}°'$ for acetate derivatives based on a pK_a' of 4.63 ± 0.02 at ionic strength 0.2 to 1.0[29] are 180 cal./mole more negative. [c] Based on the equilibrium with N,S-diacetyl-β-mercaptoethylamine. [d] This work. [e] Based on the equilibrium with acetylimidazole. [f] For the dianions of acetyl phosphate and phosphate. [g] For hydrolysis of the ester. [h] For N,S-diacetyl-β-mercaptoethylamine. The value for S-acetylmercaptoacetate is 320 cal./mole less negative. [i] Based on (closely similar) equilibrium constants with several thiol esters and the $\Delta F°$ for N,S-diacetyl-β-mercaptoethylamine. [j] From the data of De la Mare in acetic acid containing traces of water[22a] and an ionization constant of 4.1×10^{-8} for hypochlorous acid.[30] [k] At 25–37° and an ionic strength near 0.2 in the presence of excess Mg^{+2}; see ref. 3.

(24) (a) M. R. Atkinson, E. Johnson, and R. K. Morton, *Nature* (London), **184**, 1925 (1959); (b) E. A. Robbins and P. D. Boyer, *J. Biol. Chem.* **224**, 121 (1957); (c) T. Benzinger, C. Kitzinger, R. Hems, and K. Burton, *Biochem. J.*, **71**, 400 (1959).

(25) (a) M. E. Jones, *Federation Proc.*, **12**, 708 (1953); (b) P. Hele, *J. Biol. Chem.*, **206**, 671 (1954).

(26) H. Morawetz and P. S. Otaki, *J. Am. Chem. Soc.*, **85**, 463 (1963).

(27) A. Dobry, J. S. Fruton, and J. M. Sturtevant, *J. Biol. Chem.*, **195**, 149 (1952).

(28) W. P. Jencks, M. Caplow, M. Gilchrist, and R. G. Kallen, *Biochemistry*, **2**, 1313 (1963).

(29) J. Bjerrum, G. Schwarzenbach, and L. G. Sillén, "Stability Constants, Part I," The Chemical Society, London, 1957.

(30) R. P. Mariger and F. G. Soper, *J. Chem. Soc.*, 71 (1946).

Free Energies of Hydrolysis.—In Table IV are summarized the values of ΔF° and $\Delta F_{pH\ 7}{}^{\circ\prime}$, the standard free energies of hydrolysis to free acetic acid and to acetate ion at pH 7.0, respectively, calculated from the results of this work and from related equilibria. The values of ΔF° refer to a standard state of 1 M reactants and products as the nonionized species, except for acetyl phosphate, which refers to the dianions of acetyl phosphate and inorganic phosphate. The vaues of $\Delta F_{pH\ 7}{}^{\circ\prime}$ refer to a standard state of 1 M concentrations of the sum of all ionic species of the reactants and products at pH 7.0, except for hydrogen ion. The activity of pure water is taken as 1.0. The values for acetic acid derivatives are based on the thermodynamic pK_a for acetic acid of 4.76; the values based on a pK_a' of 4.63 ± 0.02 at ionic strength 0.2 to 1.0[29] are 180 cal./mole more negative. The calculations for ester hydrolysis, with the exception of acetylcholine, are based upon data which were extrapolated to zero ionic strength. The results for acetylcholine and for the acetyl-transfer reactions are not extrapolated to zero ionic strength, but these equilibria would not be expected to show a large dependence upon ionic strength, because increasing salt concentration should have a similar effect on the activity coefficients of the reactants and products. The values of −9490 and −4460 cal./mole for the free energies of hydrolysis of acetylimidazole and the thiol ester group of N,S-diacetyl-β-mercaptoethylamine may be compared to the values of −7280 and −910 cal./mole, respectively, for the heats of hydrolysis of the same compounds in water.[31] The greater part of the difference between the ΔF° and ΔH values reflects the fact that the standard state for water is taken as 1.0, rather than 55.5 M; this convention contributes −2400 cal./mole to the ΔF° values.

It has been suggested that phosphorylimidazoles and possibly acylimidazoles may be involved in biological oxidative phosphorylation.[32] The rates and equilibria for acyl-transfer reactions involving acetylimidazole and phenolates reported here may conceivably be pertinent to the uncoupling or inhibitory action of acidic phenols on oxidative phosphorylation.[33]

(31) I. Wadso, *Acta Chem. Scand.*, **16**, 479, 487 (1962).

(32) P. D. Boyer, D. E. Hultquist, J. B. Peter, G. Kreil, R. A. Mitchell, M. DeLuca, J. W. Hinkson, L. G. Butler, and R. W. Moyer, *Federation Proc.*, **22**, 1080 (1963).

(33) V. H. Parker, *Biochem. J.*, **69**, 306 (1958); H. C. Hemker, *Biochim. Biophys. Acta*, **63**, 46 (1962).

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA, MINNEAPOLIS 14, MINNESOTA]

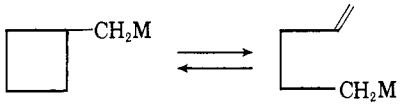
Kinetics of the Ring Opening of Cyclobutylmethylorganomagnesium Compounds[1]

BY E. ALEXANDER HILL AND JOHN A. DAVIDSON[2]

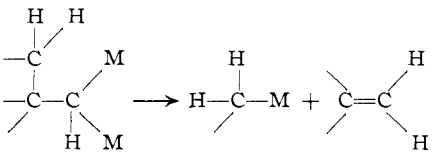
RECEIVED MARCH 28, 1964

The rearrangement of cyclobutylmethyl Grignard and dialkylmagnesium reagents to 1-pent-4-enylorganomagnesium compounds was studied kinetically. The solvent dependence and the effects of α-methyl and α-deuterium substitution are most readily interpreted on the basis of a synchronous four-center process. Contribution of a π-complex structure to the transition-state resonance hybrid is proposed.

In a previous communication, we have reported ring-cleavage reactions of the cyclobutylmethylorganometallic derivations of sodium, lithium, and magnesium.[3] These cleavages, and the analogous very facile ring opening of the corresponding cyclopropylmethylorganometallics,[4] undoubtedly derive their driving force from the relief of ring strain. The analogous acyclic organometallic cleavage should be endothermic by approximately 20 kcal. Some recently reported cleavages of acyclic organopotassium and organosodium compounds are apparently observed owing to vaporization of volatile products or metallation and polymerization of the olefin formed.[5]



M=Na, Li, MgX



In the present paper, we report a kinetic study of the ring cleavages of the cyclobutylmethylorganomagnesium compounds. This study was undertaken for the purpose of distinguishing between several alternative mechanisms which have been suggested for these rearrangements.[3]

Results

The rearrangement of cyclobutylmethylmagnesium chloride to 1-pent-4-enylmagnesium chloride in tetrahydrofuran was studied kinetically at 61.5°. The corresponding dialkylmagnesium was also studied.

(1) Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research.

(2) (a) Based in part upon a thesis submitted by J. A. Davidson in partial fulfillment of the requirements for the M.S. degree; (b) American Chemical Society Petroleum Research Fund Fellow, summer, 1963.

(3) E. A. Hill, H. G. Richey, Jr., and T. C. Rees, *J. Org. Chem.*, **28**, 2161 (1963).

(4) (a) M. S. Silver, P. R. Shafer, J. E. Nordlander, C. Ruchardt, and J. D. Roberts, *J. Am. Chem. Soc.*, **82**, 2646 (1960); (b) P. T. Lansbury, *ibid.*, **85**, 1886 (1963); (c) H. G. Richey, Jr., and E. A. Hill, *J. Org. Chem.*, **29**, 421 (1964).

(5) (a) R. A. Finnegan, *Chem. Ind.* (London), 895 (1962). (b) R. A. Finnegan, *Tetrahedron Letters*, 1303 (1962). (c) The Wittig and Stevens rearrangements, as well as several other recently reported reactions, may involve cleavages of heteroatom-substituted organometallics or organometallics in which a resonance-stabilized carbanion may be the cleavage product.[6]

(6) (a) G. Kobrick, *Angew. Chem. Intern. Ed. Engl.*, **1**, 382 (1962), and references therein; (b) P. T. Lansbury and V. A. Pattison, *J. Am. Chem. Soc.*, **84**, 4295 (1962); *J. Org. Chem.*, **27**, 1933 (1962); (c) E. Grovenstein, Jr., and G. Wentworth, *J. Am. Chem. Soc.*, **85**, 3305 (1963); (d) P. K. Freeman, D. E. George, and V. N. M. Rao, *J. Org. Chem.*, **28**, 3234 (1963).