Aminolysis of α-Acetoxystyrenes. The pK_a of Acetophenones in Aqueous Solution

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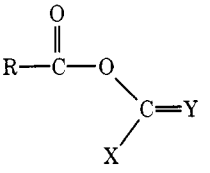
Abstract: Despite a literature pK_a for acetophenone enol of about 15.5, the acetate ester of this species, α-acetoxystyrene, aminolyzes with about the same rate as phenyl acetate; this rate is over 1000 times faster than the aminolysis rate observed for the saturated analogue, α-phenethylacetate. Because of the relationship of enol esters to active esters used in peptide synthesis, a mechanistic investigation of this aminolysis was undertaken to determine whether a special aminolysis mechanism was required to account for these results. The ester was found to conform in its aminolysis behavior to the mechanistic patterns which have been well established for aryl acetates. Thus, aminolysis terms in the rate law included terms in [amine], [amine]^2, [amine][amine·H$^+$], and [amine][OH$^-$] of about the same magnitude, in many cases, as those observed for aryl acetates. A leaving group susceptibility, β_{lg}, was found to be near unity for the term in [amine], and the susceptibility of the same term to the nucleophilicity of the amine, β_{nuc}, was found also to be near unity. Direct comparisons of rates, as well as the use of the Ritchie N_+ correlation as applied to ester aminolysis reactions, suggest that the oxygen pK_a of acetophenone enol is close to 11; the known amount of enol in acetophenone thus places the carbon pK_a for this substance *in water* at about 16, about four orders of magnitude lower than that estimated from determinations in nonaqueous solution. The aqueous solution reactivity of active esters used in peptide synthesis is discussed in the light of these and other findings.

The rapid development of the important area of peptide synthesis has led to the discovery of a series of coupling agents which have been able to bring about the efficient dehydrative linkage between an amine and a carboxylic acid to form a new amide (peptide) bond. Among these agents are a broad class of substances, the use of which generates a type of "active ester" intermediate of the general structure **1**; these materials



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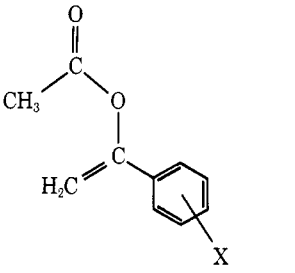
are exceptionally labile toward aminolysis or toward coupling with other nucleophiles. Among the coupling agents which yield such intermediates are carbodiimides, *N*-alkylisoxazolium salts, ethoxyacetylene, cyanamides, and others.[2] Although there have been a number of mechanistic investigations of the various features of the reactions of these compounds,[3] there has been only one detailed kinetic examination of the mechanism of aminolysis of esters of type **1**, using a cyclic analogue.[4] We felt that a study of the aminolysis of other noncyclic species of this type would shed more light on the generality of compounds of structure **1** as acyl transfer agents, would be of interest in the general problem of aminolysis mechanisms of esters,[5] and would help to define more fully the variables which are potentially under experimental control in the use of such reagents.

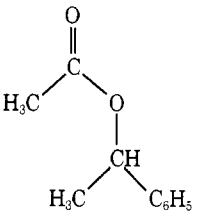
For example, it occurred to us that when X = phenyl and Y = CH_2, there would be present a particularly poor leaving group (a ketone enolate); since carbon pK_a values of ketones (e.g., acetophenone) are on the order of 20,[7-10] and enols typically exist in about 1 part in 10,[4] the oxygen pK_a of the corresponding enols would be about 16. Since it has been found for a wide variety of esters that, in aminolysis reactions, loss of the leaving group is kinetically significant[6a] so that the logarithms of the rates of aminolysis of such esters closely parallel the pK_a of their respective leaving groups, simple enol esters should not be very good acyl transfer agents on this basis. If, in fact, these esters were found to aminolyze rapidly, then interesting new mechanisms might be operative.

We were quite intrigued to find, therefore, that a series of



2a, X = *p*-OCH_3 **d**, X = *p*-Cl
b, X = *p*-CH_3 **e**, X = *m*-Cl
c, X = H **f**, X = *p*-NO_2

α-acetoxystyrenes (**2a–f**) aminolyzed rather rapidly, with one member **2e** of the series reacting at about the same rate with a given amine as phenyl acetate. As we shall document below, these esters showed many of the characteristics of "active esters". Aminolysis proved to be considerably faster than competing hydrolysis and was >1000 times faster than aminolysis of the saturated analogue, α-phenethyl acetate (**3**). Hydrolysis



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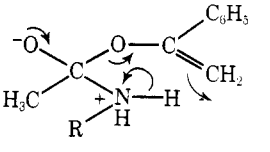
of **2c** by OH$^-$, however, was only a factor of 10 faster than the corresponding hydrolysis of **3**. There seemed to be two general alternatives for explaining this unexpectedly high reactivity of **2c**. One was a "special" mechanism, of which transition state **4** might be an attractive example. The other explanation was



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to revise downward the conventional estimates of ketone pK_a values for at least aqueous solutions, so that enolate ions could

Table I. Hydrolytic Rate Constants for α-Acetoxystyrenes (**2a–f**) and α-Phenethyl Acetate (**3**)

Compd	k_{OH},[a] M^{-1} min^{-1}
2a	54 ± 1
2b	55 ● 1
2c	63 ± 2
2d	95 ± 3
2e	102 ± 3
2f	158 ± 4
3	5 ± 1

[a] Second-order rate constants for hydrolysis by OH^-, reported with their standard deviations.

be placed generally in the category of good leaving groups. This paper reports our observations which clearly require the latter alternative.

Experimental Section

The α-acetoxystyrenes **2a–f** were prepared from the corresponding acetophenones and isopropenyl acetate by the method of Noyce and Pollack,[11] except that 0.1 equiv of *p*-toluenesulfonic acid was used instead of a truly catalytic amount. This modification led to a considerably faster reaction rate and a higher overall yield of the enol ester. All the esters were fractionally distilled to achieve separation from the unreacted acetophenones. The *p*-methoxy and *p*-nitro analogues were recrystallized from ethanol to a constant melting point prior to use. α-Acetoxystyrene was further purified by preparative GLC on a ⅜ in. × 8 ft SE-30 column at 140 °C; the other three esters were redistilled just prior to use. Melting points and boiling points were in excellent agreement with the literature[11] values, and spectra were equally consistent with the desired structures.

The amines used in the kinetics were distilled from CaH_2 or CaO just prior to use after preliminary drying over molecular sieves or KOH.

Fresh doubly distilled, deionized water that had been thoroughly flushed with argon was used in all the kinetics. Absolute ethanol was introduced from a container of commercial material after the argon flush.

Kinetic Measurements. All kinetics were done in 5 vol % ethanol–water solution at an ionic strength of 0.5 M (KCl) and a temperature of 29.9 ± 0.1 °C. Hydrolysis and aminolysis rates were measured by the appearance of acetophenones on a Cary Model 1605 uv-visible spectrophotometer or a Gilford 2220 adapter interfaced to a Beckman DU monochromator equipped with a Gilford 222A photometer and 2458-A automatic cuvette positioner. Both spectrophotometers were thermostated at the same cuvette temperature (see above).

The concentration of the esters used in the kinetic study varied from (1.0 to 2.5) × 10^{-4} M. The appearance of the acetophenones was followed at 280 nm for **2a** and **2b**, 279 nm for **2c**, 280 nm for **2d**, 292 nm for **2e**, and 268 nm for **2f**. Measurements of pH were made on a Radiometer Model 26 pH meter using the relation

$$\text{pH} = \text{meter reading} - 0.06$$

determined from pH measurements of known concentrations of acid in the solvent system employed. A pK_w of 13.72 was determined by pH measurements of known concentrations of KOH in the same solvent at 30 °C.

The amine buffer solutions were prepared just prior to use by titration of a 1 or 2 N solution of the amine with standardized HCl to the desired concentration of free amine. KCl and ethanol were added in the quantities necessary, and the solutions were diluted to volume with deionized distilled water. Dilutions were prepared by diluting aliquots of the master buffer to volume with a 0.5 M KCl solution which was 5 vol % in ethanol. A minimum of five serially diluted buffer solutions were employed at each pH. The dilutions agreed to within 0.02 pH unit, and were adjusted to agree exactly with the buffer of highest concentration by addition of small drops of concentrated HCl or KOH, as necessary. Reactions were initiated by injection of 25 μl of an ethanolic solution of the appropriate ester of the appropriate concentration into the cuvette containing the buffer solution. The kinetics of aminolysis were followed at between four and seven different pH values for each amine employed. All amines except morpholine were used in the concentration range from about 0.05 to about 0.40 M total amine; morpholine was used in the range 0.16 to 0.80 M.

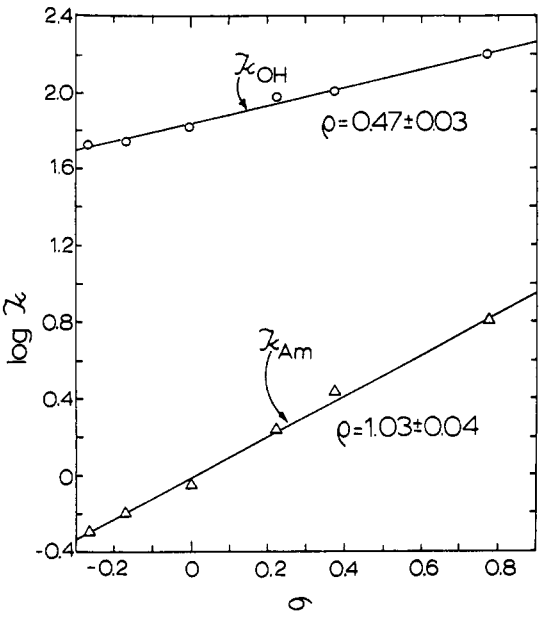


Figure 1. Plots of the logarithm of the second-order hydrolytic rate constants, k_{OH}, and the aminolysis rate constants, k_{Am} (butylamine) vs. the Hammett σ value for the substituent in the leaving group.

The rates of hydrolysis were determined using solutions of KOH in 5 vol % ethanol at 0.5 M ionic strength (KCl) in the KOH concentration range 0.008 to 0.08 M.

The pK_a values of the amines used in this study in the solvent system employed herein were determined by half-neutralization under conditions of the kinetic experiments.

Calculations. Rate constants were calculated on a Hewlett-Packard Model 9810A desk calculator using a nonlinear least-squares program developed after a method by Wentworth.[12] The rate constant for hydrolysis of a given ester was determined from the calculated intercept of a log k_{obsd} vs. log $[OH^-]$ plot. Aminolysis rate constants were determined from analysis of the dependence of the observed rate constants, k_{obsd}, on amine concentration. The values of k_{obsd} were nonlinear in total amine, $[amine_T]$, having a dependence on both $[amine_T]$ and $[amine_T]^2$ (see below). A nonlinear least-squares program also after Wentworth[12] was used on an IBM 370-65 computer to determine the coefficients of each of the above terms in amine concentration. Plots of $k_{Am,T}/f_{Am}$, where $k_{Am,T}$ is the observed coefficient of the $[amine_T]$ term and f_{Am} is the fraction of the amine in the free base form, vs. $[OH^-]$ gave k_{Am} on the intercept and $k_{Am,OH}$ as the slope, where these rate constants are defined in eq 1 in the Results section. Plots of $k_{Am^2,T}/f_{Am}$, where $k_{Am^2,T}$ is the coefficient of the $[amine_T]^2$ term, gave k_{gb} as the slope and k_{ga} as the intercept, where again these last two rate constants will be defined in conjunction with eq 1 below. This method was checked against the graphical methods of others[3a,12] and found to give the same results.

Product Analysis. Solutions of *n*-butylamine at a buffer ratio of 1:1 amine:amine·H^+ were prepared in an identical manner to that described for the kinetic solutions. These solutions (50 ml) were incubated in a 30 °C water bath for 0.5 h before they were made 10^{-3} M in α-acetoxystyrene by injection of 0.41 ml of a 0.12 M ethanolic solution of this compound. These conditions duplicate the kinetic conditions except for a somewhat higher concentration of the ester. The solution was incubated for 11 half-lives (as calculated from the rate data under identical conditions), and it was cooled and brought to pH 3 with 5% HCl. The solution was extracted five times with 50-ml portions of ether after addition of 15 g of NaCl to the aqueous portion to provide salting-out assistance in the extraction. The ethereal solution was dried ($MgSO_4$) and then fractionally distilled until the amount of material remaining in the flask was about 1 ml. At this point the residue was analyzed on a ¼ in. × 8 ft 20% Carbowax on Chromosorb W column by GLC at 140 °C. The presence of *n*-butylacetamide was proven by comparison with an authentic sample; the

Table II. Aminolysis Rate Constants for *m*-Chloro-α-acetoxystyrene[a,b]

Amine	pK_a	k_{Am}, M^{-1} min^{-1}	k_{gb}, M^{-2} min^{-1}	k_{ga}, M^{-2} min^{-1}	$k_{Am,OH}$, M^{-2} min^{-1}
Pyrrolidine	11.32	19.6 ± 1.0	99 ± 9	0	$(4.3 \pm 0.5) \times 10^{-3}$
n-Butylamine	10.57	2.76 ± 0.06	4.1 ± 0.4	0.4 ± 0.2	600 ± 60
Ethanolamine	9.57	0.204 ± 0.001	0.68 ± 0.02	0	37 ± 7
2-Methoxyethyl-amine	9.48	0.138 ± 0.001	0.312 ± 0.008	0.059 ± 0.006	0
Morpholine	8.57	0.0149 ± 0.0007	0.0080 ± 0.0002	0.0015 ± 0.0001	50 ± 40

[a] The rate constants, defined in eq 1, are given with their standard deviations. [b] α-Phenethyl acetate (**3**) was incubated with a 0.20 M *n*-butylamine buffer in which the fraction free amine was 0.8. After a time corresponding to 9 hydrolysis half-lives (Table I), the reaction mixture was assayed by GLC for *n*-butylacetamide, using the procedure which was used for the product studies in Table V; controls showed that we could have detected ≤2% of this product; none was observed. This experiment is the basis for the statement in the introduction about the relative aminolysis rates of **2c** and **3**.

only other peak observed (aside from solvent) corresponded to that produced by authentic acetophenone. The ratio of aminolysis to total reaction could then be determined from the ratio of *n*-butylacetamide to acetophenone after correction for the differential detector response.

Results

Rate constants for the alkaline hydrolysis of the α-acetoxystyrenes **2a–f**, as well as that for α-phenethyl acetate, are given in Table I. Since the spectrophotometric changes accompanying the hydrolysis of the latter compound are rather small, this number displays a rather large relative uncertainty; however, its accuracy is sufficient to establish the point raised in the introduction, that there is not much difference between the hydrolysis rates of **2c** and **3**. The pH–rate profiles for hydrolysis of all the esters had slopes of approximately 1.0, with only a slight deviation of questionable significance from unit slope observed for the *p*-nitro compound **2f** (slope 0.95). A plot of log k_{OH}, the specific second-order rate for hydrolysis, vs. σ gave a ρ value of 0.47 ± 0.03. The σ value of the *p*-nitro substituent used for phenyl acetates[13] and related compounds[14] has generally been taken as 1.00. However, since the substituent in the cases studied here does not interact directly with the oxygen atom of the leaving group, the conventional σ value of 0.778 is used in the correlations described in this work.

The aminolysis of **2a–f** by a variety of primary and secondary amines follow the rate law given by eq 1, where k_{Am} is

$$k_{obsd} - k_{OH} = k_{aminolysis} = k_{Am}[Am] + k_{gb}[Am]^2 + k_{ga}[Am][Am \cdot H^+] + k_{Am,OH}[Am][OH^-] \quad (1)$$

interpreted as the nucleophilic rate constant for first-order aminolysis, k_{gb} and k_{ga} are the rate constants for general base and general acid catalysis of aminolysis, respectively, $k_{Am,OH}$ is the rate constant for OH^--catalyzed aminolysis, [Am] is the concentration of free amine, and [Am·H^+] is the concentration of the conjugate acid of the free amine. No term in hydronium ion-catalyzed aminolysis was observed under the conditions of our experiments. This rate law is similar to that observed for aminolysis of other esters.[4,5b,15b] Figure 2 illustrates the expected nonlinear dependence of k_{obsd} on free amine concentration, and also shows that at moderate amine concentrations the aminolysis can compete quite favorably with hydrolysis. Table II summarizes the values of the various catalytic rate constants determined for aminolysis of **2e** with five primary and secondary amines. Table III, in the microfilm edition of this journal, is a complete tabulation of all the rate data obtained in this work.

Plots of the logarithm of k_{Am} and k_{gb} vs. the pK_a of the amine are shown in Figure 3. The slope (β_{nuc}) of this plot for k_{Am} is 1.1. Although the correlation for k_{gb} has a slope of about 1.4, the uncertainty of this number is quite high. The observation of considerable scatter is rather expected in view of the results of Bruice et al.[13] in the aminolysis of phenyl acetates.

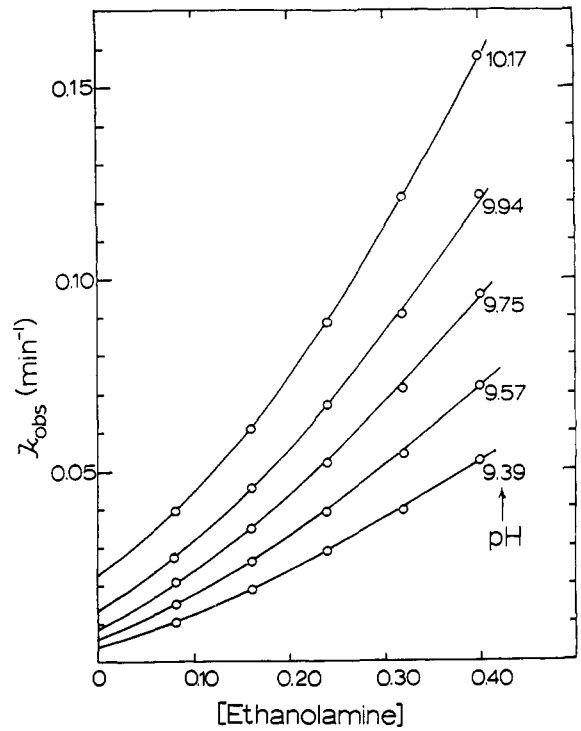


Figure 2. The observed rate constants for appearance of *m*-chloroacetophenone for *m*-chloro-α-acetoxystyrene as a substrate as a function of total ethanolamine concentration. The data were fit to a polynomial $k_{obsd} = k_0 + k_{Am,T}[amine_T] + k_{Am,^2T}[amine_T]^2$, as described in the Experimental Section, and the pH dependence of these empirical rate constants was used to extract the parameters of eq 1, also as described in the Experimental Section. The points are observed, the lines calculated.

Values of k_{Am} for all six enol esters in their reaction with *n*-butylamine are shown in Table IV. The plot of these log k_{Am} values vs. σ, also shown in Figure 1, gives a slope (ρ) of 1.03.

Compound **2c** was examined for the presence of any reaction with or catalysis by a tertiary amine. The values of k_{obsd} for the overall appearance of acetophenone vs. concentration of a 4:1 *N*-methylpyrrolidine buffer ([Am]:[Am·H^+]) at pH 11.20 up to 0.2 M in [$amine_T$] were unaffected; thus, this highly nucleophilic and relatively unhindered[44] tertiary amine does not catalyze the hydrolysis of **2c**.

Product studies on the *n*-butylaminolysis of **2c** were performed, and the results are tabulated in Table V. These studies show that the terms in amine are aminolysis rather than amine-catalyzed hydrolysis.

Discussion

Alkaline hydrolysis of esters is generally considered to proceed via a tetrahedral intermediate with rate-limiting attack of hydroxide ion.[15a,16] Hydrolysis data for various phenyl esters

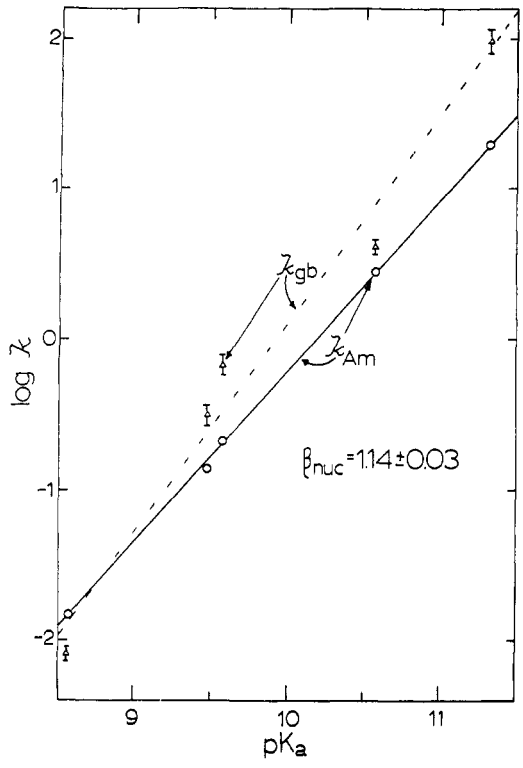


Figure 3. A nucleophilic Bronsted plot for k_{Am} and k_{gb} (eq 1) as determined for *m*-chloro-α-acetoxystyrene. The lines are the least-squares fits, and the points are observed.

Table IV. Aminolysis Rate Constants k_{Am} (Eq 1) for the Reaction of *n*-Butylamine with Esters **2a–f**

Compd	k_{Am}, M^{-1} min^{-1}
2a	0.62 ± 0.02
2b	0.68 ± 0.02
2c	0.90 ± 0.03
2d	1.77 ± 0.05
2e	2.76 ± 0.06
2f	6.52 ± 0.05
3	See footnote b, Table II

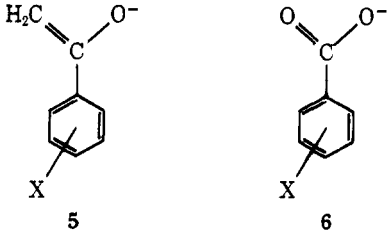
Table V. Results of a Product Study for *n*-Butylaminolysis of α-Acetoxystyrene[a]

	n-Butylacetamide/acetophenone		
[Amine], M	Aminolysis[b]	Hydrolysis[c]	Obsd
0.05	0.42	0.10	0.44 ± 0.02
0.075	0.53	0.14	0.57 ± 0.03
0.10	0.61	0.17	0.64 ± 0.02
0.12	0.65	0.19	0.67 ± 0.03
0.15	0.71	0.22	0.73 ± 0.02
0.20	0.77	0.27	0.80 ± 0.04

[a] Conditions: [*n*-$BuNH_2$]/[*n*-$BuNH_2$·HCl] = 1.0; solvent and temperature conditions identical with those in the kinetic experiments. [b] The prediction for this ratio if all terms in eq 1 in amine are aminolysis. [c] The prediction for this ratio if the k_{Am} term in eq 1 corresponds to amine-catalyzed hydrolysis.

give ρ values in the range 0.8–1.0;[15a,17,18] aminolysis of phenyl esters, on the other hand, usually give ρ values of at least 2.0.[13,15b,19] The ρ for the pK_a of phenols is 2.1–2.2,[14,20] so that the slope of a correlation of log k for aminolysis vs. pK_a of the conjugate acid of the leaving group, β_{lg}, is thus about unity. These data indicate that hydrolysis reactions of phenyl esters are much less susceptible to the nature of the leaving group (β_{lg} = 0.3–0.5) than are the aminolyses, which presumably proceed via rate-limiting loss of the leaving group.[10a] Direct plots of log k_{OH} vs. the pK_a of the leaving group for both alkyl and phenyl esters give β values of about 0.3–0.4, indicating only modest sensitivity to the pK_a of the leaving group.[21,22] Phenylbenzoates also show no ^{18}O exchange upon alkaline hydrolysis.[23]

Presumably, then, the ρ of 0.47 for the hydrolysis of α-acetoxystyrenes, if this hydrolysis corresponds to the cases cited above, should represent rate-determining attack of the nucleophile. An estimate of the ρ value for the pK_a numbers of acetophenone enols would then be in order as a partial test of this hypothesis. Although no such pK_a data exist, we can compare the deprotonation of acetophenone enols with a well-known isoelectronic reaction. Structures **5** and **6** show the



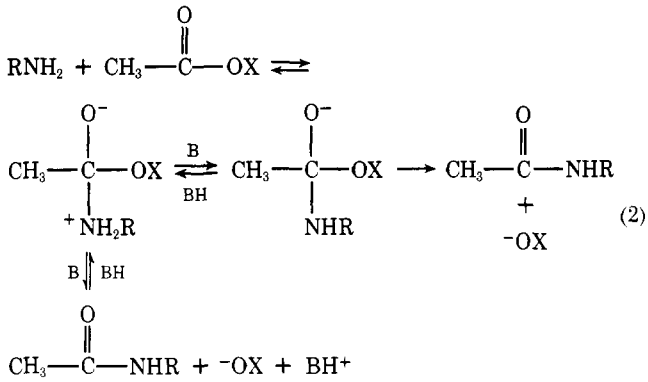
5 **6**

obvious similarity between the acetophenone enolates and the carboxylates of substituted benzoic acids. The analogy is, of course, not complete since symmetry prevents any direct through-bond interaction between the nonbonding orbital of the carboxylate moiety and the π system of the benzene ring, while no such restriction holds in the enolates. However, the contribution from the central carbon to the highest occupied orbital of the enolate is certainly expected to be quite small.[24] Substituent effects in the corresponding unionized species would similarly be expected not to be greatly different. The ρ for pK_a of benzoic acids is, of course, unity;[20] that for the deprotonation of acetophenone enols should be close to unity as well. The value of 0.47 can therefore be taken, as a first approximation, as β_{lg} for the hydrolysis of **2a–f.** In view of the uncertainties just expressed concerning the ρ for acetophenone enol pK_a values, this value is in excellent agreement with the β_{lg} for hydrolysis of phenyl acetates and indicates that bond breaking in the leaving group is far from complete in the rate-limiting attack of hydroxide to form a tetrahedral intermediate which rapidly breaks down to product.

Recent observations of high $k_{OH}/k_{exchange}$ ratios for ^{18}O carbonyl-labeled alkyl benzoates and formates with leaving groups whose pK_a values are comparable with that of hydroxide suggest the possibility that there is no tetrahedral intermediate, at least in the hydrolysis of alkyl esters.[5c,25] Since the leaving groups of our esters may have pK_a values (see below) intermediate between the leaving groups of phenyl and alkyl esters, there is then some question whether a tetrahedral intermediate exists in the hydrolysis of these compounds. Exchange of ^{18}O into unreacted esters may shed some light on this matter. Our ρ value is, therefore, not necessarily indicative of rate-determining attack *to form a tetrahedral intermediate,* but it is indicative of some form of rate-limiting nucleophilic attack. More important, the value is very similar to that observed for alkaline hydrolysis of a wide variety of other esters.

There is some direct kinetic evidence that a variety of aminolysis reactions proceed through tetrahedral intermediates.[5b,10a,26] The reactions of phenyl acetates with amines go via uncatalyzed, general base, general acid, and OH^--catalyzed pathways[5a,16] as do the aminolysis reactions for the α-acetoxystyrenes discussed herein. The ρ values for the uncatalyzed reaction (k_{Am}) are generally quite large for the aryl

acetates, usually about as large as the ρ for the pK_a of the phenols corresponding to the respective leaving groups in these reactions. In addition β_{nuc} and β_{lg} values for phenyl acetate aminolysis are close to unity[10a,13,16,27] (the latter is, more to the point of the present investigation, about double the value of β_{lg} observed in the corresponding hydrolysis reactions). These observations have led to the interpretation that the uncatalyzed aminolysis term for phenyl acetates represents rate-determining breakdown of the zwitterionic tetrahedral intermediate formed by attack of the amine on the ester.[10a] Similar structure-reactivity observations lead to the conclusion that the general base catalyzed pathway proceeds via either a rate-limiting proton transfer or breakdown of the anionic tetrahedral intermediate, as shown in:



(2)

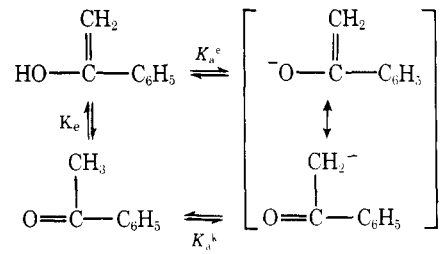
The value of ρ for the uncatalyzed reaction of α-acetoxystyrenes with *n*-butylamine is 1.03, approximately what we might expect for the ρ value of the pK_a's of the corresponding acetophone enols (see above). This value indicates a β_{lg} close to unity or a large sensitivity to the nature of the leaving group. The large β_{nuc} observed for the uncatalyzed aminolysis of **2e** is indicative of rather complete bond formation between the amine nitrogen and the acyl carbon in the transition state of the rate-determining step. A mechanism such as that depicted in structure **4,** involving some form of internal catalysis, would result in lower β_{nuc} values, since both the acidity of the protonated amine and the basicity of the amine itself are kinetically important, and lower ρ values (or β_{lg} values), since the anionic form of the leaving group is never fully developed. Similar observations have been made for one case of internal catalysis in the aminolysis of phenyl quinoline-8-carboxylates.[15]

Our data show such a blatant similarity to the pattern established for the aminolysis of aryl acetates that we must conclude that there exists no reason for postulation of a "special" mechanism, such as that shown in structure **4.** Not only are the structure-reactivity parameters for aminolysis of α-acetoxystyrenes very similar to those for the aryl acetates, but also even the rate constants themselves are quite similar.[5a,13,16] A direct comparison between the aminolysis rates of **2e** and phenyl acetate, shown in Table VI, is indicative of this correspondence. Although tertiary amines catalyze phenyl acetate hydrolysis, these amines are considerably weaker catalysts than primary amines of identical pK_a.[10] If this result is due to steric effects, the failure of *N*-methylpyrrolidine to catalyze the hydrolysis of **2c,** in which the leaving group is considerably more bulky than a phenol, is understandable.

Recently, an attempt has been made to correlate the reactions of nucleophiles with phenyl esters[28] using the unifying N_+ parameter that has been useful with other nucleophilic reactions. It was found that the nucleophilic reactions of amines with phenyl esters could be nicely correlated by

$$\log k_{obsd} = \log k_0 + N_+ - \log[1 + k_{-x}/k_{-y}] \qquad (3)$$

in which k_{obsd} is the observed rate constant, k_0 is dependent solely on the ester identity, and the k_{-x} and k_{-y} parameters reflect the relative leaving ability of the "nucleophile" and "leaving group", respectively, from the tetrahedral intermediate. These last two parameters were developed under the assumption that they are mutually independent. Using hydrolysis data for *m*-chloro-α-acetoxystyrene and aminolysis data for 2-methoxyethylamine and morpholine with the same ester, we were able to calculate a log k_{-y} value for this ester of approximately −0.5; phenyl acetate is defined to have on this scale log k_{-y} = 0.0, and *p*-nitrophenyl acetate and 2,4-dinitrophenyl acetate have log k_{-y} values of 2.0 and 3.8, respectively.[28] Since the k_{-y} values were found to correlate with the pK_a of the leaving groups for a variety of oxygen leaving groups, we thus have a basis for using this correlation in reverse for calculation of the pK_a for acetophenone enols. As additional support for the number obtained we can also compare the actual k_{Am} values with those for phenyl acetates, since the sensitivity to leaving group and the mechanism in each case appear to be about the same. These comparisons yield a pK_a for *m*-chloroacetophenone enol of 11.5. From known data[9] for the pK_a of acetophenone in nonaqueous solvents (based, however, on lengthy extrapolations to the dilute aqueous reference state), we can estimate the carbon pK_a of *m*-chloroacetophenone at approximately 18.5–19.0. The thermodynamic cycle relating enols, ketones, and enolates is given by eq 4, in which K_e =



(4a)

$$pK_a^k = pK_a^e - \log K_e \qquad (4b)$$

[enol]/[ketone]. Titration for the enol content of acetophenone in methanol solution has led[9] to a value of $K_e = 3.5 \times 10^{-4}$. Using a value of log K_e of 4.0, we would expect a pK_1 for *m*-chloroacetophenone enol of 14.5 to 15.0, a number in serious disagreement with the estimate of 11.5 from our kinetic data. This result would suggest that the pK_a values determined for ketones in nonaqueous solvents are not necessarily applicable in aqueous solution. Working in reverse from our data, our results suggest a carbon pK_a for *m*-chloroacetophenone in water of about 15.5, a value considerably lower than that expected from the conventional data cited in nonaqueous solution.

We emphasize that previous estimates of ketone pK_a values are undoubtedly correct within the confines of the assumptions and lengthy extrapolations used in their determination. It is the tacit assumption that these pK_a values are valid in aqueous solution which requires revision; this assumption has been made by nearly everyone who needs these pK_a values at one

Table VI. Comparison of the k_{Am} Terms (Eq 1) for Aminolysis of *m*-Chloro-α-acetoxystyrene (k_{Am}, *m*-ClAS) and Phenyl Acetate (k_{Am}, PA)

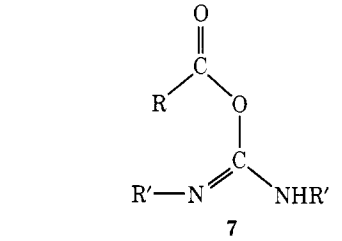
Amine	k_{Am}, *m*-ClAS, M^{-1} min^{-1}	k_{Am}, PA,[a] M^{-1} min^{-1}
n-Butylamine	2.76	4.5
2-Methoxyethylamine	0.138	0.25
Morpholine	0.0149	0.0315

[a] From ref 4a; conditions: water, ionic strength = 1.0 M (KCl), 25 °C.

time or another and naturally found aqueous solution data unavailable. The aqueous solution pK_a of cyclohexanone or cyclopentanone cited by Bell[29] of 16.7 is probably more typical of many ketones, and to place acetophenone in the pK_a range of 15–16 would appear to be quite reasonable.

Our data show that the high reactivity of activated esters derived from enols is due to the surprisingly low pK_a of enols, as well as to the term in [amine]^2 characteristic of many aminolysis reactions. However, the mechanisms of aminolysis seem to be quite the same as the mechanisms invoked for phenyl acetate aminolysis, and our work further supports the generality of these mechanisms.

Although to state that the aminolysis mechanisms of all activated esters fall into the now-emerging pattern[10] of which this work forms a small part would be a statement lacking thorough experimental support at present; nevertheless, the consequences of such a generalization are interesting. The active esters **7** derived from carbodiimide activation of a carboxylic acid are an interesting case. The oxygen pK_a of the leaving group (pK_{OH}) should be about 9, based on the N–H pK_a of the corresponding ureas[30,31] (ca. 14) and estimates of the fraction (ca. 10^{-5}) pseudoacid form present in the ureas from bond-energy considerations. According to this work, as well as the studies referenced herein on aminolysis of phenyl esters, an ester with a leaving group whose conjugate acid has a pK_a of 9 will be no more reactive than a mildly activated aryl ester, and this statement is not in accord with experience with *O*-acylisoureas. Hegarty and Bruice[4] have resolved this problem by finding experimentally with a cyclic analogue of **7** that the active form of the ester is undoubtedly the N-protonated form, which was shown to aminolyze with a basic amine about 10^5 faster tha the unprotonated form. Protonated *O*-methylisourea (pK_a 9.8) and the effect of O-acylation[4] lead one to an estimate of pK_{NH^+} for **8a** of about 5.5 to 6. Since most aqueous carbodiimide reactions are carried out in the vicinity of pH 4, the ester as it is generally used is in its protonated form. The α-acetoxystyrenes studied here could, in principle, also be activated by protonation; the rates of this type of reaction are known, however,[11] and in the region of pH in which a reasonable amount of amine is available in its unprotonated form, the protonation rates are impossibly slow. An interesting intermediate case in this regard would be the "active esters" derived from alkoxyacetylenes (**9**); it might not be surprising to find rates of proton transfer (path b, eq 6) which compete with rates of direct aminolysis (path a, eq 6).



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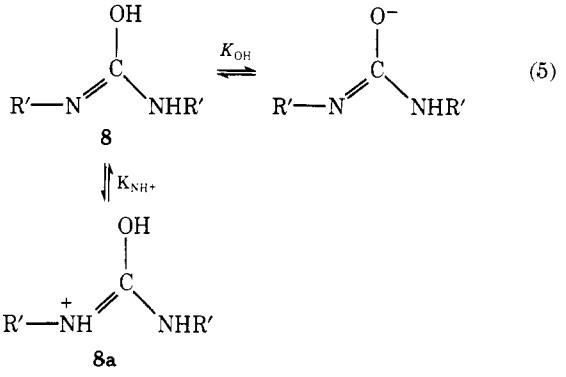

(5)

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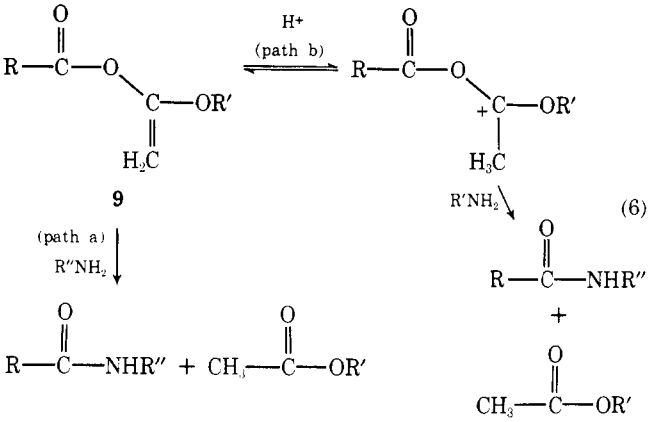
8a

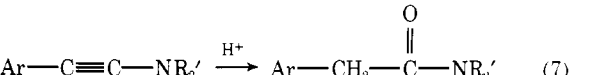


(6)

9

Another feature of highly active esters is their lack of selectivity, particularly with respect to various basic amine nucleophiles. This can now be clearly understood from the observations of Jencks,[16] Bruice,[4] and their colleagues. Downwardly curved nucleophilic Bronsted plots are observed at high values of the pK_a of the conjugate acid of the nucleophile; such plots have been observed, for example, in the aminolysis of 2,4-dinitrophenyl acetate, esters of 4-methoxypyridine *N*-oxide, as well as for aminolysis of the protonated (but not the unprotonated) form of the cyclic *O*-acylisoureas studied by Bruice and Hegarty.[4] The enol esters studied in this work show no tendency of this type, an observation consistent with their "phenyl acetate-like" behavior. Oxygen nucleophiles also show, in many cases, such reactivity–selectivity patterns.[32] Such a lack of selectivity would lead to the expectation that aminolysis might be accompanied by competing reactions of other nucleophiles, such as carboxylates (anhydride formation), intramolecular amide groups (azlactone formation and racemization), etc. Such side reactions have not been studied systematically in water (where, in any case, water present in high concentration is an effective competing nucleophile), but are well known in nonaqueous or partially aqueous solvents. Thus, the use of less activated but nevertheless reasonably electrophilic esters (e.g., *p*-nitrophenyl esters) has found favor because of the virtual absence of these side reactions.[34-35]

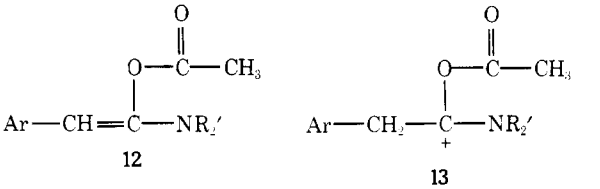
An interesting case in the literature which is pertinent to this study[36] is the mechanism of the acid-catalyzed hydrolysis of ynamines (**10**). The authors of this study found that conversion



(7)

10 **11**

of the ynamines to give amides **11** was accompanied by interesting effects of added acetic acid. Increasing low concentrations of acetic acid precipitously retarded the rate of appearance of amide; higher concentrations of acetic acid, however, accelerated amide appearance linearly and less dramatically. To account for this behavior, the authors proposed a "complex" of the ynamine and acetic acid which hydrolyzed more slowly than the ynamine itself; this complex was observable spectrophotometrically. An active ester **12** would be consistent with



12 **13**

the observations and would be expected to hydrolyze more slowly than the ynamine. The conjugate acid of the ynamine–acetic acid "complex" was observed to have an apparent pK_a

of 4.3, a number not unreasonable for the pK_a of **13.** Breakdown of this species required a term in acetic acid and a proton, which would be consistent with anhydride formation from **13;** the authors, however, invoked the intermediacy of a vinyl cation. In the formation of **12,** the pK_a of **13,** and the formation of anhydrides even in aqueous solution, ynamines are thus quite similar to carbodiimides, and it is interesting that these species have been found to be of some utility as coupling agents for peptide synthesis as well.[2,37]

There thus appears to be a spectrum of active esters available which so far falls within one mechanistic class. At the least reactive end of the spectrum are alkyl esters (which were used in the early days of peptide synthesis[38]); phenyl esters and enol esters of the type studied herein represent a viable compromise of reactivity and selectivity. Somewhat more reactive, and still reasonably selective, would appear to fall *N*-hydroxysuccinimide, 1-hydroxybenzotriazole, and related esters, which may in addition be formed in situ because of the excellent nucleophilic properties of these groups. Finally, at the most reactive end of the spectrum are the protonated *O*-acylisoureas and 1-acyloxyvinylamines which, although very reactive, also may be rather unselective in many cases.

Although it is undoubtedly a large jump between the idealized studies of active esters in aqueous solution and the use of these versatile intermediates in peptide synthesis and related operations, nevertheless conclusions and observations in one situation appear to hold at least qualitatively for the other. The increasing use of water-soluble activating agents for peptide degradation,[39] protein modification,[40] linkage of ligands to insoluble supports for affinity chromatography and related operations,[42] and analytical procedures[3a,41] suggest that studies of this type will assume even more practical utility in the near future.

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Supplementary Material Available. A listing of all primary rate data for the aminolysis and hydrolysis of esters **2a–f** (Table III) (13 pp). Ordering information is given on any current masthead page.

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