Formation, Rearrangement, and Hydrolysis of Enol Esters derived from Isoxazolium Salts

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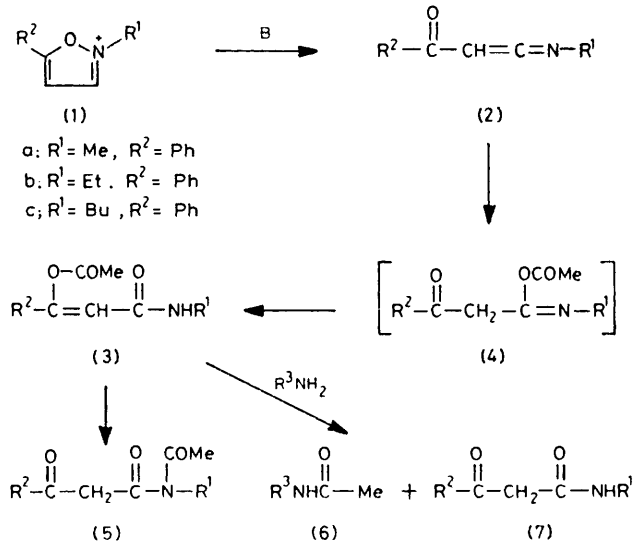
The hydration of *N*-t-butylbenzoylketenimine (2c) in water at 25° [to give the amide (7c)] occurs *via* acid-catalysed (at pH < 6) and pH-independent pathways. General acid catalysis by acetic acid is noted leading to the enol esters (3). The further reactions of these esters (3; R = Me, Et, or Bu^t) in aqueous solution have been investigated. In basic solution (pH > 6) (3; R^1 = Me or Et) undergo HO$^-$-catalysed neighbouring amide group participation to give the *N*-acyl analogues (5), which are subsequently hydrolysed to the amides (7) at a rate which can be slower or faster than the rate of formation of (5). However when R = Bu^t, no O ⟶ N acyl group rearrangement is observed [*i.e.* (3c) ⟶ (5c)]; instead slow base-catalysed hydrolysis of the enol ester occurs to give (7c) directly. In acid solution all the enol esters studied are hydrolysed at much the same rate. The implications of these results for the use of the isoxazolium salts (1) [the precursors of the enol esters (3)] as reagents for peptide synthesis are commented on.

SINCE its introduction in 1961, the amide bond-forming reagent (1; R^1 = Et, R^2 = *m*-sulphonatophenyl) (Woodward's Reagent K) [1] has been widely used in peptide synthesis.[2-8] It activates a carboxylic acid by converting it to an enol ester (3), which then reacts with an amine to form the amide (6).

The advantages and disadvantages of Reagent K in amide bond formation have been discussed by Olofson and Marino.[9] In addition to very high product yields, even in difficult systems, the by-products are all water soluble and therefore easily removed from the product peptide. The coupling also takes place with little or no racemization under optimum conditions. A major disadvantage (apart from the high cost of the reagent) is a side rearrangement and the formation of some racemic peptide in certain useful reaction environments.



The α-oxoketenimine (2) has been identified as an intermediate [formed by base-catalysed ring opening of the isoxazolium salt (1)]; [10,11] addition of the carboxylic acid presumably gives the *O*-acylisoamide (4) as an intermediate (although this has never been detected) followed by O ⟶ O′ acyl-group migration to give the 'active reagent' (3). The competing reaction is an O ⟶ N acyl-group migration which leads to the formation of the *N*-acetyl derivative (5), which does not readily transfer the acyl group intermolecularly.

In this study we present a detailed account of the factors which affect (a) the formation of the enol ester (3), (b) the intramolecular O ⟶ N acyl-group migration (3) ⟶ (5), and (c) the intermolecular acyl transfer from the enol ester (3) to water, in order to define the structural features which would constitute the optimum reagent.

RESULTS AND DISCUSSION

Hydrolysis and Addition of Acetic Acid to the Oxoketenimine.—*N*-t-Butylbenzoylketenimine (2c) was chosen for study since it is relatively stable towards polymerisation; it was prepared by treating the isoxazolium salt (1c) with triethylamine.[12] Repetitive scans of the u.v. spectrum of (2c) at pH 3—8 in aqueous buffers show rapid conversion into the corresponding amide (7c) on reaction with water. The hydrolysis of (2c) showed marked buffer catalysis (see below) and the rate of reaction with water was obtained by extrapolating the observed rate constants (k_{obs}) at a given pH to zero buffer concentration. The results are summarised in Figure 1 which shows a plot of log k_0 against pH. Clearly two competing mechanisms of hydration are operating, one which is dependent on the acidity of medium and the other which is pH-independent. The solid line in Figure 1 has been drawn using equation (1) with $k_{H_3O^+}$ 1.0 × 10^2 and k_{H_2O} 7.0 × 10^{-6} l mol^{-1} s^{-1}.

$$k_0 = k_{H_3O^+}[H_3O^+] + k_{H_2O}[H_2O] \quad (1)$$

For related simple ketenimines [such as (8)], it has recently been shown that hydration occurs *via* rate-determining proton transfer from H_3O^+ or H_2O to the ketenimine at all pH values (up to 13). It is likely that the same mechanism also operates in the present instance since the rate of acid-catalysed hydration of (2a) is reduced 22-fold [relative to (8; Ar = Ph)]. This rate reduction is entirely explicable in terms of the presence of the electron-withdrawing benzoyl group in (2c), since

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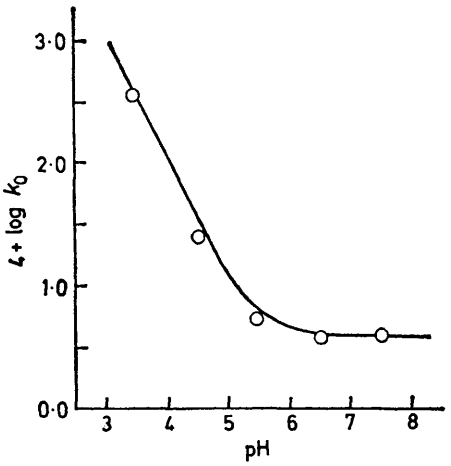


FIGURE 1 Plot of log k_0 for the reaction of *N*-t-butylbenzoylketenimine (2c) in water at 25° against pH (μ 1.0, KCl). The rate constants k_0 were obtained by extrapolation at zero buffer concentration. The line is theoretical from equation (1) with $k_{H_3O^+}$ 100 and k_{H_2O} 7 × 10^{-6} l mol^{-1} s^{-1}

it has been shown that substituents in Ar in (8) show a Hammett ρ value of −0.8 for this reaction [13] (the rate of hydronium-catalysed hydration of ketenimines shows little sensitivity to the nature of the *N*-alkyl substituent).[14]

General acid catalysis was also noted in the addition of acetic acid to (2c) in aqueous buffer solutions. Catalysis was noted over the pH range 3.5—6.5, the slopes of the second-order plots being proportional to the fraction of undissociated HOAc present. The behaviour of (2c)

$$ArCH{=}C{=}N{-}Pr^i$$

(8)

therefore parallels that of (8) and again rate-determining proton transfer from the undissociated acid to the oxoketenimine is the most likely reaction pathway; the *O*-acylisoamide (4c) is then formed by attack of AcO^- on the nitrilium ion in a subsequent step.

At pH values above the pK_a of acetic acid a simple dependence of k_{obs} on [HOAc] over a wide buffer range was no longer observed; a typical result is shown in Figure 2. Such behaviour, although not investigated in detail, is typical of a change in mechanism with changing buffer concentration (resulting possibly from competing direct attack of AcO^- in this case). In any event, even at the highest [HOAc] and [AcO^-] used, there was no evidence for the intermediacy of the *O*-acylisoamide (4c) as a long-lived intermediate; repetitive scans of the u.v.

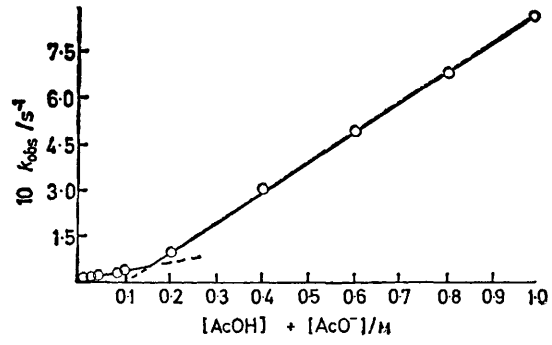


FIGURE 2 Plot of log k_{obs} against the concentration of an acetate buffer at pH 5.5 for the reaction of *N*-t-butylbenzoylketenimine (2c) in water at 25°

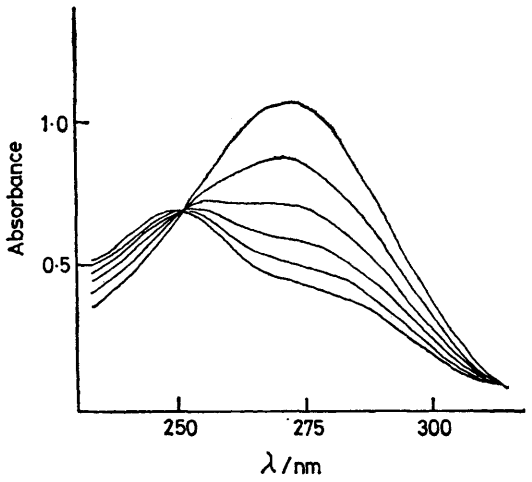


FIGURE 3 Repetitive scans in the u.v. region of the reaction of β-acetoxy-*N*-ethylcinnamamide (3b) at 25° in water (pH 6.35); the time intervals between scans is 30 min

spectrum showed tight isosbestic points at all pH values. The only product detected was the enol ester (3c) together with the amide (7c) (at low [HOAc]); under these conditions (see below) the enol ester is stable toward further reaction in aqueous solution.

Reaction of Enol Acetates (3) *in Aqueous Solution.* *β-Acetoxy-N-ethylcinnamamide* (3b). Repetitive scans in the u.v. region for (3b) in aqueous solution shows complex behaviour. At pH 6.35 (Figure 3) there is no evidence for the presence of relatively long-lived intermediates as shown by the tight isosbestic point at 252 nm. However at pH 8.8, this isosbestic point is

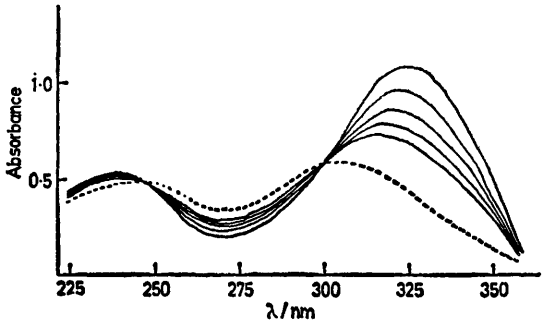


FIGURE 4 Repetitive scans in the u.v. region for the reaction of (3b) at 25° in water (pH 10.05); the time interval between scans is 4 min; the broken line is the spectrum obtained at infinite time

absent and typical A ⟶ B ⟶ C type behaviour is shown; for example at 325 nm the absorbance shows an initial increase followed by a slower subsequent decrease. At higher pH (>10) the B ⟶ C portion of the reaction can be followed as the reaction again shows tight isosbestic points, (Figure 4). In acid solution the enol ester

shows acid-catalysed hydrolysis, but the product is different from that initially formed in base. The observed kinetic results are summarised in Figure 5; it was not possible to obtain data for the B ⟶ C reaction at pH <10 since the A ⟶ B reaction occurs more slowly (or at a comparable rate) in this region.

The results can be analysed in terms of the empirical Scheme 1 from which the observed rate of disappearance

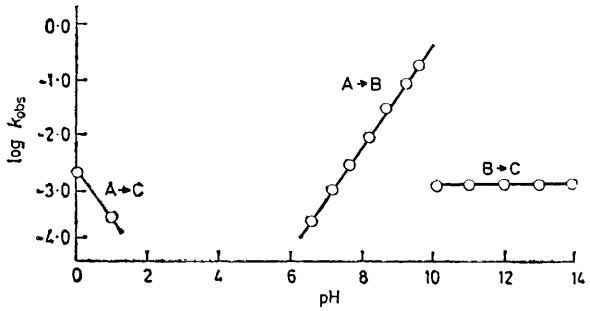
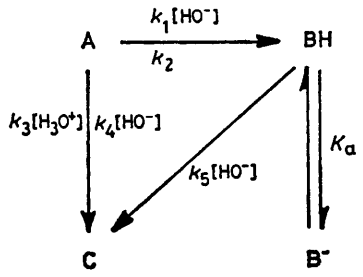


FIGURE 5 A plot of $\log(k_{obs}/s^{-1})$ *versus* pH for the reaction of (3b) in water at 25° (μ 1.0, KCl); the points are experimental and the lines theoretical using equation (2) with the values for the constants given in Table 1. The line for the B ⟶ C portion of the reaction has been drawn with k_{obs} 9.31 × 10^{-4} s^{-1}

of A can be expressed as equation (2) with k_1 5.0 × 10^3 and k_3 2.0 × 10^{-3} l mol^{-1} s^{-1} and $k_4 = k_2 = 0$. The

$$k_{obs.} = k_1[HO^-] + k_2 + k_3[H_3O^+] + k_4[HO^-] \quad (2)$$

B ⟶ C process is apparently pH-independent (see Figure 5); such a pH-independent reaction can arise in several ways, *e.g.* spontaneous reaction of BH (if the pK_a of BH is >14) or reaction of the conjugate base B^- with



SCHEME 1

H_2O or of BH with HO^- (if the pK_a of B is ≤10). We favour (see below) the latter mechanism.

β-*Acetoxy*-N-*methylcinnamamide* (3a). The observed rate constants for the reaction of (3a) are summarised in Figure 6. The reactivity is broadly similar to that of (3b) except that (a) the A ⟶ B process now clearly also shows a pH-independent rate (pH 2—5) and (b) the B ⟶ C process is shown not only to be pH-independent above pH 10 but to be proportional to $[HO^-]$ at pH <9. The disappearance of the enol ester follows equation (2) (solid line in Figure 6) with the values given for the constants in Table 1.

The intermediate BH was clearly identified as the *N*-acylamide (5a) in this case. The observed pH-dependence of the B ⟶ C process (Figure 6) implies that BH has a pK_a of 9.23 [the observed rate constants fit equation (3) with k_5 38 l mol^{-1} s^{-1} and K_a 5.92 × 10^{-10}]. This is reasonable when compared with the pK_a values of

$$k_{obs} = k_5K_w/(K_a + a_H) \quad (3)$$

related materials: acetylacetone (pK_a 9.0) and methyl acetoacetate (pK_a 10.0). Finally *N*-acetyl-*N*-methylbenzoylacetamide (5a) was synthesised and its hydrolysis investigated over the pH region 8—14; it showed similar behaviour to that observed for the intermediate B derived from the enol ester (3a), the observed rate constants fitting equation (3) with the same values of k_5 and K_a.

β-*Acetoxy*-N-*t-butylcinnamamide* (3c). This enol ester

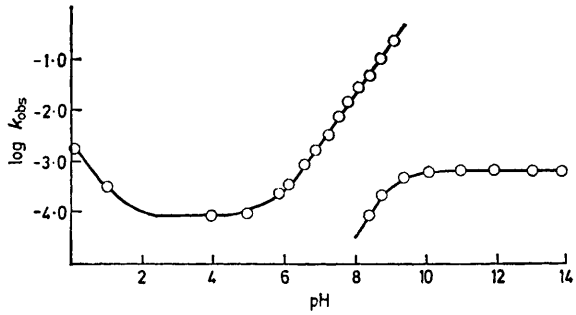


FIGURE 6 A plot of $\log(k_{obs}/s^{-1})$ for the reaction of β-acetoxy-*N*-methylcinnamamide (3a) in water at 25°. The lines are theoretical using equation (2) and the Table for A ⟶ B reaction and equation (3) (with k_5 38 l mol^{-1} s^{-1} and K_a 5.92 × 10^{-10}) for the B ⟶ C reaction

was found to react only at high and low pH (see Figure 7); it was unreactive in the region pH 2—7. Moreover repetitive scans of the u.v. at the pH where reaction occurred showed that no relatively long-lived intermediates were present. Thus direct hydrolysis of the enol ester (3c) to the amide (7c) occurred at all pH, without the intermediacy of (5a). Moreover base-catalysed

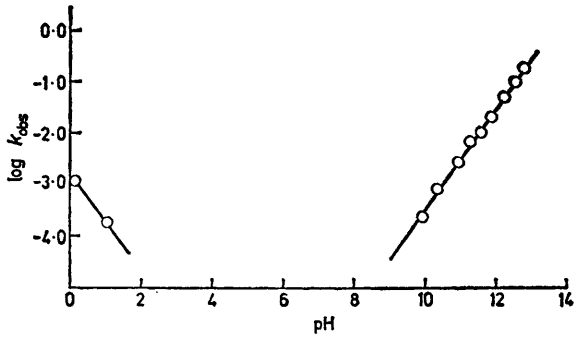


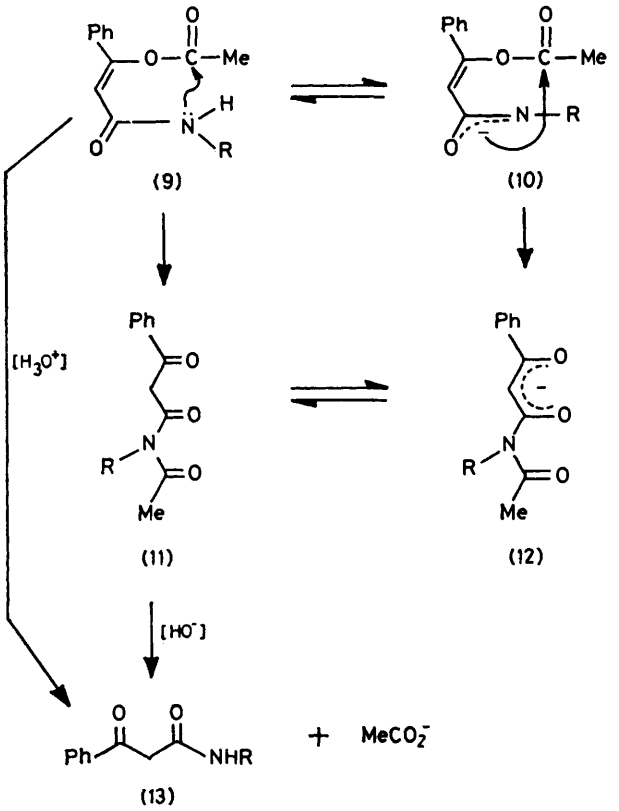
FIGURE 7 Plot of $\log(k_{obs}/s^{-1})$ against pH for the hydrolysis of β-acetoxy-*N*-t-butylcinnamamide (3c) in water at 25°. The theoretical line [equation (2)] is followed with the values for the constants given in the Table

hydrolysis of the enol ester (3c) occurs at a markedly slower rate (see Table 1) than the base catalysed O ⟶ N acyl-group rearrangement for (3a or b).

These results can be summarised in terms of Scheme 2. In acid solution all the enol esters (9) undergo acid-catalysed reaction at much the same rate (see Table);

the product formed is the oxoamide (13) (product C). The observed rates of hydrolysis are similar to those observed by Noyce and Pollack [15] for α-acetoxystyrenes which are known to hydrolyse by an $A_{AC}2$ mechanism in dilute acid.

The rearrangement of the enol ester (9) to the imide (11) shows a high sensitivity to the group R attached to



SCHEME 2

nitrogen. When R = Me (the smallest group studied) both neutral (9) and the conjugate base (10) undergo this rearrangement. With R = Et only reaction *via* the anion (10) is observed but at a reduced (four-fold relative to R = Me) rate. Finally when R = Bu^t no rearrangement of (9) or its conjugate base (10) is observed; in default, direct hydroxide ion-catalysed hydrolysis of the enol ester (9; R = Bu^t) occurs giving (13), directly. The decrease in the rate of rearrangement of (10) to (12) on replacing R = Me by R = Bu^t is therefore 5 600-fold at a minimum. Such a large effect is clearly steric in origin, either by restricting the transition state for the formation of (12) or holding the anion (10) in a configuration unsuitable for intramolecular nucleophilic attack.

TABLE

Summary of rate constants for hydrolysis of enol esters (3) [a]

Compound	k_1/ l mol^{-1} s^{-1}	k_2/s^{-1}	10^3k_3/ l mol^{-1} s^{-1}	k_4/ l mol^{-1} s^{-1}
(3a)	2.11×10^4	8.97×10^{-5}	2.07	
(3b)	5.01×10^3		1.98	
(3c)			1.53	3.55

[a] In water at 25° (μ 1.0, NaCl).

In Scheme 2 we have depicted nucleophilic attack *via* the neutral amide and the conjugate base as occurring *via* N-attack alone (in spite of the well recognised ambident character of this group).[16] However, oxygen attack may very well be competing but since this would give the *O*-acylisoamide (4) which spontaneously regenerates (9), this reaction pathway is not observed.

The hydrolysis of the imide (11) (which corresponds to BH in Scheme 1) most likely occurs *via* HO^- attack on the neutral material (11) rather than *via* the kinetically indistinguishable spontaneous reaction of the conjugate base (12). Since (11) was observed only when R = Me or Et and the hydrolysis of (11) was faster than its formation at pH <10 when R = Et, it is not possible to comment in detail on this step. However the pH-independent 'plateau' rate observed at high pH is almost the same in both cases [9.21×10^{-4} for (11; R = Me) and 9.31×10^{-4} s^{-1} for (11; R = Et), see Figures 5 and 6]; since this represents k_5K_w/K_a [from equation (3) with $K_a \gg a_H$], both k_5 and K_a respond in the same way to the substituent change (or most likely show no great variation).

The imides (11) have two positions for possible cleavage yielding (13) and acetic acid or benzoylacetic acid and an *N*-alkylacetamide. We have confirmed that attack occurs at the acetylcarbonyl under our conditions in base, both by actual isolation of (13) and by spectral comparison at various pH values with an authentic sample of (13; R = Et). Competing attack at both sites may occur under different conditions (*e.g.* in ethanol) [10,11] and four products have been isolated in varying amounts for the reaction of (13; R = Ph) with benzylamine.[17]

The key finding is therefore the progressive elimination of the competing intramolecular rearrangement of (10) to (12) when the bulk of R is increased. This is an obvious advantage in peptide synthesis where the desired reaction involves acyl transfer from (9) to an external nucleophile. For intermolecular reaction to compete with base-catalysed rearrangement of (9; R = Me or Et), the pH of the solution would effectively have to be maintained <8 unless high concentrations of the nucleophile can be used. At these pH common amine nucleophiles are present as their conjugate acids, so that no further gain is observed by using solutions of greater acidity.

Difficulty has previously been encountered in attempts to actually isolate enol esters such as (8; R = Bu^t); [11] however we have found that the ester is readily formed on reaction of the oxoketenimine (2c) with acetic acid and triethylamine in acetonitrile solution, so that the use of the reagent with NBu^t has obvious advantages. The observed kinetic results also show that rearrangement to (10) occurs largely through the conjugate base (9) when R = Me or Et. An obvious way to block this pathway would be to ensure that the nitrogen of the enol ester (8) was disubstituted. This has in fact been

recently done by Gais and Lied [18] using 4-dimethylaminobut-3-yn-2-one (an ynamine); this forms an adduct with carboxylic acids (at low temperature) which is structurally analogous to (9). However the presence of the NMe_2 group ensures that the enol ester only undergoes *inter*molecular transfer of the acyl group.

EXPERIMENTAL

Substrates.—The following materials were prepared by methods described in the literature: *N*-methyl-5-phenylisoxazolium hydrogensulphate, m.p. 133—133.5° (lit.,[11,19] 135, 133—134.5°); *N*-t-butylbenzoylketenimine (from *N*-t-butyl-5-phenylisoxazolium perchlorate [20]) as a yellow oil, $\lambda_{max.}$ (dioxan) 247 and 290 nm [lit.,[12] 242 and 288 nm (cyclohexane)]. β-Acetoxy-*N*-methyl- and -*N*-ethyl-cinnamide, and *N*-acetyl-*N*-methylbenzoylacetamide were prepared by the method of Woodward and Olofson.[11]

β-*Acetoxy*-N-*t-butylcinnamamide* was prepared from *N*-t-butyl-5-phenylisoxazolium perchlorate [20] using the method described for the preparation of 2-acetoxy-3,4,5,6-tetrahydro-*N*-methylbenzamide; [9] the *enol ester* was obtained as a light yellow oil (Found: C, 68.8; H, 7.4; N, 5.4. $C_{15}H_{19}NO_3$ requires C, 69.0; H, 7.3; N, 5.4%); $\bar{\nu}_{max.}$ (KBr disc) 3 290 (NH) and 1 768 cm^{-1} (C=O); $\lambda_{max.}$ 268 nm (dioxan); δ ($CDCl_3$) 1.39 (9 H, s), 2.38 (3 H, s), 6.12 (1 H, s), and 7.45 (5 H, s).

N-*Ethylbenzoylacetamide*. *N*-Ethyl-5-phenylisoxazolium fluoroborate (0.68 g) was dissolved in water (10 ml). Barium carbonate (0.59 g) was added and the mixture stirred at room temperature for 24 h. The solution was then heated to 100°, filtered and the *amide* precipitated on cooling, m.p. 89.5—91° (Found: C, 68.3; H, 6.9; N, 7.2. $C_{11}H_{13}NO_2$ requires C, 69.1; H, 6.8; N, 7.3%). $\bar{\nu}_{max.}$ (KBr) 3 270 (NH), 1 685 (ketone C=O), and 1 645 (amide C=O) cm^{-1}; δ ($CDCl_3$) 1.14 (3 H, t), 3.35 (2 H, q), 3.94 (2 H, s), and 7.5—8.2 (5 H, m).

Kinetic Methods.—The reactions were followed spectrophotometrically by following changes in absorbance at appropriate wavelengths (established by initial repetitive scans) using methods and apparatus which have previously been described.[21] The solvent used throughout for kinetic experiments was doubly distilled water and ionic strength was maintained constant by the addition (where necessary) of up to 1M-KCl. Low concentrations (5×10^{-3}M) of appropriate buffers (carbonate, borate) were used for experiments outside the buffering range of hydroxide ion for the reactions of the enol esters. Preliminary experiments established that no appreciable buffer catalysis occurred under these conditions and that pH drift was minimal (>0.05 pH unit).

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