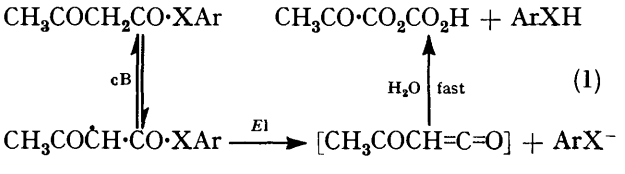
Leaving Group Effects in Thiolester Alkaline Hydrolysis. Part 1. A Keten-mediated (*E*1cB) Pathway for Basic Hydrolysis of *S*-Acetoacetylcoenzyme A and Analogues

By **Kenneth T. Douglas** * and **Norbert F. Yaggi**, Department of Chemistry, University of Essex, Colchester, and Department of Chemistry, Duquesne University, Pittsburgh, Pennsylvania 15219, U.S.A.

The basic hydrolysis of a series of leaving-group substituted acetothiolacetates (CH_3COCH_2COSR) has been studied in aqueous media. Hydrolysis of *N*-acetyl-*S*-acetoacetylcysteamine follows a kinetic ionisation curve with an inflexion corresponding to the p*K* of this ester as determined by spectrophotometric and electrometric titrations. The rate constant at high pH was shown to follow a Brønsted relationship with $\beta_{L.G.}$ −1.13, where $\beta_{L.G.}$ is the slope of a plot of the logarithm of the rate constant *versus* the pK_a of the conjugate acid of the leaving group. This, and other evidence from rate comparisons, activation parameters, and kinetic solvent isotope effects, indicated an *E*1cB hydrolytic mechanism involving unimolecular collapse of the ester enolate ions *via* a ketenoid transition-state. *S*-Acetoacetylcoenzyme A was also hydrolysed in base by this mechanism. Direct comparison of rates of leaving group expulsion for ArS and ArO was possible by means of this unimolecular process. For a leaving group with $pK_{L.G.}$ 10, the oxyanion departs *ca.* 1 or 2 orders of magnitude faster than the thiolate anion; for $pK_{L.G.}$ 6.0, the advantage of oxygen over sulphur is 10^3—10^4 fold. In a direct structural comparison, PhS departs 32 times as rapidly as PhO. The contribution of steric release in the *E*1cB transition-state for *S*-t-butyl acetothiolacetate hydrolysis is discussed. The pK_a values of some acetothiolacetates were measured.

It has long been accepted that thiolesters have pronounced acidic properties and many of the coenzymic activities of S-acetylcoenzyme A have been rationalised on this basis.[1-4] In non-enzymic thiolester systems, α-hydrogen lability has been suggested on the basis of the condensation reactions of *O*- and *S*-esters,[5-8] comparisons of keto–enol equilibria,[5] and pK_a measurements on acetoacetates.[7,9]

Aryl acetoacetates with powerful leaving groups and acidic α-hydrogen atoms have been shown to undergo hydrolysis and acyl transfer by means of an elimination–addition mechanism [equation (1)] involving a ketenoid



a; X = O
b; X = S

transition state.[10] Recently, reviews of various aspects of such elimination–addition (EA) mechanisms of acyl transfer have appeared and established the route as important in many reactions of biologically and economically significant molecules.[11-13] In spite of the centrality of *S*-acetoacetylcoenzyme A to many metabolic processes, *e.g.* ketone body formation, lipid biogenesis, and amino-acid degradations, little attention [14] appears to have been paid to such a keten pathway [equation (1b)] for this acidic thiolester. Nor has heavy emphasis been given in the past by physical organic chemists to the possibility of such elimination–addition pathways in the many biological reactions of thiolacetates, *e.g.* the stereochemical course of enzyme-catalysed (*S*)-malate synthesis [15,16] from glyoxalate and *S*-acetylcoenzyme A, to be discussed in a subsequent paper.

In view of the acidity of thiolesters and the excellent leaving ability [17] of RS^-, we considered the possibility that important biological thiolesters, including *S*-acetoacetylcoenzyme A and *S*-acetylcoenzyme A, respectively, might undergo hydrolysis and acyl transfer in aqueous solution by an elimination route. Thus, in addition to the usual associative, bimolecular route for base-catalysed ester hydrolysis [18-20] of α-acidic esters either an *E*1cB (as for the acetoacetates [10]) or an *E*2 mechanism is possible.

A prerequisite for the complete understanding of any enzyme mechanism is a detailed knowledge of the intrinsic properties of the participants. Consequently, we have undertaken a kinetic analysis of the alkaline hydrolysis of a series of leaving-group-substituted acetothiolacetates (1) to discover the participation, or otherwise, of a keten route in the non-enzymic cleavage of *S*-acetoacetylcoenzyme A.[21]

CH_3COCH_2COR
(1)

a; R = SEt
b; R = SPr^n
c; R = SBu^t
d; R = Scoenzyme A
e; R = SCH_2CH_2NHAc
f; R = $SC_6H_4CH_3$-*p*
g; R = SC_6H_5
h; R = SC_6H_4Cl-*p*

In addition to its biochemical significance, such a study of *E*1cB ester hydrolysis potentially [10] may provide a *direct* comparison of the relative leaving tendencies of oxygen and sulphur(II) anionic leaving groups if conditions can be obtained such that the rate-determining step for both types of ester is the elimination of leaving group from the ester conjugate base.

EXPERIMENTAL

Materials.—Deuterium oxide (99.8 atom % D) and [$^2H_3$]acetonitrile (99 atom % D) were from the Aldrich Chemical Co. *N*-Acetyl-*S*-acetoacetylcysteamine was obtained from Sigma. Diketen (Tridom) and aniline were distilled before use and stored in the cold under nitrogen.

S-Acetoacetylcoenzyme A was obtained as the tetralithium salt ($C_{25}H_{36}Li_4N_7O_{18}P_3S,5H_2O$) from P-L Biochemicals, Inc. Thiols were commercially obtained. Acetonitrile (Fisher Pesticide Grade) was further purified by molecular sieve (Linde 4A) treatment, followed by distillation from a small quantity of phosphorus pentaoxide and finally by distillation off calcium hydride.

Alkyl acetothiolacetates were synthesised as liquids from the appropriate thiol and diketen under basic catalysis (sodium acetate) as described in the literature.[22] Ethyl acetothiolacetate (1a) showed b.p. 50—56 °C at 2 Torr (lit.,[6] 110° at 30 Torr); n_D^{25} (1.494 2). n-Propyl acetothiolacetate (1b) showed b.p. 56.5—62° at 0.4 Torr; $n_D^{21.5}$ 1.492 9 (lit.,[22] b.p. 102—103° at 12 Torr; n_D^{25} 1.494 4) and the t-butyl ester (1c) had b.p. 48.5—52° at 0.7 Torr; n_D^{22} 1.485 0 (lit.,[22] b.p. 100° at 15 Torr; n_O^{25} 1.481 8).

However, this procedure was unsatisfactory for the aryl esters (1f—h), 2-acetyl-5-hydroxy-3-oxohex-4-enolactone being isolated on attempted work-up These esters were prepared by triethylamine (trace) catalysed reaction of the thiol with diketen in dichloromethane followed by removal of volatile components at room temperature by Rotovap. p-*Chlorophenyl acetothiolacetate* (1h) was found to be a solid and was purified by recrystallisation, m.p. 45—46 °C (Found: C, 52.5; H, 3.8; Cl, 14.8. $C_{10}H_9ClO_2S$ requires C, 52.2; H, 4.0; Cl, 15.5%). Esters (1f and g) were liquids and decomposed on distillation. Attempted chromatography on a silica gel column caused further decomposition, the eluted ester being further contaminated with dehydroacetic acid. However, elemental analysis of the *phenyl ester* (1g) obtained directly as the Rotovap residue above was satisfactory (Found: C, 61.8; H, 5.2. $C_{10}H_{10}O_2S$ requires C, 62.0; H, 5.2%). The phenyl ester (1g) has been reported in the literature to be a solid,[23] m.p. 174.5—175.5°, but this solid product does not have structure (1g). Further information has been reported elsewhere along with synthetic details of the reactions of aromatic thiols with diketen.[24]

Esters (1a—c and f—h) all showed satisfactory i.r. spectra and the n.m.r. spectra were characteristic of the keto–enol mixtures expected for acetoacetates,[25] *viz.*, δ(CCl_4) *ca.* 2.18 (CH_3, keto), *ca.* 1.98 (CH_3, enol), *ca.* 3.68 ($-CH_2-$, keto), *ca.* 5.35 (=CH–, enol).

Kinetic Measurements.—Rate measurements were performed either by spectrophotometric or pH-stat recording procedures. U.v.–visible spectrophotometry was carried out using a GCA-McPherson 707-K double beam spectrophotometer, whose reaction chamber was thermostatted to 25.00 ± 0.05 °C by means of water circulating from a Haake FJ thermoregulator unit. Substrate (10—50 μl stock solution) was added on the flattened tip of a Teflon stirring rod to 3.0 ml of appropriate medium, equilibrated to 25 °C in a 10 mm path length cuvette. Correct temperature equilibration was readily ascertained as the instrument was equipped with a thermocouple for immersion in the cuvette. More rapid reactions were studied by means of stopped-flow spectrophotometry using a Durrum–Gibson apparatus at 25.00 ± 0.05 °C (model 2095, Forma thermostat bath). For stopped-flow study of the acetothiolacetates, ester stock solutions were prepared (immediately before use) in 10^{-3}M-hydrochloric acid containing 5% (v/v) acetonitrile. This medium was chosen as the esters were shown to be hydrolytically stable therein over the time period required. Equal volumes of ester stock solution and sodium hydroxide solutions of twice the required final base concentration (0.05—0.10M) and ionic strength were mixed rapidly and the change in transmittance corresponding to the hydrolysis reaction recorded as a function of time on a storage oscilloscope. Photographs were taken of three or more superpositions for any given ester–base combination. Such data were analysed by computer using a program written by E. Subak.

The Radiometer recording pH stat and titration system used consisted of a PHM 64 pH meter (0.001 pH readability) with a TTT 60 titrator and REC 61 chart recorder. A calomel electrode was used in conjunction with a type G-2222B glass electrode in more basic media. The glass reaction vessel was kept at 25.00 ± 0.02 °C by means of a model 1 420 Thermomix. A type ABU 12 autoburette of 0.25 ml (for kinetic studies) or 2.50 ml (for measurement of dissociation constants) capacity was used to deliver titrant. All reactions were carried out under a stream of scrubbed, carbon-dioxide free, dry nitrogen. The pH meter was standardised against Fisher standard buffers: titrant normality was checked against standard acid. Measured pH values were corrected, when necessary, for the sodium ion error.

Acetothiolacetate hydrolyses were followed spectrophotometrically by means of the absorption band (*ca.* 300 nm) corresponding to the ester carbanions. The acetoacetate esters of *p*-chlorothiophenol, thiophenol, and *p*-thiocresol were followed (by stopped-flow) at 306 nm, esters of coenzyme A (Δε 2.15 ± 0.03 × 10^4 l mol^{-1} cm^{-1}) and t-butyl thiol (Δε 2.39 ± 0.15 × 10^4 l mol^{-1} cm^{-1}) were followed at 303 nm and esters of *N*-acetylcysteamine (Δε 1.34 ± 0.06 × 10^4 l mol^{-1} cm^{-1}), ethanethiol and propane-1-thiol were studied at 313 nm. The extinction changes reported above (log Δε *ca.* 4.1—4.3) are close to those previously reported [10] for oxygen esters of acetoacetic acid (log Δε *ca.* 4.67 at 295 nm). In determinations of the rates of cleavage of the ester conjugate bases, conditions were chosen to avoid interference from buffer components. Thus, by working in sodium hydroxide solutions, the process recorded was simply collapse of the ester anions, whereas at lower pH, in the presence of buffers, one would expect C–H cleavage (to form the carbanion) to become at least partly rate determining with consequent buffer effects as for oxygen esters of acetoacetic acid [10] and phenylmethanesulphonic acid.[26]

The acid dissociation constant of *N*-acetyl-*S*-acetoacetylcysteamine was determined electrometrically from the pH at half-neutralisation using the Radiometer apparatus described above. In addition, the acid dissociation constants of esters (1a, c, and e) were determined spectrophotometrically by means of the absorption (at 300 nm) of the ester conjugate bases. The background absorbance of the neutral ester at this wavelength was corrected for by subtracting the reading obtained in 0.1M-hydrochloric acid for that ester. Where reaction of substrate [only (1e)] caused the absorbances to change with time, these were extrapolated to zero time.

In pH 7.00 phosphate buffer containing excess of Ellman's thiol reagent, addition of 4-chlorophenyl acetothiolacetate (1h) resulted in an instantaneous release of 98.5 ± 1.5% of the theoretical quantity of thiolate anion at 410 nm using an extinction coefficient ε_m of 1.36 × 10^4 l mol^{-1} cm^{-1}.

One of the products of hydrolysis of t-butyl acetothiolacetate was shown to be t-butyl thiol by trapping with 1-fluoro-2,4-dinitrobenzene (FDNB). *S*-t-Butyl acetothiolacetate (2 ml of 0.446M) and sodium hydroxide (89 ml of 0.1M) were stirred together for 1 h at 25 °C. FDNB (1 ml

of a 20% solution in acetonitrile) was added, along with an additional 8 ml of acetonitrile (for solubility), and the mixture stirred for 5 h at 25 °C. From this procedure an 86.7% yield of t-butyl 2,4-dinitrophenyl sulphide was isolated and identified by mixed m.p. determination with an authentic sample [27] of the sulphide. In a control experiment, identical to that above, but using pure t-butyl thiol in place of the acetothiolacetate ester, 91.5% of the theoretical amount of sulphide was isolated. These results indicate production, during hydrolysis of 94.8% (=86.7 × 100/91.5) of the theoretical amount of $-\overset{O}{\overset{\|}{C}}-S-$ cleavage product of t-butyl acetothiolacetate.

Reactions were followed under pseudo-first-order conditions and plots of ln $(A_\infty - A_t)$ *versus* time were linear to >90% of reaction for the spectrophotometric studies; in pH-stat work analogous plots were linear to >85% of reaction. Rate data were analysed by fitting values of $(A_\infty - A_t)$ and t to an equation, $y = ae^{bx}$, by a least squares regression analysis, to give a rate constant of $-b$; correlation coefficients were high ($\geqslant$0.998). Random checks using a variant of the Guggenheim method gave identical results. Very slow reactions were followed by the method of initial rates using infinity values calculated from the measured extinction change of the reaction. The products of hydrolysis of t-butyl acetothiolacetate were transparent in the region of 300 nm at the ester concentrations used.

Stock solutions of esters for conventional kinetic studies were prepared in acetonitrile, except for *S*-acetoacetylcoenzyme A and *N*-acetyl-*S*-acetoacetylcysteamine, which were dissolved in degassed water. Such stock solutions were prepared immediately prior to use. All water used in kinetic studies was glass distilled and degassed before stock solution preparations. Kinetics were studied in the presence of 10^{-5}M-disodium ethylenediaminetetra-acetate to minimise interference by metal ions. Ionic strength was held at 0.1M using sodium chloride as support electrolyte unless otherwise stated.

Routine linear and exponential regression analyses were performed on a programmable Wang 720C calculator; we are grateful to the Duquesne University Department of Pharmacy for use of the calculator and n.m.r. facilities. M.p.s are uncorrected. Analyses were performed by Chemalytics Inc.

RESULTS

(i) *Acetothiolacetate Hydrolysis.*—In phosphate buffer (pH 7.98) no buffer effect on rate was detectable for *N*-acetyl-*S*-acetoacetylcysteamine from 0.03 to 0.10M-phosphate concentration. However, the rate of hydrolysis of this ester was pH dependent, values of $k_{obs.}$ (the observed pseudo-first-order rate constant) from pH 7—14 being collected in Table 1. These data are quantitatively described by equation (2), which describes the kinetic ionisation of a monobasic acid.[28] The pH profile is shown

$$k_{obs.} = k'/(1 + [H^+]/K_a) \qquad (2)$$

as a plot of $\log_{10} k_{obs.}$ *versus* pH in Figure 1 and corresponds to a $pK_{app.}$ of 8.71. From pH 10.8 to 12.7 the value of $k_{obs.}$ is constant and equal to k', which will be referred to, hereafter, as the alkaline plateau rate constant. Thus, by working in dilute sodium hydroxide solutions we can study the k' term in isolation. In Table 2, we have collected rate data for the alkaline plateau terms of esters (1a—h). It is readily seen that for these esters the rate of hydrolysis in base (0.01—0.10M-sodium hydroxide) is independent of hydroxide ion concentration.* If these data are plotted

TABLE 1

pH Profile data for the hydrolysis of *S*-acetoacetyl-*N*-acetylcysteamine in aqueous solution at 25.0 °C; the ionic strength was 0.1M except where noted and 10^{-5}M-ethylenediaminetetra-acetate was present [a]

pH [b]	$10^4 k_{obs.}/s^{-1}$
	3.80 [c]
12.69	3.98
11.82	4.02
11.52	4.03
10.80	4.00
10.00 [d]	2.95
9.00	2.87
8.99 [d]	2.30
7.98	0.939 [e]
7.05	0.104 [e]

[a] Rates determined spectrophotometrically in 0.8% (v/v) acetonitrile, except where noted. [b] The pH-readings are likely to reflect closely the true pH value as low levels of acetonitrile can be assumed to exert negligible influence on the electrode reading (F. J. Kezdy and M. L. Bender, *Biochemistry*, 1962, **1**, 1097. [c] In 1.0M-sodium hydroxide solution, ionic strength 1.0M. [d] Measured by means of a pH-stat in 0.5% (v/v) acetonitrile using a Type C electrode, with appropriate sodium ion correction from a nomogram. [e] By the method of initial rates.

against $pK_{L.G.}$ (the pK_a of the conjugate acid of the corresponding leaving group), a Brønsted-type plot for leaving group dependence is obtained with a slope of $\beta_{L.G.}$. The

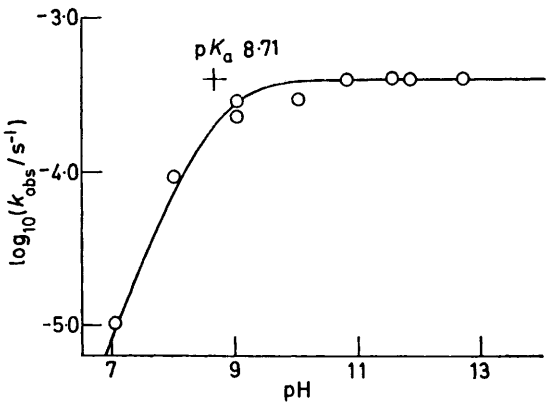


FIGURE 1 pH profile for the rate of hydrolysis of *N*-acetyl-*S*-acetoacetylcysteamine, using data from Table 1. Points are experimental: line is theoretical for an acid of dissociation constant of 8.71 and $k' = 4.01 \times 10^{-4}$ s^{-1}

best line is obtained when the point for the *S*-t-butyl ester is excluded from the correlation, under which circumstances equation (3) describes the data.

* Any measurement of the rate of hydrolysis of an acetoacetyl thiolester must, therefore, take into account the pK_a of the ester itself in calculation of an apparent second-order rate constant. This can be done using the relationship, $k_{HO^-} = K_w k'/K_a$. For *S*-acetoacetylcoenzyme A itself, Jaenicke and Lynen [3] quote the hydrolysis rate as 0.24 l mol^{-1} min^{-1}: their value is in error because of the ionisation correction required. The value they quote is derived from a half-life of 30 min at pH 13, which presumably refers to 0.1M-hydroxide ion. Assuming a pK_a of 8.6 (see Table) for *S*-acetoacetylcoenzyme A, the correct apparent second-order rate constant is 0.93 l mol^{-1} s^{-1} as the half-life of 30 min at pH 13 corresponds to $k_{obs.}$ 3.71×10^{-4} s^{-1}, close to the value of k' we report in Table 2.

The Brønsted plot is shown in Figure 2, along with data from the literature [10] for the corresponding term in the oxygen ester series, $CH_3COCH_2CO{\cdot}OR$. In the latter,

$$\log_{10}k' = 7.60 - 1.13\ pK_{L.G.} \quad (r\ 0.997) \qquad (3)$$

alkyl acetoacetates with poorer leaving groups ($pK_{L.G.} > 11.3$) are considerably less sensitive to leaving group due to a change from an elimination–addition mechanism for the more activated esters to a bimolecular mechanism for the alkyl esters.[10] If a line parallel to this 'bimolecular' Brønsted correlation for the alkyl (oxygen) acetoacetates is drawn through the points for the least reactive thiolacetoacetates (1a and b), the *S*-t-butyl ester is seen to deviate positively. The positive deviation of the *S*-t-butyl ester is even greater (*ca.* 150 fold rate increase) if one compares it with the line fixed by equation (2) for the other thiolacetoacetates.

TABLE 2

Alkaline plateau rate constants (k') for acetothiolacetates (1) at 25.0 °C, ionic strength 0.1M and, except where stated, in the presence of 0.8% (v/v) acetonitrile

Ester	$[HO^-]$/M	$k_{obs.}$/s^{-1}	average k'/s^{-1}	pK_{RSH}
(1h)	0.10	4.38		
	0.05	4.43	4.405 [a]	6.14 [d]
(1g)	0.10	1.78		
	0.05	1.79	1.785 [a]	6.62 [d]
(1f)	0.10	0.822		
	0.05	0.862	0.84 [a]	6.82 [d]
(1e)	0.10	3.98×10^{-4}		
	0.01	4.02		
	0.005	4.03	4.01×10^{-4}	9.92 [e]
(1d)	0.10	3.79×10^{-4}		
	0.05	4.12		
	0.01	3.59	3.88×10^{-4} [b]	9.6 [f]
(1c)	0.05	1.29×10^{-3}		
	0.01	1.30	1.295×10^{-3}	11.22 [e]
(1b)	0.10	6.90×10^{-5}		
	0.05	6.67		
	0.01	6.32	6.63×10^{-5} [c]	10.65 [g]
(1a)	0.010	7.80×10^{-5}		
	0.005	7.62		
	0.001	7.60	7.67×10^{-5}	10.61 [e]

[a] 2.5% v/v acetonitrile, by stopped flow. [b] 0% v/v acetonitrile. [c] Although we have quoted k' as the average of the three values of $k_{obs.}$ reported, this is not strictly correct as $k_{obs.}$ apparently shows a small hydroxide ion dependence. We have not investigated this small change further. [d] Ionic strength 0.04M; P. De Maria, A. Fini, and F. M. Hall, *J.C.S. Perkin II*, 1973, 1969. [e] Ionic strength 0.015M; R. J. Irving, L. Nelander, and I. Wadso, *Acta Chem. Scand.*, 1964, **18**, 769. [f] H. Beinert, R. W. von Korff, D. E. Green, D. A. Buyske, R. E. Handschuhmaker, H. Higgins, and F. M. Strong, *J. Biol. Chem.*, 1953, **200**, 385; ionic strength not given. [g] D. L. Yabroff, *Ind. Eng. Chem.*, 1940, **32**, 257.

Arrhenius parameters and deuterium kinetic solvent isotope effects on the alkaline plateau rate constants of esters (1e and c) are recorded in Table 3. For t-butyl acetothiolacetate the ratio k'_H/k'_D at 49.0 °C was 1.43: this was the ratio of the mean of two values at different lyoxide concentrations in each medium with a deviation in each of <2%. There is a slight temperature dependence of k'_H/k'_D for the plateau rate constant of *N*-acetyl-*S*-acetoacetylcysteamine: the values at 25.0 and 40.0 °C were 1.55 and 1.48, respectively (from least squares regression analysis of $\log_{10}k_{obs.}$ *versus* $1/T$).

Increasing aniline concentration causes a significant decrease in the alkaline plateau rate constant for breakdown of the anion of *N*-acetyl-*S*-acetoacetylcysteamine at 39.0 °C, rates being collected in Table 4.

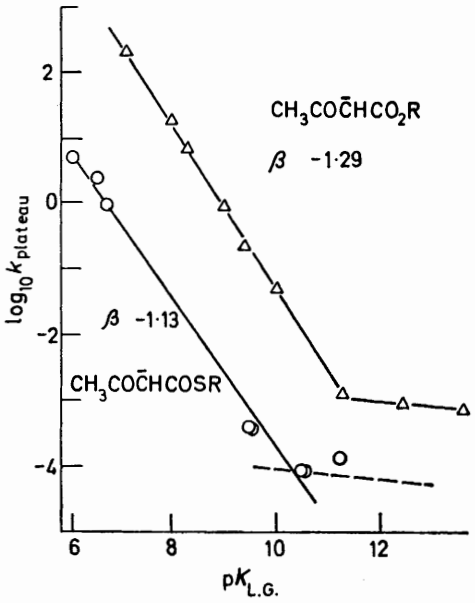


FIGURE 2 Leaving-group dependences for the hydrolyses of acetoacetate esters. The data for the thiolacetoacetates (○) are from Table 2, whilst the data for the oxo esters (△) are from ref. 10. The alkaline plateau rate constants are plotted *versus* the pK_a of the conjugate acid of the leaving group

(ii) pK_a *Measurements.*—The pK_a of ethyl acetothiolacetate at 24.7 °C, ionic strength 0.1M, measured electrometrically in the presence of 10% acetonitrile (v/v) was found to be 9.12 ± 0.01. Spectrophotometric titration of esters (1a, c, and e) at ionic strength 0.1M and 25.0 °C in the presence of 0.8% v/v acetonitrile yielded the pK_a

TABLE 3

Alkaline plateau rate constants at several temperatures and in H_2O and D_2O media for t-butyl acetothiolacetate (1c) and *N*-acetyl-*S*-acetoacetylcysteamine (1e) [a]

	$10^4k_{obs.}$/s^{-1}		
Temperature (°C)	(1e) (H_2O)	(1e) (D_2O)	(1c) (H_2O)
22.9	3.95		
23.3			12.8
25.0	5.27	3.40	
30.0	10.2		
31.0			31.5
39.0			61.8
39.3		25.7	
39.9	38.0		
51.5	186		
52.1		135	
49.0			154 [b]
$\Delta H^‡$/kcal mol^{-1}	25.0 ± 0.5	25.6 ± 0.0	17.5 ± 0.6
$\Delta S^‡$/cal K^{-1} mol^{-1}	+10.4 ± 1.5	+11.3 ± 0.0	−12.6 ± 2.1

[a] Ionic strength 0.1M, acetonitrile 0.8% v/v. [b] At ionic strength 0.05M, $k_{obs.}$ was 1.52×10^{-2} s^{-1} for this ester under these conditions.

TABLE 4

Inhibitory effect of aniline on k' term for *N*-acetyl-*S*-acetoacetylcysteamine [a]

10^2[$PhNH_2$]/M	$10^3k_{obs.}$/s^{-1}
0.000	3.45
0.417	3.30
1.25	3.07
1.67	2.95

[a] Measured in 0.15M-sodium hydroxide solution at 39.0 °C, 0.2M ionic strength in the presence of 10^{-5}M-EDTA and 10% acetonitrile (v/v).

values recorded in Table 6. Also tabulated are some literature pK_a values for acetoacetate derivatives.

DISCUSSION

The pH profile obtained for *N*-acetyl-*S*-acetoacetylcysteamine (Figure 1) indicates complete conversion of substrate to its conjugate base at high pH ($\gtrsim$9). The pK_a of ester (1e), determined either spectrophotometrically by means of the conjugate base absorbance at *ca.* 300 nm or electrometrically (Table 5) agrees closely with the position of the kinetic inflexion observed in Figure 1.

TABLE 5

Acid dissociation constants for acetoacetate derivatives [a]

CH_3COCH_2COX

X	pK_a
SBu^t	9.16
SEt	8.93
$S(CH_2)_2NHAc$	8.65 [b]
$OC_6H_4NO_2$-*p*	8.5 [c]
$OCH_2C{\equiv}CH$	9.83 [c]
OEt	10.7 [d]

[a] Measured at 25.0 °C and ionic strength 0.1M unless otherwise stated. [b] Lynen [9] reported a value of 8.54 for this ester at 0 °C; the ionic strength was not given and a good fit to the Henderson–Hasselbach equation could not be obtained as no allowance was made for the decay of the enolate ion with time. [c] From ref. 10. [d] R. P. Bell, 'The Proton in Chemistry,' Cornell University Press, Ithaca, 1959.

This indicates rapid pre-equilibrium carbanion formation for all the buffers and pH values studied here (see the discussion of pH profiles for this type of carbon acid system in ref. 10).

The plateau rate constant for these thiolacetoacetates probably refers to an *E*1 collapse of the enolate ion with leaving group expulsion for the following reasons. The value of $\beta_{L.G.}$ for the plateau rate constants (k') is

TABLE 6

Brønsted leaving group sensitivities ($\beta_{L.G.}$) for the k' (elimination of leaving group from substrate) term for various carbonyl species

Conjugate base	$\beta_{L.G.}$ (for k' term)
$CH_3C\bar{O}N{\cdot}CO{\cdot}OAr$ [a]	−1.02
p-$NO_2C_6H_4\bar{N}{\cdot}COOAr$ [b]	−1.4
p-$NO_2C_6H_4\bar{N}{\cdot}CO{\cdot}Ooximes$ [c]	−1.4
$^-O{\cdot}CO{\cdot}OAlkyl$ [d]	−1.1
$CH_3C\bar{O}CH{\cdot}CO{\cdot}OAr$ [e]	−1.29
$CH_3C\bar{O}CH{\cdot}COSR$ [f]	−1.13

[a] Calculated from the data of M. Bergon and J.-P. Calmon, *Bull. Soc. chim. France*, 1976, 797. [b] A. F. Hegarty and L. N. Frost, *J.C.S. Perkin II*, 1973, 1719. [c] Calculated from data of J. Hladká, J. Mindl, and M. Večeřa, *Coll. Czech. Chem. Comm.*, 1977, **42**, 3316. [d] C. K. Sauers, W. P. Jencks, and S. Groh, *J. Amer. Chem. Soc.*, 1975, **97**, 5546. [e] Ref. 10. [f] This work.

−1.13, a very large and negative value. This is close to the value reported for aryl acetoacetates undergoing *E*1cB hydrolysis ($\beta_{L.G.}$ = 1.29).[10] Large, negative $\beta_{L.G.}$ values for such k' terms are characteristic of *E*1cB reactions, and some $\beta_{L.G.}$ (k') values for various carbonyl systems are collected in Table 6.

For an ionizing substrate, such as an acetoacetate, the apparent second-order rate constant for hydroxide ion $k_{HO^-} = k'K_w/K_a$ and thus, $\beta_{L.G.}$ (k_{HO^-}) for the apparent second-order term is $\beta_{k'} - \beta_{K_a}$ (where β_{K_a} is the negative of the slope of a plot of the pK_a of the substrate *versus* the pK_a of the conjugate acid of the leaving group, pK_{XH}). Figure 3 shows such a plot for both acetoacetates and thiolacetoacetates (data from Table 5). Although the data are limited it is apparent that the sensitivity of ester pK_a to the leaving group is low on this basis and β_{K_a} is in the range 0.25—0.30. Thus

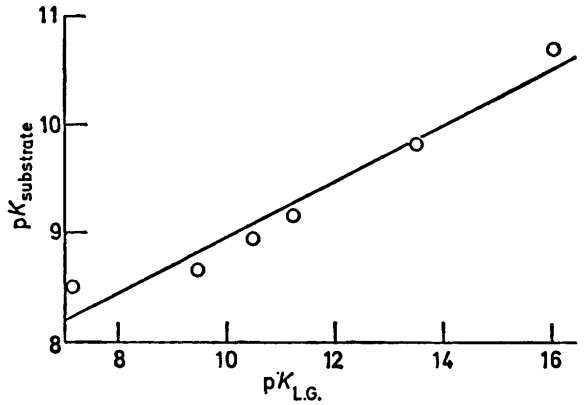


FIGURE 3 Plot of the pK_a values of acetoacetates and acetothiolacetates (CH_3COCH_2XR) *versus* the pK_a of the conjugate acid of RX^-: data are from Table 6

$\beta_{L.G.}$ (k_{HO^-}) is *ca.* (−1.13 − 0.25) or −1.38. This value is considerably more negative than the sensitivity of esters to bimolecular nucleophilic attack by hydroxide ion. For example $\beta_{L.G.}$ (k_{HO^-}) for $CH_3CO{\cdot}OR$ [29] and CH_3COSR [30] is *ca.* −0.3.

Such leaving group sensitivities have proven to be among the most dependable criteria of mechanism in this area. Other lines of evidence are also consistent with an *E*1cB route. The entropy of activation for the k' term of ester (1e) is +10.4 cal mol^{-1} K^{-1}, consistent with a unimolecular transition state. Kinetic deuterium solvent isotope effects for esters (1e and c) are also comparable to values previously reported for similar *E*1cB solvolyses *via* solvated anions.[10,12]

The very slight inhibition of the rate of breakdown of the anion of (1e) by aniline could be ascribed to a kinetically significant shunting of the anion by aniline attack on the β-keto site. We detected no significant spectral shifts and, because of the numerous, kinetically indistinguishable pathways possible under such circumstances as well as the small size of the effect, did not investigate this aspect of the reaction in more detail.

At low pH Pratt and Bruice [10] found saturation dependence of rate for the acetoacetates on aniline

concentration indicating a rate-determining step. However, the product was the anilide indicating that rate-determining and product-determining steps were different. Our aniline studies were carried out at high pH and no anilide was formed because 0.01M-aniline cannot effectively compete with $>10^{-5}$M-HO^-.

It is important to note that Pratt and Bruice [10] detected a change in mechanism for oxygen esters of acetoacetic acid. For poorer leaving groups, a break in the $\beta_{L.G.}$ (k') plot occurred at $pK_{L.G.}$ *ca.* 11 and for the less reactive esters a bimolecular mechanism was proposed to account for the markedly reduced $\beta_{L.G.}$ value in this region. It is worthwhile estimating the location at which the corresponding break-point for mechanism should occur for acetothiolacetates. For the acetothiolacetates one can calculate k_{HO^-} values based on the measured k' terms (Table 2) and the correlation for β_{K_a} (using data from Table 6 and Figure 3). These k_{HO^-} values can be plotted on the same axes as observed values [30] of k_{HO^-} for thiolacetate esters (see Figure 4).

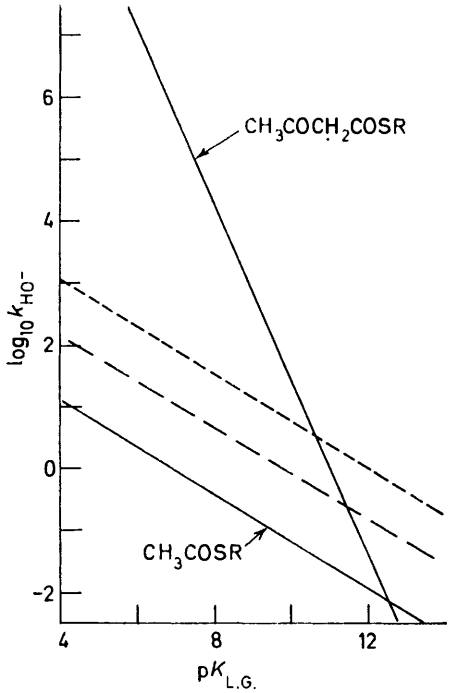


FIGURE 4 Brønsted plot of (apparent) second-order rate constants *versus* $pK_{L.G.}$ for hydroxide-ion catalysed hydrolysis of acetothiolacetate esters and thiolacetates (solid lines). The dotted lines are explained in the text

One cannot simply compare these two lines and look at the point of intersection, because an α-acetyl group should activate the ester carbonyl group to a (hypothetical) attack by hydroxide ion. However, allowance for this activating influence of the α-acetyl group can be made as follows. The Brønsted (leaving group) plots can be compared for k_{HO^-} terms for acetates [29] (CH_3COX) and for alkyl acetoacetates, $CH_3COCH_2COOAlkyl$ (reasonably suggested by Pratt and Bruice [10] to follow a bimolecular pathway). In the former case the observed values of k_{HO^-} can be used, in the latter k_{HO^-} must be calculated from the observed values [10] of k' and the estimates of ester pK_a in Figure 3. The line for acetoacetates undergoing bimolecular attack by HO^- lies 1—2 orders of magnitude above that for the acetates.* Using a similar factor for the acetothiolacetates one can construct the two dotted lines on Figure 4. The intersection point lies between $pK_{L.G.}$ *ca.* 10.8 and *ca.* 11.6, *i.e.* the mechanism is predicted to change from *E*1cB to bimolecular for esters derived from thiols slightly less acidic than ethanethiol (actually at about the basicity limit of currently available thiols!). If one compares rates from *E*1cB and bimolecular routes, conveniently done using Figure 4, one sees that although there is a rate advantage by the *E*1cB route of *ca.* 10^3—10^4-fold when $pK_{L.G.}$ *ca.* 6, for the thioethyl esters there is no substantial difference.

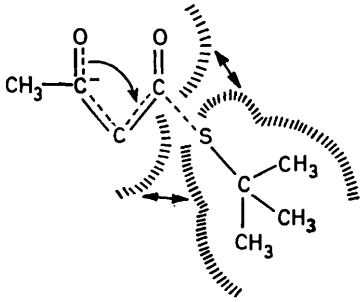
Acetoacetyl transfer from *S*-acetoacetylpantatheine and *S*-acetoacetyl-CoA to glutathione in basic media has been shown to depend on the concentration of glutathione.[31] If this transthiolation followed an *E*1cB route no dependence of rate on [glutathione] would be expected and therefore it is likely that its mechanism is bimolecular. Apparently, the change to a powerful sulphur nucleophile has dictated this mechanistic shift. It is likely therefore that in situations where thiols or other activated nucleophiles are involved, a bimolecular transition state will be used. This is presumably a consequence of the low rate advantage (*E*1cB over bimolecular attack of HO^- on neutral ester) of only 1—2 orders of magnitude for *S*-acetoacetylcoenzyme A hydrolysis (see Figure 4).

The positive deviation of t-butyl acetothiolacetate from either the '*E*1cB line' or the 'bimolecular line' (see Figure 2) is notable. In spite of the greater steric bulk in the leaving group and hence around the ester carbonyl group, this ester hydrolyses *ca.* 16 times faster than ethyl acetothiolacetate. In contrast, t-butyl thiolacetate hydrolyses,[32] *via* bimolecular attack of HO^-, five-fold slower than ethyl thiolacetate,[33] using data at 30 °C in 43% acetone–H_2O for both esters. A reasonable explanation of the apparent high reactivity of the bulky acetothiolacetate is that the steric effect of the t-butyl group can be relaxed in a dissociative transition state (steric acceleration of the *E*1cB pathway). The magnitude of this steric acceleration can be estimated as *ca.* 150-fold by comparing the observed k' for the *S*-t-butyl ester to the 'normal' *E*1cB value of k' [either from equation (5) or the extrapolated line in Figure 2].

A steric acceleration in nucleophilic addition to an activated double bond has been reported for 2-t-butylbenzenethiolate ion; this was ascribed to steric inhibition of solvation of the thiolate ion.[34] However, a referee has pointed out the similarity in pK_a values of Bu^tSH and n-alkyl thiols as evidence for lack of steric inhibition of solvation in water.

* The uncertainty arises largely because the $\beta_{L.G.}$ plot for CH_3COX is dispersed into two families (one for *O*-aryl, one for other *O*-groups) while that for CH_3COSR is not. The origins of this dispersion difference will be discussed subsequently.

As the rate-determining step in the hydrolyses of activated-aryl acetoacetate and acetothiolacetates is cleavage of the bond restraining the leaving group one can make a direct comparison of the leaving tendencies of various species by means of this reaction. For a leaving group whose conjugate acid has pK_a 10, the oxyanion departs some one or two orders of magnitude faster than the thiolate species; for $pK_{L.G.}$ 6.0, the advantage of oxygen over sulphur is *ca.* 10^3-fold.*



Although considerable use has been made in the literature [17,35] of the *greater* leaving ability of RS^- than RO^- from sp^3 carbon, a direct comparison of leaving tendencies has been lacking until recently.[21,36] A source of difference in leaving group effects between thiols and alcohols–phenols lies in the very much lower pK_a values of thiols compared with alcohols–phenols for a given structure (*cf.* EtOH and EtSH). Stirling's group [36] has measured relative leaving abilities in the elimination reactions of an extensive series of sulphones of the type $PhSO_2(CH_2)_2Z$, using the $(E1cB)_R$ mechanism in the manner described above. For Z = OPh the rate of expulsion of leaving group is 16.7 times faster than for Z = SPh. We observe, by comparison with Pratt and Bruice's data,[10] that for CH_3COCH_2COZ [in an $(E1cB)_R$ reaction also] that Z = SPh departs 32 times as rapidly as Z = OPh (the OPh was measured at ionic strength 1.0M and 30 °C, whereas the SPh was measured at ionic strength 0.1M and 25 °C; no correction has been made for this). Thus, the change of departure site from a $-CH_2$ group to a $>C=O$ group apparently inverts the relative leaving tendencies of OPh and SPh. Unfortunately, deeper analysis of this is precluded at present as the solvents for two substrate systems differ (aqueous for the acetoacetates, ethanolic for the sulphones). The complexity of the concept of ' leaving ability ', as discussed in detail by Marshall *et al.*,[36] is, at the very least, underscored by the above. However, for a given solvent system and chemical substrate type the concept is useful as long as changes in hybridisation state between ground and transition states are the same. Our comments above concerning relative leaving abilities of XR for X = O and S either on the basis of equal $pK_{L.G.}$ or by isostructural comparison (PhS^- *versus* PhO^-) refer to departure from an enolate anion to form a ketenoid transition state ($\simeq sp^2 \longrightarrow \simeq sp$). Different relative values may be expected for departure from tetracoordinate adducts, such as those formed in the course of bimolecular ester hydrolyses, where the change is $\simeq sp^3 \longrightarrow \simeq sp^2$.

* These values were calculated using equation (3) for the *S*-acetoacetate esters and $\log_{10}k' = 11.50—1.28\ pK_{L.G.}$ (calculated in ref. 21) from the data of Pratt and Bruice.[10]

We are grateful to the Research Corporation for financial support and to Duquesne University for the purchase of a spectrophotometer.

[9/1620 *Received*, 12*th October*, 1979]

REFERENCES

[1] T. C. Bruice and S. J. Benkovic, ' Bioorganic Mechanisms,' Benjamin, New York, 1966, vol. I.
[2] F. Lynen, *Fed. Proc.*, 1953, **12**, 683.
[3] L. Jaenicke and F. Lynen in ' The Enzymes,' eds. P. D. Boyer, H. Lardy, and K. Myrback, Academic Press, New York, 1960, 2nd edn., vol. 3, p. 3.
[4] P. Goldman and P. R. Vagelos in ' Comprehensive Biochemistry,' eds. M. Florkin and E. H. Stotz, Elsevier, Amsterdam, 1964, vol. 15, p. 71.
[5] R. B. Baker and E. E. Reid, *J. Amer. Chem. Soc.*, 1929, **51**, 1567.
[6] M. W. Cronyn, M. P. Chang, and R. A. Wall, *J. Amer. Chem. Soc.*, 1955, **77**, 3031.
[7] G. E. Lienhard and W. P. Jencks, *J. Amer. Chem. Soc.*, 1965, **87**, 3863.
[8] J. C. Sheehan and C. W. Beck, *J. Amer. Chem. Soc.*, 1955, **77**, 4875.
[9] F. Lynen, *Fed. Proc.*, 1953, **12**, 685.
[10] R. F. Pratt and T. C. Bruice, *J. Amer. Chem. Soc.*, 1970, **92**, 5956.
[11] A. Williams and K. T. Douglas, *Chem. Rev.*, 1975, **75**, 627.
[12] K. T. Douglas in ' Progress in Bioorganic Chemistry,' eds. E. T. Kaiser and F. J. Kezdy, Wiley–Interscience, New York, 1976, vol. 4.
[13] (*a*) K. T. Douglas and A. Williams, *J. Chem. Educ.*, 1976, **53**, 544; (*b*) A. Williams and K. T. Douglas, *Chem. and Ind.*, 1977, 679.
[14] A keten form of ' active-acetate ' was proposed by C. Martius, *Hoppe-Seyler's Z. Physiol. Chem.*, 1943, **279**, 96, before the discovery of coenzyme A and the possibility of a keten intermediate in *S*-acetylcoenzyme A reactions was suggested (ref. 3, p. 62) before experimental demonstration of elimination–addition hydrolytic routes for carbon acid esters.[10] Lactone intermediacy in the action of citrate synthase was also advanced (A. Eschenmoser and D. Arigoni, quoted in ref. 3, p. 62). The keten possibility was also advanced in an early review, J. W. Cornforth, *J. Lipid Res.*, 1959, **1**, 1.
[15] (*a*) J. W. Cornforth, J. W. Redmond, H. Eggerer, W. Buckel, and C. Gutschow, *Nature*, 1969, **221**, 1212; (*b*) J. Luthy, J. Retey, and D. Arigoni, *ibid.*, p. 1213.
[16] F. Lynen, *Biochem. Soc. Symp.*, 1970, **31**, 1.
[17] A. Frankfater and F. J. Kezdy, *J. Amer. Chem. Soc.*, 1971, **93**, 4039.
[18] (*a*) W. P. Jencks in ' Catalysis in Chemistry and Enzymology,' McGraw–Hill, New York, 1969, pp. 500—501; (*b*) R. Hershfield and G. L. Schmir, *J. Amer. Chem. Soc.*, 1972, **94**, 1263.
[19] M. L. Bender, *Chem. Rev.*, 1960, **60**, 53.
[20] S. L. Johnson, *Adv. Phys. Org. Chem.*, 1967, **5**, 237.
[21] Preliminary communication, N. F. Yaggi and K. T. Douglas, *J. Amer. Chem. Soc.*, 1977, **99**, 4844.
[22] F. Duus, P. Jakobsen, and S.-O. Lawesson, *Tetrahedron*, 1968, **24**, 5323.
[23] K. Konishi, H. Umemoto, M. Yamamoto, and T. Kitao, *Nippon Kagaku Kaishi*, 1973, 118.
[24] N. F. Yaggi and K. T. Douglas, *J.C.S. Chem. Comm.*, 1977, 609.
[25] E. J. Drexler and K. W. Field, *J. Chem. Educ.*, 1976, **53**, 392.
[26] M. B. Davy, K. T. Douglas, J. S. Loran, A. Steltner, and A. Williams, *J. Amer. Chem. Soc.*, 1977, **99**, 1196.
[27] E. A. M. F. Dahmen, R. Dijkstra, and A. J. Verjaal, *Erdoel. Kohle*, 1963, **16**, 768 (*Chem. Abs.*, 1964, **60**, 340d); R. W. Bost, J. O. Turner, and R. D. Horton, *J. Amer. Chem. Soc.*, 1932, **54**, 1985.
[28] A. Williams and K. T. Douglas, *J.C.S. Perkin II*, 1972, 1454; 1974, 1727.
[29] J. F. Kirsch and W. P. Jencks, *J. Amer. Chem. Soc.*, 1964, **86**, 837.

[30] K. T. Douglas, C. Mervis, and N. Y. Yaggi, unpublished results.
[31] G. I. Drummond and J. R. Stern, *Arch. Biochem. Biophys.*, 1961, **95**, 323.
[32] P. N. Rylander and D. S. Tarbell, *J. Amer. Chem. Soc.*, 1950, **72**, 3021.
[33] J. R. Schaefgen, *J. Amer. Chem. Soc.*, 1948, **70**, 1308.
[34] D. Semenow-Garwood, *J. Org. Chem.*, 1972, **37**, 3797.
[35] M. L. Bender, *Chem. Rev.*, 1960, **60**, 63; A. Frankfater and F. J. Kezdy, *J. Amer. Chem. Soc.*, 1971, **93**, 4039; R. B. Martin and R. I. Hedrick, *ibid.*, 1962, **84**, 106; K. A. Connors and M. L. Bender, *J. Org. Chem.*, 1961, **26**, 2498; H. Hirohara, M. L. Bender, and R. S. Stark, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 1643; W. P. Jencks, 'Catalysis in Chemistry and Enzymology,' McGraw-Hill, New York, 1969, pp. 500—501; D. J. Hupe and W. P. Jencks, *J. Amer. Chem. Soc.*, 1977, **99**, 451.
[36] D. R. Marshall, P. J. Thomas, and C. J. M. Stirling, *J.C.S. Perkin II*, 1977, 1898.