Malonylcoenzyme A Models. Part 1. *E*1cB (Keten) Pathway for Acyl Transfers of Malonic Acid Thiolmonoesters including *S*-Malonylcoenzyme A

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The aminolysis of a series of malonate thiolmonoesters (HO_2CCH_2COSR) has been studied kinetically at 25 °C. No indication of substrate ionisation was observed for the *S*-4-chlorophenyl and *S*-phenyl esters up to 0.45M-sodium hydroxide. Morpholinolysis showed saturation kinetics, *i.e.* the observed pseudo-first-order rate constant became insensitive to morpholine concentration at higher morpholine concentrations. This behaviour was analysed in terms of the rate equation $k_{obs} = k_{max.}[B]/(K + [B])$, where $k_{max.}$ is the limiting rate constant at higher buffer (B) concentrations and K the concentration of buffer required for $k_{obs} = 0.5\,k_{max.}$ Saturation aminolysis was also observed for *S*-malonylcoenzyme A. Arrhenius parameters were determined for the above kinetic parameters ($k_{max.}$, K, $k_{max.}/K$) for the *S*-benzyl ester. Trapping experiments with aniline showed that rate-determining and product-determining steps differed. The results were interpreted in terms of an intermediate keten formed from the ester dianion ($^-O_2CCHCOSR$). However, to explain leaving-group dependences of $k_{max.}$, K, *etc.*, a degree of leaving-group protonation in the transition-state had to be invoked, although mechanisms involving the zwitterion-anion $[^-O_2C\bar{C}H{\cdot}CO\dot{S}(H)R]$ could be excluded.

The biochemistry of *S*-acylcoenzyme A derivatives has been rationalised partly on the basis of nucleophilic attack by acceptor species on the acyl carbonyl group.[1] An alternative (*E*1cB) process has been demonstrated recently for *S*-acetoacetylcoenzyme A[2] and related thiolesters [equation (1)],

$$CH_3COCH_2COSCoA \rightleftharpoons CH_3CO\bar{C}HCOSCoA \longrightarrow CH_3COCH{=}C{=}O \xrightarrow{fast} \text{products} \quad (1)$$

although *S*-acetylcoenzyme A follows a bimolecular associative pathway.[3] Many biological situations involve thiolesters, including the mechanisms of action of sulphydryl proteases, aldehyde dehydrogenases, glyoxalase, thiolase, *etc.* Acyl coenzyme A intermediates (especially acetyl, malonyl, and acetoacetyl) are pivotal intermediates in many pathways for amino-acids, carbohydrates, and fatty acids.[1]

S-Malonylcoenzyme A functions as a donor of two-carbon units in the synthesis of β-ketoacyl acyl carrier protein, an intermediate in *de novo* fatty acid biosynthesis [4] and in microsomal elongation of fatty acids.[5] It is also a precursor of 6-methylsalicylate in aromatic biosynthesis [6] and is probably also involved in fatty acid regulation *via* its inhibitions of fatty acid synthetase and citrate activation of acetylCoA carboxylase [7] and participates in bacterial degradation of propionic acid.[8] The chemistry of malonyl transfer from thiomalonate half-esters is therefore fundamental for a molecular understanding of several biological processes.

Consequently, we have considered the possibility of a keten (*E*1cB) pathway for acyl transfer from *S*-malonylcoenzyme A. This route has been established for *S*-[2] and *O*-aryl [9] acetoacetates, aryl malonates [10] (aminolysis), and two thiomalonate diesters.[9] In contrast to *S*-acetoacetates (*E*1cB), the *S*-acetates follow a $B_{AC}2$ route.[3] Thus, we have studied the aminolyses of a series of thiolmonoesters of malonic acid (1)—(5) including *S*-malonylcoenzyme A. A preliminary report of some of these results has appeared.[11]

HO_2CCH_2COSR
(1) R = C_6H_5
(2) R = C_6H_4Cl-4
(3) R = $C_6H_4CH_3$-4
(4) R = $CH_2C_6H_5$
(5) R = CoA

Experimental

S-Malonylcoenzyme A (trilithium salt) was purchased from P. L. Biochemicals Inc. Other thiolmonoesters of malonic acid (analytical data in Table 1) were prepared by the method of Howard *et al.*[12] Malonic acid monoanilide was prepared by refluxing malonic acid monochloride [10,13] with aniline in dry diethyl ether and was recrystallised from diethyl ether–ethanol, m.p. 130—132 °C (lit.,[13] 132 °C).

S-4-Chlorophenyl deuteriothiomalonate was prepared by refluxing together the deuteriated acid chloride [prepared from deuteriomalonic acid (Sigma Chemical Co.; 99% D)] and 4-

Table 1. Analytical data for malonic acid thiolmonoesters $HO_2C{\cdot}CH_2COY$

		Calculated (%)				Molecular	Found (%)			
Y	M.p. (°C)	C	H	S	Cl	formula	C	H	S	Cl
SC_6H_5	67—70	55.1	4.1			C_9H_8OS	55.0	4.4		
SC_6H_4Cl-4	123—125	46.9	3.1	13.9	15.4	C_9H_7ClOS	47.0	3.0	14.2	15.4
$SC_6H_4CH_3$-4	85—86.5	57.1	4.8			$C_{10}H_{10}OS$	56.9	4.7		
SCH_2Ph	73.5—75	57.1	4.8	15.3		$C_{10}H_{10}OS$	57.3	5.0	15.5	

chlorodeuteriothiophenol in dry diethyl ether for 6 h. Removal of solvent left a solid, which was recrystallised from dry 1 : 4 toluene–light petroleum (b.p. 60—80 °C) to give product (13%, 0.6 g), m.p. 120—125 °C. The ester was 90% α-deuteriated (by ^{1}H n.m.r. spectrometry) and 70% pure (by Ellman's reagent for SH titration after base cleavage), the main impurity being malonic acid (t.l.c.). Further purification was not undertaken to avoid further deuterium loss.

S-4-Chlorophenyl hydrogen-(2,2-dimethyl)thiomalonate was prepared by refluxing equivalent quantities of 2,2-dimethylmalonyl monochloride,[13] 4-chlorothiophenol, and pyridine in dry benzene for 3 h. The reaction mixture was cooled, filtered, and solvent removed from the filtrate to leave a solid, which was taken up in diethyl ether, the solution filtered, shaken with an equal volume of water and then with aqueous sodium hydrogencarbonate. The resulting aqueous layer (adjusted to pH 7) was acidified (HCl) and extracted with diethyl ether. The solid obtained on ether removal was recrystallised from toluene–light petroleum (yield 0.35 g. 13%), m.p. 133—140 °C. Examination of the product by t.l.c. (silica gel; $CHCl_3$) showed the presence of small amounts of the starting materials. Purification of all thiomalonate monoesters was difficult because of the similar properties of starting materials and products.

Kinetic Methods.—Rates of hydrolysis (k_{obs}) were measured spectrophotometrically under pseudo-first-order conditions using a Pye–Unicam SP8-100 instrument or a Canterbury SF-3A stopped-flow spectrophotometer at an appropriate wavelength determined by repetitive spectral scanning of a reacting mixture. In the case of the *S*-benzyl thiomonoester and malonylcoenzyme A, rate constants were obtained, as described below, by a titration method using Ellman's reagent [14] to detect thiol liberated during the course of reaction.

A sample tube containing morpholine-buffered solution (2 ml) was placed in a carefully thermostatted oil bath. After temperature equilibration, ester stock solution (50 μl) was added and the stopwatch started. At intervals, portions (50 μl) of reaction mixture were injected into a sample cuvette containing Ellman's reagent (3 ml) solution in pH 7 buffer, and absorbance changes (A_t) measured at 412 nm.

Results

(1) *Hydroxide Ion Kinetics.*—For both esters (1) and (2) k_{obs} (the observed pseudo-first-order rate constant) showed first-order dependence on [HO^-] with no indication of saturation, even at 0.45M-sodium hydroxide for the SPh ester (1), indicating that the pK_a of this ester must be >14. Both esters were studied at 264 nm, 25 °C, and ionic strength 1.0M (held with KCl) in the presence of 10% v/v acetonitrile. In these and all other kinetic studies reported below 10^{-4}M-EDTA was present to minimise metal-ion catalysed interference with thiolester hydrolysis. The second-order rate constants for hydroxide ion attack (k_{HO^-}) for (1) and (2) were 43.1 and 72.0 l mol^{-1} s^{-1}, respectively.

(2) *Amine Buffer Catalysis.—Morpholine.* Morpholinolysis of (1) in buffered media obeyed good pseudo-first-order kinetics up to 90% of reaction and was studied in detail to delineate the kinetic behaviour of the system. Plots of pseudo-first-order rate constants (k_{obs}) for (1) in morpholine buffers *versus* free morpholine base concentration showed saturation at higher base levels. Similar behaviour was observed for (2) and (3). Such curved behaviour followed equation (2) where [B] is the concentration of the free base form of the buffer. Rearrangement gives (3) which predicts a straight line for a plot

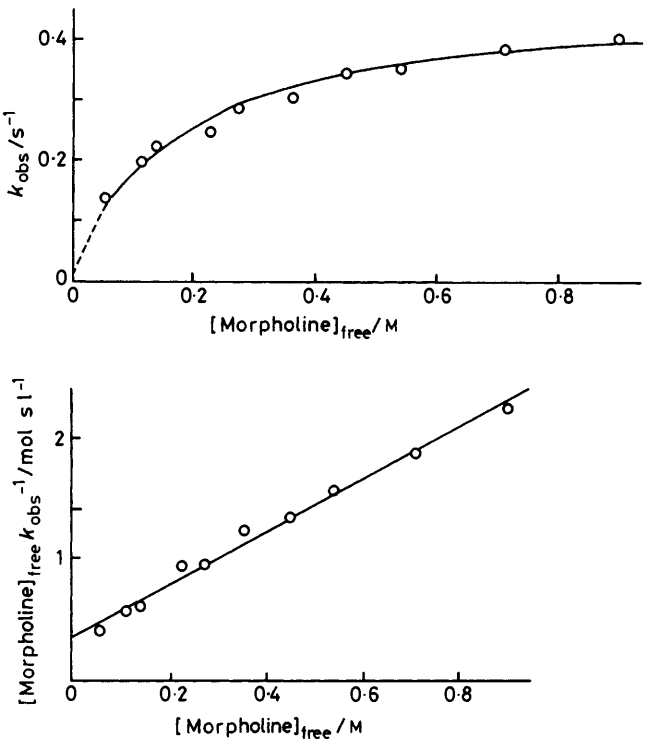


Figure 1. Upper: a plot of k_{obs} *versus* [morpholine] as 90% free base form for *S*-*p*-chlorophenyl hydrogenthiomalonate at 25 °C and ionic strength 1.0M (held with KCl), in the presence of 10^{-4}M-EDTA and 10% v/v acetonitrile, studied at 280 nm. Lower: the same data as above as a linearized plot. Points are experimental: lines are theoretical using equations (2) and (3) with $k_{max.}$ 0.467 s^{-1} and K 0.169 mol l^{-1}

$$k_{obs} = k_{max.}[B]/(K + [B]) \quad (2)$$

$$[B]/k_{obs} = [B]/k_{max.} + K/k_{max.} \quad (3)$$

of $[B]/k_{obs}$ *versus* [B] (see Figure 1) from which values of K and $k_{max.}$ are readily extracted by least-squares linear regression analysis. Rate constants for (1) and (3) in morpholine buffers of varying free base concentration but constant total buffer concentration were collected as K, $k_{max.}$, and $k_B (= k_{max.}/K)$. For each of these terms the dependence on the fraction of buffer present as free base form was obtained (Table 1). The $k_{max.}$ term depends on terms in both [B] and [BH^+]; k_B depends only on [B] and K is approximately independent of free base fraction. Intercepts at $[B]/[B_{TOT}]$ 1 and 0 for each of these terms are recorded in Table 2 for (1) and (3).

The morpholinolysis of (4) at 25 °C was very slow. Therefore the reaction was studied at higher temperatures by Ellman titration to minimise the problem of oxidation of liberated thiol. Rate constants ($k_{max.}$, K, and k_B) for (4) at 25 and 55 °C were obtained by interpolation into the appropriate Arrhenius equation, determined at four temperatures (see Table 3 for collected Arrhenius parameters). Values of rate parameters for 25 °C are in Table 4, along with such data for the other thiolesters (1)—(3) and (5). The value of $k_{max.}$ at 55 °C was calculated to be 1.66×10^{-3} s^{-1}. Rate constants were measured for *S*-malonylcoenzyme A (5) in 90% free base morpholine buffers at 55 °C by titration with Ellman's reagent. A plot of k_{obs} *versus* $[morpholine]_{free}$ is shown in Figure 2. An estimate of $k_{max.}$ for this ester at 25 °C was obtained by comparison with $k_{max.}$ for (4) at 55 °C [$k_{max.}$ (4)/(5) at 55 °C 1.34];

Table 2. Contributions from B and BH^+ forms of morpholine buffer to the aminolysis of thiolmonoesters of malonic acid ($^-O_2CCH_2COY$) at 25 °C

	Y = SC_6H_5		Y = $SC_6H_4CH_3$-4	
Kinetic parameter	B	BH^+	B	BH^+
$k_{max.}$/s^{-1}	0.23 ± 0.03	0.027 ± 0.004	0.087 ± 0.005	0.032 ± 0.002
k_B/l mol^{-1} s^{-1}	0.6 ± 0.2	0.02 ± 0.09	0.26 ± 0.09	0.05 ± 0.02
K/mol l^{-1}	0.36	0.44	0.33	0.43

Table 3. Activation parameters for *S*-benzyl hydrogenthiomalonate

	Kinetic parameters		
	$k_{max.}$	K	k_B
$\Delta E^‡$/kJ mol^{-1}	45.9 ± 2.9	−26.7 ± 12	70.9 ± 17.9
$\Delta H^‡$/kJ mol^{-1}	43.5 ± 2.9	−29.3 ± 12	68.4 ± 17.9
$\Delta S^‡$/J K^{-1} mol^{-1} 25 °C	−157.8 ± 8.8	−338.2 ± 37.6	−78.2 ± 56

Table 4. Kinetic constants for morpholinolysis of thiolmonoesters of malonic acid at 25 °C, 90% free base, 10% v/v acetonitrile, [EDTA] 10^{-4}M, ionic strength 1.0M (held with KCl)

$HOOCCH_2COX$ X =	$pK_{l.g.}$[c]	$k_{max.}$/s^{-1}	k_B/l mol^{-1} s^{-1}	K/mol l^{-1}
SC_6H_4Cl-4	6.135	0.467	2.79	0.169
SC_6H_5	6.62	0.210	0.606	0.348
$SC_6H_4CH_3$-4	6.82	8.17×10^{-2}	0.256	0.319
$SCH_2C_6H_5$ [a]	9.43 [d]	3.2×10^{-4}	5.4×10^{-4}	0.640
SCoA [b]	9.60 [e]	4.29×10^{-4}	7.24×10^{-4}	0.860

[a] Interpolated from the Arrhenius correlation. [b] By comparison with the *S*-benzyl ester. [c] From P. De Maria, A. Fini, and F. M. Hall, *J. Chem. Soc., Perkin Trans. 2*, 1973, 1969 unless otherwise stated. [d] M. M. Kreevoy, E. T. Harper, R. E. Duvell, H. S. Wilgus, and L. T. Ditsch, *J. Am. Chem. Soc.*, 1960, **82**, 4899. [e] H. Beinert, R. W. von Korff, D. E. Green, D. A. Buyske, R. E. Handschumaker, H. Higgins, and F. M. Strong, *J. Biol. Chem.*, 1953, **200**, 385.

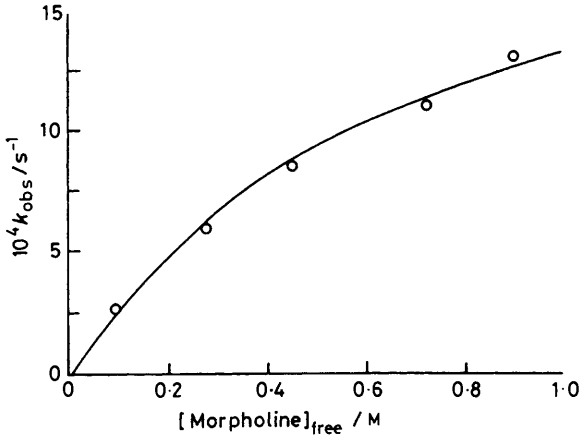


Figure 2. Saturation kinetics in the plot of k_{obs} *versus* [morpholine] as free base (90% as free base) for *S*-malonylcoenzyme A at 55.0 °C and ionic strength 1.0M (held with KCl) in the presence of 10^{-4}M-EDTA and 10% v/v acetonitrile. Points are experimental; line is theoretical from equation (2) with $k_{max.}$ 2.23×10^{-3} s^{-1} and K 0.701 mol l^{-1}

assuming similar activation parameters for both compounds gives $k_{max.}$ 4.29×10^{-4} s^{-1} at 25 °C for (5).

The Brønsted leaving-group correlations for the various rate parameters are given by equations (4)—(6) where $pK_{l.g.}$ is the pK_a of the conjugate acid of the appropriate leaving group, RSH.

$$\log_{10} k_B = 6.67 - 1.04\ pK_{l.g.}\ (r\ 0.996) \quad (4)$$

$$\log_{10} K = 0.16\ pK_{l.g.} - 1.61\ (r\ 0.940) \quad (5)$$

$$\log_{10} k_{max.} = 5.26 - 0.91\ pK_{l.g.}\ (r\ 0.997) \quad (6)$$

Trapping Experiments.—For trapping the (putative keten) intermediate, produced in this system, aniline was chosen as a nucleophile as it does not react directly with the mono-4-nitrophenyl ester of malonic acid.[10] The hydrolysis of *S*-4-chlorophenyl hydrogenthiomalonate in phosphate buffer was carried out in the presence of aniline as follows.

(i) *General trapping procedure and identification of product.* *S*-4-Chlorophenyl hydrogenthiomalonate (27 mg) was dissolved in 0.4M-phosphate buffer (5 ml, 80% free base) containing aniline (0.07M). The solution was incubated at 40 °C for 2 h, then extracted with CH_2Cl_2 to remove aniline and 4-chlorothiophenol. The aqueous phase was acidified with HCl (pH 1.2) and diluted with degassed water to 10 ml. The u.v. spectrum of this solution showed the absorbance ($\lambda_{max.}$ 242 nm) expected for the monoanilide. The u.v. spectrum of authentic malonic acid monoanilide in phosphate buffer also showed maximum absorbance at 242 nm [10]). Control experiments indicated that the efficiency and reproducibility of monoanilide extraction by this procedure was 84% (based on $\varepsilon_{max.}$ 11 600 at 242 nm for the monoanilide [10]).

(ii) *Effect of [buffer] on anilide formation.* *S*-4-Chlorophenyl hydrogenthiomalonate (100 µl of a solution in dioxan containing 12.9 mg ml^{-1}) was injected into phosphate buffer (5 ml, 80% free base, various concentrations) containing aniline (0.07M), and hydrolysed for 3 h at 40 °C. The concentration of

monoanilide produced was estimated (after CH_2Cl_2 extraction and acidification) and the yield of anilide was found to be independent of phosphate concentration (69.1% anilide at 0.24M-HPO_4^{2-} and 70.1% at 0.12M-HPO_4^{2-}.

(iii) *Dependence of anilide formation on* [*aniline*]. The procedure was repeated under the conditions described in (ii) but at various concentrations of aniline at constant concentration of phosphate buffer and the yield of monoanilide measured. A plot of the percentage anilide produced against aniline concentration is shown in ref. 11, along with k_{obs} for 4-chlorophenyl hydrogenthiomalonate in phosphate buffer solution at 25 °C in the presence of various concentrations of aniline. The rate constants were independent of the aniline concentration.

Comparing k_{obs} 5.62×10^{-4} s^{-1} measured for 4-chlorothiophenyl ester in phosphate buffer in the absence of $PhNH_2$ with k_{obs} 5.64×10^{-4} s^{-1} at that buffer concentration in the presence of $PhNH_2$ shows that the aniline does not catalyse the reaction under these conditions.

Discussion

Possible Mechanisms.—Morpholinolysis of malonate thiolmonoesters showed that the reaction is catalysed at low buffer concentration, whereas at higher buffer concentration this catalysis gradually disappears so that saturation kinetics were observed. Similar behaviour [15] was observed with primary, secondary, and tertiary amines, phosphate buffer, and some thiols as buffers but not with borate, carbonate, acetate, and hydroxide ions. In this latter group of buffers (borate, *etc.*), k_{obs} showed first-order dependence on buffer concentration. Kirby and Lloyd [10] reported similar behaviour for the hydrolysis of *m*- and *p*-nitrophenyl hydrogenmalonates in 4-methylmorpholine and phosphate buffers also with no indication of saturation kinetics in carbonate or hydroxide ion solution. Holmquist and Bruice [16] reported that the plots of k_{obs} *versus* the buffer concentration for catalysed hydrolysis of ethyl *o*-nitrophenyl malonate and ethyl *o*-nitrophenyl methylmalonate in morpholine, phosphate, glycine ethyl ester, and *N*-ethylmorpholine were curved, whereas they observed linear dependence for hydrolysis with carbonate and acetate buffers.

Such curvature often indicates a change in rate-determining step with buffer concentration.

One possible explanation involves an intermediate keten [equation (7)]. The first step (at low base concentration) is

$$\bar{O}_2CCH_2COSR \underset{k_{-1}[BH^+]}{\overset{k_1[B]}{\rightleftharpoons}} \bar{O}_2C\bar{C}HCOSR \qquad (7)$$

$$\bar{O}_2C\bar{C}HCOSR \xrightarrow{k_2} \text{products} \xleftarrow{\text{fast}} [\bar{O}_2CCH{=}C{=}O] + RS^-$$

general-base catalysed α-deprotonation to yield a carbanion; the second step is the decomposition of this dianion, for which a change in base concentration would have no rate effect. Steady-state analysis yields (8) or, as $[BH^+] = [B][H^+]/K_a$,

$$k_{obs} = \frac{k_1k_2[B]}{(k_2 + k_{-1}[BH^+])} \qquad (8)$$

where K_a is the acid dissociation constant of the buffer used, (9). This is of the same form as equation (2) with $k_{max.} = k_1k_2K_a/k_{-1}[H+]$ and $K = k_2K_a/k_{-1}[H^+]$. Or, one can write $k_B = k_1 = k_{max.}/K$ and equations (10) and (11).

$$k_{obs} = \frac{\frac{k_1k_2}{k_{-1}} \cdot \frac{K_a}{[H^+]}[B]}{\left(\frac{k_2}{k_{-1}} \cdot \frac{K_a}{[H^+]} + [B]\right)} \qquad (9)$$

$$k_{max.} = \frac{k_1k_2}{k_{-1}} \cdot \frac{[B]}{[BH^+]} \qquad (10)$$

$$K = \frac{k_2}{k_{-1}} \cdot \frac{[B]}{[BH^+]} \qquad (11)$$

Equation (12), with a non-obligatory complex, also yields a kinetic expression of the correct algebraic form for the observations with $k_{obs} = kK_b[B]/(K_B + [B])$.

$$^-O_2CCH_2COSR^1 + R^2NH_2 \overset{K_b}{\rightleftharpoons} {}^-O_2CCH_2COSR^1 \cdot R_2NH_2$$
$$\downarrow k \qquad (12)$$
products

However, in the same aniline concentration region, the percentage of product derived from aniline (monoanilide) is dependent on the concentration of aniline,[11] while there is no rate effect by added aniline, *i.e.* rate- and product-determining steps differ. The mechanism of equation (12) is excluded.

Details of the Keten Mechanism.—Considering leaving-group dependences for the simple *E*1cB mechanism of equation (7), we find from equation (11) that where $\beta_K^{l.g.}$, $\beta_{k_2}^{l.g.}$, and $\beta_{k_{-1}}^{l.g.}$ are the slopes of plots of $\log_{10}$ (rate parameter) *versus*

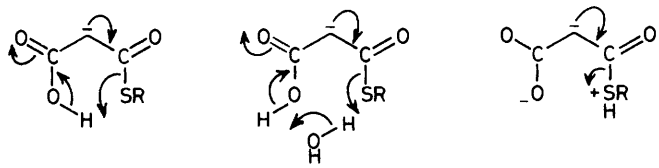
$$\beta_K^{l.g.} = \beta_{k_2}^{l.g.} - \beta_{k_{-1}}^{l.g.} \qquad (13)$$

the pK_a of the conjugate acid of appropriate leaving group (RSH), then equation (13) holds. If, as seems likely, k_{-1} (for reprotonation by protonated amine) is close to or at diffusion control then it should exhibit a low or zero leaving-group dependence and $\beta_K^{l.g.} \sim \beta_{k_2}^{l.g.}$. In this case $\beta_{k_2}^{l.g.}$ would be +0.16, a value obviously not in line with a simple *E*1 transition state for k_2. For example, the *E*1 process for analogous thiolacetoacetates [2] has $\beta_{k_2}^{l.g.}$ −1.1. Alternatively, if we assume, by analogy with the thiolacetoacetates,[2] that $\beta_{k_2}^{l.g.}$ −1.1 for the thiolmalonates, then the calculated value of $\beta_{k_{-1}}^{l.g.}$ is −1.26, which is not in agreement with k_{-1} referring to a diffusion-controlled process. Either interpretation seems highly unreasonable. A referee points out that even if morpholine cation is donating its proton slowly to the delocalised anion in the k_{-1} step (≪diffusion), $\beta_{k_{-1}}$ −1.26 seems large.

An alternative possibility involves the dianionic intermediate ($^-O_2C\bar{C}HCOSR$) reprotonating at the $^-CO_2^-$ site [to give (6) or (7) as the eliminative steps] or at the leaving group (8). All would decrease the rate dependence on leaving group basicity. Analogous processes have been proposed in the decompositions of phosphate monoanions,[17] ($\beta_{l.g.}$ −0.27), benzoylphosphate monoanions [18] ($\rho_{l.g.}$ 0.2), carbamyl phosphate monoanions [19] ($\rho_{l.g.}$ 0.24) and, under acid catalysis, sulphate monoesters [20] ($\beta_{l.g.}$ +0.3).*

* A referee notes that a possible alternative explanation of a low $\beta_{k_2}^{l.g.}$ depends on the relative stabilities of carbanion and keten. These will converge, and might even cross over, as electron withdrawal is decreased (on going for example from the acetoacetate to the malonate). Consequently the position of the transition state for C–S cleavage could shift considerably, thus accounting for a substantial fall in the sensitivity of k_2 to the leaving group.

This suggests that the observed β_K of 0.16 could represent the difference between the two moderate values for the k_2 and k_{-1} steps. Proton transfer to carbon at one end of an ambident system is intrinsically slow, as evidenced by many β values well below unity for general base catalysis of enolisation (including β 0.59 for this system), suggesting that the structure of the carbanion does indeed influence the rate of ketonisation significantly.



(6) (7) (8)

Route (8) is readily removed as a possibility as the pK_a of the α-CH_2 site is >14 (our estimate [15] being of the order of 19) and the pK_a of the $^{-}CO—\overset{+}{S}\begin{matrix}R\\H\end{matrix}$ site must be very low, no greater than −1 at most. Thus at intermediate pH (*ca.* 9—10), the fraction of ester present as species (8) will be miniscule ($<10^{-7}$) and the observed value of K along with a value [21] of 1.4×10^{11} l mol^{-1} s^{-1} for a diffusion-controlled k_{-1} step would lead to an elimination rate constant for process (8) of $>10^{16}$ s^{-1}, much greater than the, so-called, ' vibration limit ', approximately set by the vibration frequency of the chemical bonds in (8). Concentrations of CO_2^{-}-protonated forms (6) and (7) must also be small so that the actual elimination whether *via* (6), or the water-mediated analogue (7), must be very rapid.

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