Malonylcoenzyme A Models. Part 2.[1] The Methylene Deprotonation Step of the *E*1cB Acyl Transfer of Malonic Acid Thiolmonoesters

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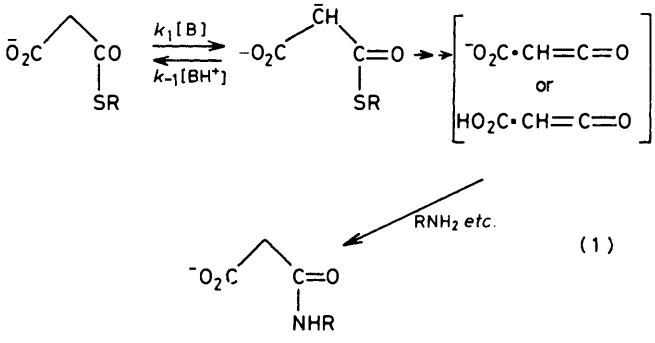
The deprotonation of the α-methylene site in *S*-aryl hydrogenthiomalonates (HO_2CCH_2COSR) was general-base catalysed. Nitrogen bases fit a single Bronsted correlation for this deprotonation step with slope 0.59 for primary, secondary, and tertiary amines. In general, amines followed saturation kinetics, but hydroxylamine followed the rate law $k_{obs} = k_1[NH_2OH] + k_2[NH_2OH]^2$; nevertheless, the k_1 parameter fitted the Bronsted correlation for nitrogen bases. Oxygen-based buffers fitted an independent Bronsted correlation with β 0.42 and were slightly less reactive than nitrogen bases for a given catalyst pK_a. Saturation kinetics were observed for thiolysis of *S*-4-chlorophenyl hydrogenthiomalonate, implying a ketenoid (*E*1cB) pathway for malonyl transfer. The primary isotope effect (H/D) for *N*-methylmorpholine catalysed deprotonation (dedeuteriation) of $HO_2C.CH_2COSPh$ and $DO_2C.CD_2COSPh$ was *ca.* 3.6. The second-order rate constant for ethylamine attack on *S*-4-chlorophenyl hydrogen-(2,2-dimethyl)-thiomalonate was 600-fold smaller than that estimated at lower buffer concentrations for *S*-4-chlorophenyl hydrogenthiomalonate, in accord with a general-base catalysed deprotonation mechanism for the latter. The 2,2-dimethylmalonate ester showed a marked rate of attack of water on the monoanion probably reflecting intramolecular general-base catalysis by the $-CO_2^-$ group. Second-order rate constants for hydroxide ion attack on *S*-phenyl and *S*-4-chlorophenyl hydrogenthiomalonates were measured.

S-Monoesters of malonic acid, including *S*-malonylcoenzyme A, follow an *E*1cB acyl transfer mechanism [*e.g.* equation (1)] for amine acceptors (see Part 1 [1]). A similar mechanism operates for their oxy-analogues.[2] For such derivatives, saturation kinetics obeying equation (2) were observed in morpholine buffers. For the keten mechanism, operating according to equation (2) to give rise to such a rate law, it was

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

shown that the rate-determining step changes with buffer concentration from a complex elimination process at higher values of [B], to rate-determining ester deprotonation at lower buffer concentration {*i.e.* when $[B] \ll K$, equation (2) reduces to $k_{obs} = k_B[B]$ with $k_B = k_{max.}/K$}. The preceeding paper [1] investigated the elimination aspects of this system. This article probes the details of the proton transfer from the α-methylene group of malonate esters to a variety of bases. There is considerable biochemical interest in the mechanisms of malonyl transfers involving acyl carrier protein and *S*-malonylcoenzyme A and the present results shed some light on the nature of the deprotonation step for such reactions.



(1)

Experimental

Malonate thiolmonoesters were as described in Part 1,[1] which also gives details of the kinetic procedures used, *etc.*

The pK_a values of buffers were measured by the method of half-neutralisation. All pH measurements were made with a Philips PW 9409 digital pH meter, thermostatted at the appropriate temperature, ±0.1 °C, by means of circulating water.

Hydroxylamine hydrochloride (Aldrich Chemical Co.) was recrystallised from 95% ethanol and dried *in vacuo*. Amines were distilled before use as buffers or recrystallised as their hydrochlorides as appropriate.

Results

(i) *Amine Buffers.*—Aminolyses of *S*-phenyl hydrogenthiomalonate in a series of amine buffers were studied (at 262 nm) and at 90% free base, 25 °C, ionic strength 1.0M, and 10% acetonitrile (v/v) in the presence of 10^{-4}M-EDTA in degassed media to minimise oxidative interference. As reported for morpholine, all amines (except for hydroxylamine) studied showed saturation dependences of k_{obs} (the observed pseudo-first-order rate constant) on the concentration of the free base form of the buffer. For such 'saturation' buffers data were analysed in terms of equation (1), by linearisation to extract $k_{max.}$, K, and k_B ($= k_{max.}/K$), values of which are collected in Table 1, along with buffer pK_a values measured under identical conditions. A plot of $\log_{10}k_B$ *versus* the pK_a of the buffer is shown in Figure 1. Within experimental error a single correlation is adequate to fit data for primary, secondary, and tertiary amines. Equation (3) describes this Brønsted plot for nitrogen bases, including a point (see below) for hydroxylamine, where $\log_{10}k_B^{(N)}$ refers to the apparent second-order rate constant for nitrogen-buffers (*i.e.* $k_B = k_{max.}/K$) and $pK_a^{N\text{-base}}$ is the pK_a of the appropriate amine.

$$\log_{10}k_B^{(N)} = 0.59pK_a^{N\text{-base}} - 5.15 \ (r\ 0.984) \quad (3)$$

(ii) *Hydroxylamine.*—Rate coefficients for hydroxylaminolysis of *S*-phenyl hydrogenthiomalonate showed a complex rate law [equation (4)], quite distinct from the saturation behaviour of other nitrogen bases. In agreement with this rate

Table 1. Kinetic constants ($k_{max.}$, k_B, K) and pK_a values for hydrolysis of *S*-phenyl hydrogenthiomalonate at 25 °C, 90% free base, 10% v/v acetonitrile, [EDTA] 10^{-4}M, and ionic strength 1.0M (held with KCl)

No.	Buffer	pK_a [a]	$k_{max.}$/s^{-1}	k_B/l mol^{-1} s^{-1}	K/mol l^{-1}
1	Morpholine	8.32 [b]	0.21	0.606	0.348
2	Piperidine	11.24	12.38	28.72	0.43
3	Benzylamine	9.22	0.513	3.13	0.164
4	n-Butylamine	10.55	3.39	9.09	0.373
5	Methylamine	10.72	11.1	13.6	0.82
6	Triethylamine	10.86	3.89	24.92	0.156
7	*N*-Methylmorpholine	7.7 [c]	3.83×10^{-3}	0.21	0.018
8	Ethanolamine	9.71 [d]	0.461	2.57	0.179
9	Ethyl glycinate	7.65 [b]	0.125	0.098	1.27
10	Phosphate dianion	6.34 [b]	5.01×10^{-4}	0.29×10^{-3}	0.054
11	Acetate	4.55 [e]	no saturation k_B 4.79×10^{-4} l mol^{-1} s^{-1}		
12	Borate	9.2 [e]	no saturation k_B 0.178 l mol^{-1} s^{-1}		
13	Carbonate	10.33 [f]	no saturation k_B 0.092 l mol^{-1} s^{-1}		
14	Hydroxide	15.6 [b]	no saturation k_B 43.06 l mol^{-1} s^{-1}		
15	L-Cysteine [g]	8.42 [h]	0.961	12.32	0.078
16	*N*-Acetyl-L-cysteine [g]	9.52 [i]	0.699	45.9	0.015
17	Hydroxylamine	5.97 [b]	k_1 0.38 l mol^{-1} s^{-1}	k_2 0.72 l^2 mol^{-2} s^{-2}	

[a] Determined by the method of half-neutralisation (25 °C, μ 1.0M); in this study unless otherwise stated. [b] Values of pK_a taken from T. Deacon, A. Steltner, and A. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1975, 1778. [c] From L. R. Fedor, *J. Am. Chem. Soc.*, 1967, **89**, 4479. [d] From A. Williams and W. P. Jencks, *J. Chem. Soc., Perkin Trans. 2*, 1974, 1753. [e] From A. Williams and W. P. Jencks, *J. Chem. Soc., Perkin Trans. 2*, 1974, 1760. [f] From B. Holmquist and T. C. Bruice, *J. Am. Chem. Soc.*, 1969, **91**, 2993. [g] For hydrolysis of *S*-4-chlorophenyl hydrogenthiomalonate under the same conditions. [h] From D. M. E. Reuden and T. C. Bruice, *J. Am. Chem. Soc.*, 1976, **98**, 114. [i] From M. Friedman, J. F. Cavins, and J. S. Wall, *J. Am. Chem. Soc.*, 1965, **87**, 3672.

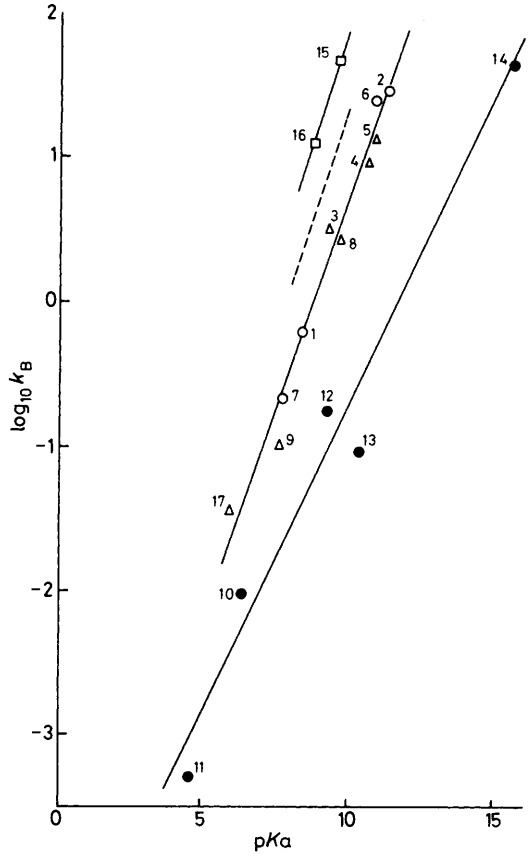


Figure 1. Brønsted plot of $\log_{10}k_B$ *versus* pK_a (base) for *S*-phenyl hydrogenthiomalonate in buffers (for key, see Table 1). Points experimental, lines are theoretical by least-squares linear regression analysis of the data in Table 1 according to the Brønsted equations given in the text

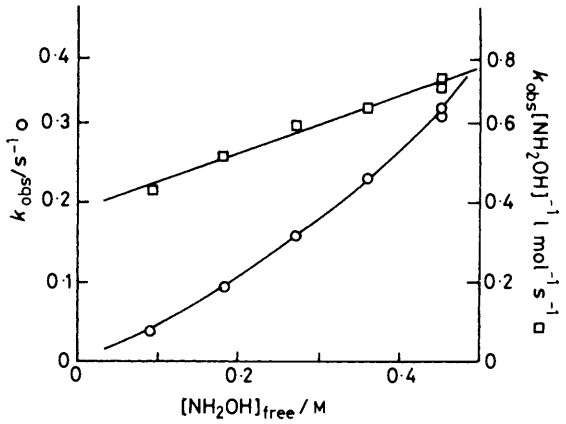


Figure 2. Hydroxylaminolysis of *S*-phenyl hydrogenthiomalonate at μ 0.1M and 25 °C. Points are experimental: lines are theoretical for the rate law given by equation (4) with k_1 0.38 l mol^{-1} s^{-1} and k_2 0.72 l^2 mol^{-2} s^{-1}

law a plot of $k_{obs}/[NH_2OH]$ *versus* $[NH_2OH]$ was linear (see

$$k_{obs} = k_1[NH_2OH] + k_2[NH_2OH]^2 \qquad (4)$$

Figure 2) and from this, values of k_1 and k_2 were calculated as 0.38 ± 0.01 l mol^{-1} s^{-1} and 0.72 ± 0.04 l^2 mol^{-2} s^{-1}, respectively. The value of $k_B^{(N)}$ used for equation (3) was taken as k_1. Omitting this from the Brønsted plot yielded only a slightly poorer correlation (r 0.983) but with a higher (=0.64) β value (β is the slope of a plot of $\log_{10}k_B$ *versus* pK_a of buffer B). In the discussion below the Brønsted equation containing the k_1 value for hydroxylamine is used. Similar rate laws with squared $[NH_2OH]$ terms have been observed for other thiolesters and it is possible that this powerful α-nucleophile has dictated a mechanistic change. However, no further analysis will yet be attempted as it has not been established whether attack by NH_2OH is from O or N.

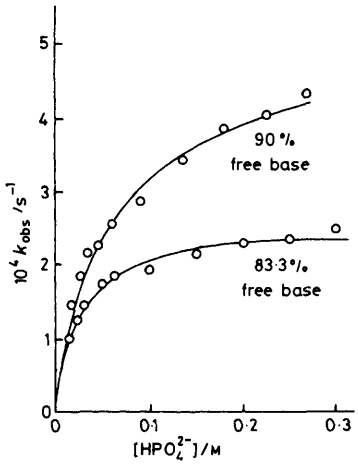


Figure 3. Saturation rate effects in phosphate buffer catalysed hydrolysis of *S*-phenyl hydrogenthiomalonate at 25 °C and ionic strength 0.1 M (held with KCl) in the presence of 10% v/v acetonitrile and 10^{-4}M-EDTA. Points are experimental; lines are calculated from equation (2) using $k_{max.}$ 5.01 × 10^{-4} s^{-1}, K 0.054 mol l^{-1} (90% free base) and $k_{max.}$ 2.58 × 10^{-4} s^{-1}, K 0.024 mol l^{-1} (83.3% free base)

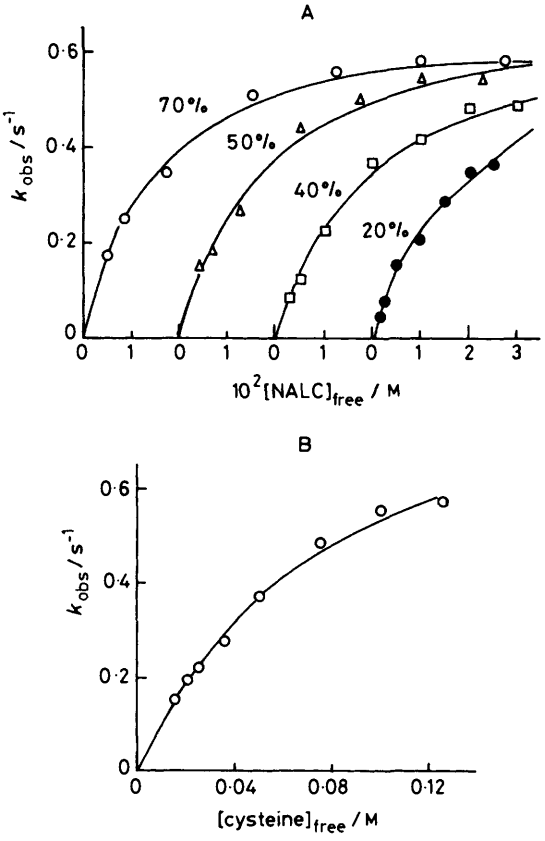


Figure 4. A. Plots of k_{obs} *versus* *N*-acetyl-L-cysteine (NALC) concentrations for *S*-4-chlorophenyl hydrogenthiomalonate at different percentages of free base. Note that the *x*-axis is staggered to accommodate the data. Points are experimental; lines are theoretical using equation (2) with data from Table 2. B. Plots of k_{obs} *versus* [cysteine]$_{free}$ (*i.e.* as RS^- form) for *S*-4-chlorophenyl hydrogenthiomalonate in L-cysteine buffers. Points are experimental; line is theoretical using equation (2) with $k_{max.}$ 0.961 s^{-1} and K 0.078 mol l^{-1}

Table 2. Rate parameters for thiolysis of *S*-4-chlorophenyl hydrogenthiomalonate in *N*-acetyl-L-cysteine buffers with varying percentages of free base at 25 °C, ionic strength 1.0M (held with KCl), and 5% acetonitrile (v/v) measured at 282 nm in the presence of 10^{-4}M-EDTA

Free base fraction ([RS^-]/[RSH]$_{total}$)	pH	$k_{max.}$/s^{-1}	K/mol l^{-1}	k_B/l mol^{-1} s^{-1}
0.70	10.04	0.699	0.015	45.9
0.50	9.70	0.714	0.018	39.7
0.40	9.50	0.705	0.020	35.3
0.20	8.95	0.620	0.017	36.4

(iii) *Oxygen Buffers.*—Hydrolysis of *S*-phenyl hydrogenthiomalonate in phosphate buffer (at different percentages of free base form) showed saturation at higher concentrations of HPO_4^{2-} (see Figure 3). A plot of [HPO_4^{2-}]/k_{obs} *versus* [HPO_4^{2-}] was linear, in agreement with equation (1) as rate law.

Hydrolysis of this ester in acetate buffer showed no indication of saturation kinetics up to 0.9M free base form. Consequently, k_B for acetate ion was taken as the slope of the first-order plot of k_{obs} *versus* acetate ion. Similarly, no saturation phenomena were detected for borate or carbonate buffers. Values of k_B are recorded in Table 1. The Brønsted equation was $\log_{10}k_B = 0.42pK^{o\text{-base}} - 4.76$ (r 0.981).

(iv) *Thiol Buffers.*—Hydrolysis of *S*-4-chlorophenyl hydrogenthiomalonate in *N*-acetyl-L-cysteine buffers, at various free base (*i.e.* RS^-) fractions, showed saturation (see Figure 4). Values of k_B, K, and $k_{max.}$ for *N*-acetyl-L-cysteine at different fractions of free base form of the thiol buffer (*i.e.* varying [RS^-]/[RSH]) are collected in Table 2. Similarly, a curved concentration dependence on [RS^-] was observed for L-cysteine as buffer with this ester. Values of k_B, $k_{max.}$, and K for L-cysteine {276 nm, μ 1.0M, 25 °C, 50% free base, 5% acetonitrile (v/v), [EDTA] 4 × 10^{-4}M} were 12.3 l mol^{-1} s^{-1}, 0.961 s^{-1}, and 0.078 mol l^{-1}, respectively.

The sulphur bases appear to follow an independent Brønsted correlation (see Figure 1), but, as only two points are available, conclusions and an estimated β^S +0.5 must be tentative. In addition, the sulphur–buffer data are for the 4-chlorophenyl ester as opposed to the phenyl ester for the O- and N-bases. (The reason for the change of ester was that the 4-chloro-ester was less prone to oxidation artefacts than the unsubstituted derivative.) The effect of the ester change is likely to be fairly small. Indeed, as $\beta_{l.g.}$ for the k_B term with morpholine as base is −1.04 (see Part 1 [1]) and $\Delta pK_{l.g.}$ (the difference in pK_a of the appropriate RSH species) is 0.48 for 4-ClC_6H_4SH and C_6H_5SH, one can estimate that the S-base 'correlation' should be moved down the $\log_{10}k_B$ axis by *ca.* 0.5 units (see dotted line), if $\beta_{l.g.}$ values for N- and S-bases are comparable.

Kinetic Isotope Effects.—Isotope effects on the aminolysis of the 2,2-diprotio and 2,2-dideuterio forms of *S*-4-chlorophenyl ester (HO_2CCH_2COSAr and DO_2CCD_2COSAr, respectively) were studied using *N*-methylmorpholine as tertiary amine buffer (90% free base form) at 25 °C, μ 1.0M, and 15% acetonitrile (v/v) in the presence of 10^{-4}M-EDTA. Saturation kinetics were observed and in all cases good pseudo-first-order progress curves were obtained (see Table 3). Comparing the thioprotio-ester studied in H_2O and the thiodeuterio-ester studied in D_2O, the kinetic isotope effects were

Table 3. Hydrolysis of $HOOCCH_2COSC_6H_4Cl$-4 and $DOOCCD_2COSC_6H_4Cl$-4 in 4-methylmorpholine buffers at 25 °C, 90% free base, 15% acetonitrile, [EDTA] 1×10^{-4}M, and ionic strength 1.0M (held with KCl) at λ_{kln} 274 nm

[4-Methylmorpholine]$_{free}$/M	$HOOCCH_2CO{\cdot}SC_6H_4Cl$-4 in H_2O $10^2k_{obs}/s^{-1}$	$HOOCCH_2CO{\cdot}SC_6H_4Cl$-4 in D_2O $10^2k_{obs}/s^{-1}$	$DOOCCD_2CO{\cdot}SC_6H_4Cl$-4 in H_2O $10^2k_{obs}/s^{-1}$	$DOOCCD_2CO{\cdot}SC_6H_4Cl$-4 in D_2O $10^2k_{obs}/s^{-1}$
0.36	1.42	1.25	1.43	1.11
0.30	1.41	1.26	1.42	1.08
0.24	1.40	1.21	1.40	1.09
0.18	1.40	1.12	1.33	1.03
0.12	1.30	1.04	1.29	0.93
0.06	1.19	0.82	1.10	0.76
0.03	1.01		0.89	0.59
0.018	0.89	0.60	0.76	0.42
0.009	0.67	0.44	0.54	0.27

$k_{max.}$ (H/D) 1.23, K(H/D) 0.38, and k_B(H/D) 3.25. These are lower limits of the rate ratios as only 90% isotopic purity was achieved for the thiodeuterio-ester. Hence, the isotope effect on k_B is probably of the order of 3.25/0.9 = 3.6 or greater.

S-4-*Chlorophenyl Hydrogen*-(2,2-*dimethyl*)*thiomalonate*.—The dependence of k_{obs} on [ethylamine] was first order in ethylamine buffer at 25 °C, μ 1.0M, 20% acetonitrile (v/v), and 90% free base, for this ester with slope ($=k_{EtNH_2}$) 9.62×10^{-2} l mol^{-1} s^{-1} and intercept (at [$EtNH_2$] 0) of k_{spon} 1.87×10^{-2} s^{-1}. The second-order rate constant for hydroxide ion attack on this ester (k_{HO^-}) was measured as 3.6×10^{-3} l mol^{-1} s^{-1} {25 °C, μ = 1.0M, 10% acetonitrile (v/v), [EDTA] 10^{-4}M}. Using k_{HO^-}, the Henderson–Hasselbach equation, and a pK_a of 10.88 for ethylamine shows that the hydroxide component under the conditions of the ethylamine experiments would be 2.4×10^{-5} s^{-1}. Consequently the observed intercept of 1.87×10^{-2} s^{-1} probably reflects water attack on the ester monoanion.*

Using equation (3) and $\beta_{l.g.}$ −1.04 for the k_B term (morpholine base) yields a value of *ca.* 60 l mol^{-1} s^{-2} as an estimate of the value of k_{EtNH_2} for *S*-4-chlorophenyl hydrogenthiomalonate. This value is *ca.* 600-fold greater than that observed (0.096 l mol^{-1} s^{-1}) for the 2,2-dimethyl ester.

Hydroxide Ion Attack on Aryl Hydrogenthiomalonates.—Both *S*-phenyl and *S*-4-chlorophenyl hydrogenthiomalonate followed first-order rate dependence on hydroxide ion concentration with k_{HO^-} (the second-order rate coefficients for hydroxide ion attack) values being 43.1 and 72.0 l mol^{-1} s^{-1} at 25 °C, μ 1.0M, 10% acetonitrile (v/v).

Discussion

The curved buffer dependences observed for hydrogenthiomalonates have been explained [1] in terms of an *E*lcB-variant mechanism [equation (1)]. Consequently, the k_B values should refer to general-base catalysed thiolester monoanion deprotonation.

The k_B term depends on the concentration of the free base form of morpholine for both the *S*-phenyl and *S*-*p*-cresyl thiomalonate half-esters, with the contribution from the BH^+ component of the buffer effectively essentially indistinguishable from zero (see Part 1 [1]). This is in accord with a general-base catalysed deprotonation of the α-carbon site of the ester being the rate-limiting step for morpholinolysis at low buffer concentrations. Further support for this view is provided below.

In Table 4 are compared some rate constants for aminolysis of malonate esters and analogues. The difference (more than two orders of magnitude) between the thiomalonate and thioacetate esters in Table 4 argues in favour of different aminolysis mechanisms. In addition the dependence of rate on [morpholine] whilst showing saturation for the former, is linear for the latter, which is known to aminolyse by attack at the ester carbonyl carbon atom.[3] The ethyl *o*-nitrophenyl malonate esters listed are both believed to aminolyse *via* *E*lcB pathways [4] and k_B for these reflects the deprotonation process. The difference between the thiomalonate half-ester and ethyl *o*-nitrophenyl malonate could lie in the difference in activation of the bridging methylene site to deprotonation; the pK_a of the diester [5] is *ca.* 9.4, whilst that for the thiomalonate is >14. As, if seems reasonable, the reprotonation of the carbanion by protonated amine is diffusion-controlled [6] for both esters, then the rates of carbanion formation would reflect such pK_a differences. The effect of steric hindrance on the deprotonation of the α-methylene site of malonates appears to be large, *ca.* 10^2-fold for a methyl group, considering the data in Table 4.

Table 4. Rates of morpholinolysis of esters and thiolesters

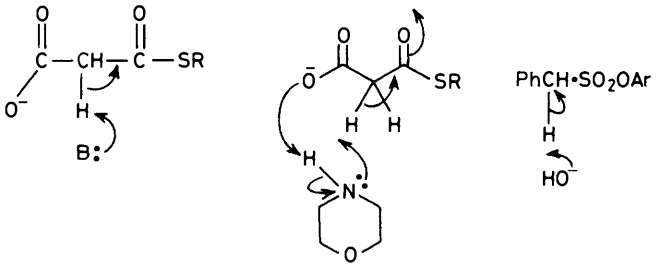
Ester	k_B/l mol^{-1} s^{-1}
$^-O_2C{\cdot}CH_2COSC_6H_4Cl$-4 [a]	2.76
$CH_3CO{\cdot}SCH_2CF_3$ [b]	0.0953
$EtOCOCH_2COOoNp$ [c]	900
$EtOCOCH(CH_3)OoNp$ [c]	8

[a] At 25 °C; this work. [b] At 30 °C; from ref. 3. [c] At 30 °C; OoNp = 2-nitrophenolate, data from ref. 4.

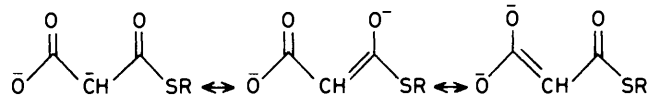
Kinetic solvent deuterium isotope effects are also in accord with k_B reflecting some form of rate-determining proton transfer. For *N*-methylmorpholine with *S*-4-chlorophenyl hydrogenthiomalonate k_B (H/D) is *ca.* 3.6, after correction for the isotopic impurity of the deuterio-ester analogue. For proton abstraction from carbon acids a maximum value of k_H/k_D of *ca.* 10 is reported.[7] The observed value of k_H/k_D appears to depend on a variety of factors for carbon acids. One correlation shows an approximate bell-shaped relationship between $\log_{10}(k_H/k_D)$ for a carbon acid and the difference in pK between the carbon acid and the appropriate base. From this we can estimate here a pK difference of *ca.* 11, indicating that the pK of the malonate could be of the order of 19. Our kinetic data indicate that the pK_a for this ester is certainly >14.

The activation parameters of $\Delta H^{\ddagger}$ 66.9 kJ mol^{-1} and $\Delta S^{\ddagger}$ −75 J K^{-1} mol^{-1} for the morpholinolysis of the *S*-benzyl ester can be compared with data for the amine-catalysed deprotonation of nitroethane [6a] for which $\Delta S^{\ddagger}$ −27.6 J K^{-1} mol^{-1} for

* Although only one point is available for the rate of attack of H_2O on $HO_2CC(CH_3)_2COSC_6H_4Cl$-*p* comparison of k_{H_2O} (1.87×10^{-2} s^{-1}) with k_{HO^-} (3.6×10^{-3} l mol^{-1} s^{-1}) for this ester shows that either the water rate is abnormally high or k_{HO^-} abnormally low.



(1a) (1b) (2)



(3) (4) (5)

dimethylamine and -74.8 J K^{-1} mol^{-1} for ammonia. It is difficult to use such data constructively in view of the high dependence of $\Delta S^{\ddagger}$ on the substitution pattern of the amine for nitroethane: the charged nature of the malonate substrate would further complicate the analogy.

The value of k_{EtNH_2} estimated for *S*-4-chlorophenyl hydrogenthiomalonate is 600-fold greater than that observed for *S*-4-chlorophenyl hydrogen-(2,2-dimethyl)thiomalonate, again in agreement with proton abstraction from carbon for the former.

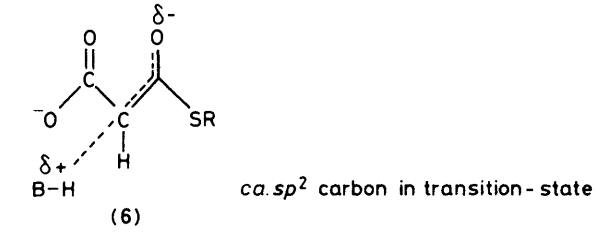
Figure 1 shows that k_B values for phenyl hydrogenthiomalonate follow independent, but almost parallel, linear free energy relationships for oxygen and nitrogen buffers, with β values of 0.42 and 0.59, respectively. Although the data are limited, sulphur buffers seem to follow a parallel, independent Brønsted plot for the *S*-4-chlorophenyl ester, with β^{S} 0.5. Such β values are comparable with those reported in the literature for base-catalysed ionisations of carbon acids, *e.g.* β 0.6 for 2-ethoxycarbonylcyclopentanone,[8] 0.52 for acetoacetate ion,[9] 0.6 for 2-acetylcyclohexanone,[8] 0.65 for ethyl nitroacetate,[8] and 0.88 for acetone.[8] In addition, the fit of primary, secondary, and tertiary amines to the same Brønsted line is in agreement with general-base catalysed proton abstraction from the thiomalonate (1a).

A contribution (1b) of the ionised carboxy-group to amine-catalysed deprotonation can be ruled out as the tertiary amines fit the same Brønsted (β_{Nuc}) correlation as do the primary and secondary amines. The leaving group dependence for the morpholinolysis of thiomalonate half-esters obeys the linear free energy relationship (5).[1] This value of -1.04 for $\beta_{l.g.}$ is large for a deprotonation process but little comparati

$$\log_{10}k_B = 6.67 - 1.04\ pK_{l.g.}\ (r\ 0.996) \qquad (5)$$

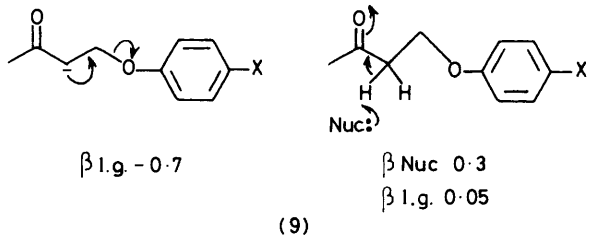
data is available in the literature. However, $\beta_{l.g.}$ for deprotonation by hydroxide of the α-methylene group of aryl phenylmethanesulphonates [10] is -0.4. For this system, the carbanion is largely stabilised by inductive effects (2) whereas extensive π-delocalisation stabilisation of the carbanion from the thiomalonates is likely, *viz.* (3)—(5).

There is an apparent conflict between the β and $\beta_{l.g.}$ values for aminolysis of thiomalonate half-esters. The value of 0.6 for β_{Nuc} indicates partial proton transfer in the transition-state with only an intermediate charge development on the malonate skeleton (charge quantitation is impossible without knowledge of the ester pK_a values as functions of base and leaving group structures). However, the highly negative (-1.04) value of



(6) *ca.* sp^2 carbon in transition-state

(7) (8)



β l.g. −0·7 β Nuc 0·3 β l.g. 0·05

(9)

$\beta_{l.g.}$ for this system implies that a large degree of negative charge development has occurred on the malonate skeleton and that this is localised largely on the ester carbonyl function [*i.e.* that a species analogous to (4) makes a major contribution to transition-state stabilisation]. This is reasonable in comparison to the hybrid (5) with two negative charges on one carboxy-group. However, such extensive charge delocalisation on the ester carbonyl seems to imply almost complete proton transfer to the base in the transition-state, *viz.* (6).

It is difficult to find appropriate models for $\beta_{l.g.}$ but for the *E*1 collapse of thioacetoacetate [11] anions $\beta_{l.g.}$ -1.13 and for the rate-determining breakdown of thiolester tetracoordinate intermediates $\beta_{l.g.}$ has been estimated [12] as -1.1. In these cases, the high leaving group dependence is easily explicable as the bonding changes in the reactions involved are largely those of the bond restraining the departing group, *i.e.* (7) and (8). This type of rationale is not, of course, available for the deprotonation step of the thiomalonates.

For 4-(*p*-X-phenoxy)butan-2-ones,[13] $\beta_{l.g.}$ for the decomposition of the substrate conjugate base is -0.7 (calculated from data in ref. 13) and β for proton abstraction is 0.3, a considerably lower value [see (9)]. The value of $\beta_{l.g.}$ for the 2-dimethylaminoethanol-catalysed deprotonation of these derivatives calculated from data in ref. 13 is very small (*ca.* 0.05).

Thus for these butanones the aryloxy substituent has a very small effect on the deprotonation rate and β is also quite small. For the malonates the effect of thiol substituent on the α-methylene deprotonation is extremely marked ($\beta_{l.g.}$ -1.04) and β is quite high (*ca.* 0.6). Comparing butanones and malonates the explanation of these differences presumably lies in two features. First, for the former the electronic substitutions (ArO) are separated from the deprotonation site by a CH_2 group whereas for the latter the separation is by a C=O group, which presumably ' transmits ' electronic effects more efficiently [some support for this lies in the $\beta_{l.g.}$ values for the elimination steps of the butanones ($\beta_{l.g.}$ -0.7) and of aryl acetoacetates [5] ($\beta_{l.g.}$ *ca.* -1.1)]. Secondly, for the esters

resonance stabilisation of the enolate-forming transition-state is presumably into the ester carbonyl bond linking the deprotonation site with the RS substitution sites, whereas in the butanones delocalisation *must* be to a distal C=O group. The occurrence of this effect is presumably dictated by the presence of a full negative charge already resident on the C=O distal to the RS functions.

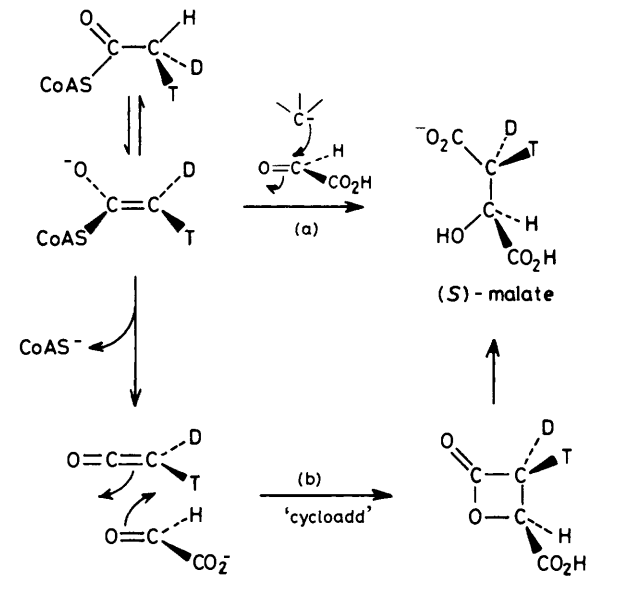
For the base-catalysed deprotonation step involved in the isomerisation of t-butyl thiobut-3-enoate [14] (CH_2=$CHCH_2$-COSBu^t) *calculated* deprotonation rate constants have β +0.52 but the value for triethylamine catalysis (2.43 l mol^{-1} s^{-1}) was less than the value predicted from Brønsted correlation, suggesting steric hindrance to proton transfer. The rates of triethylamine-catalysed deprotonation of 4-phenoxybutan-2-one [13] and *S*-phenyl hydrogenthiomalonate (Table 1) are comparable, being 0.224 and 34.9 l mol^{-1} s^{-1}, respectively. The thiol derivatives do deprotonate more rapidly, however, than the oxy-compounds but these are not direct structural analogues.

Acyl Transfer to Thiols.—Our observation of saturation kinetics with the thiolyses of *S*-4-chlorophenyl hydrogenthiomalonate by *N*-acetyl-L-cysteine and cysteine (Figure 4) is most surprising in view of the findings of Sedgwick *et al.*[15] They found that the product of thiolysis of 4-nitrophenyl malonate, chiral at the methylene site because of CHD-substitution, had not lost its chirality, thus excluding a free keten pathway as the mechanism. As they pointed out, their finding does not contradict earlier work on this ester, for which acyl transfers were to weaker nucleophiles.

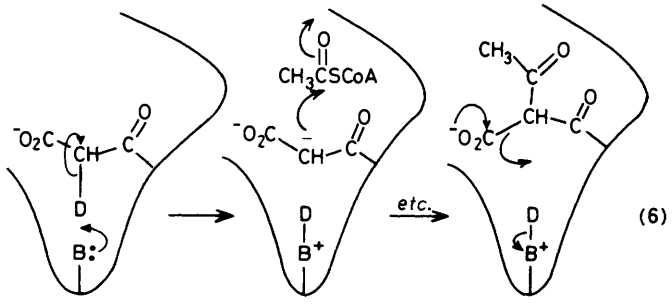
If the saturation curves which we have observed are caused by the operation of an *E*1cB pathway with thiol bases it is difficult to see why the change from a malonate thiolester to a malonate oxyester leads to a mechanistic change for a fixed thiol nucleophile. With a non-aqueous medium (hexamethylphosphoramide) and *N*-caprylylcysteamine as acceptor thiol with *S*-4-nitrophenyl hydrogenthiomalonate *S*-malonyl-*N*-caprylylcysteamine was formed, but with a considerable bridging (malonate) methylene H–T exchange. However, in aqueous media (pH 9.2) with coenzyme A or *N*-acetylcysteamine as the thiol, when solid 4-nitrophenyl hydrogenmalonate was added to give product some tritium exchange did occur (especially CoASH). However, in spite of this, little or no chirality was lost in the thiolesterification process. One possibility is formation of an intimate carbanion–acceptor complex which preserves the α-methylene chirality throughout its subsequent reactions. For such a case, the rate of reprotonation of the complex to give starting materials must be slow relative to elimination, *etc.*

Biological Implications.—In enzyme-catalysed deprotonations of *S*-malonyl derivatives (*e.g.* of coenzyme A, of acyl carrier protein) we may expect charge development to be onto the ester carbonyl bond as in (4), unless some electrophilic stabilisation of the CO_2^- moiety occurred. Such electrophilic binding to CO_2^- would deactivate the molecule to decarboxylation, to keten elimination pathways, and indeed to nucleophilic attack by the methylene group and is thus unlikely. The rate of deprotonation of the methylene group is very sensitive to the pK_a of the thiol moiety and would be markedly assisted if protonation of this group occurred in an active site because of the markedly negative value of $\beta_{l.g.}$ (−1.04) for this process.

A keten pathway is a real possibility for a number of thiol-ester systems in biochemistry. For example, consider the stereochemical course of enzyme-catalysed (*S*)-malate synthesis from glyoxalate and *S*-acetylcoenzyme A which has been shown, in some very elegant experiments,[15,16] to occur



Scheme



(6)

with inversion of configuration at the acetyl methyl centre (see Scheme), an observation usually taken as implicating a planar enolate ion.[16] Such an enolate ion might well be expected, if it possessed a 'free' existence, to collapse rapidly by leaving-group expulsion (*cf.* thioacetoacetates, thiomalonates) to an enzyme bound keten [Scheme (b)] from which products would form by addition of glyoxalate, followed by hydrolytic cleavage.*

Either a bimolecular attack of enolate ion on glyoxalate [Scheme (a)] or the elimination–addition route [Scheme (b)] are viable explanations of the observed stereochemistry. The keten route has been previously considered [18],† in lipid metabolism.

It might be suggested that the work of Lynen and his co-workers [20] argues against an enzymic keten route for the condensation reaction of fatty biosyntheses, because there was no proton exchange with the solvent when the condensation reaction of malonyl–acyl carrier protein was carried out in the presence of tritiated water under the influence of β-ketoacyl–acyl carrier protein (ACP) synthetase (condensing enzyme).[20]

* Lactone intermediacy has been suggested in the action of citrate synthase.[17]

† As long ago as 1943, before coenzyme A had become recognised as such, Martius [19] had proposed that keten ($H_2\overset{\delta+}{C}=\overset{\delta-}{C}=O \longleftrightarrow H_2\overset{+}{C}-\overset{-}{C}=O$) was the nature of 'active-acetate.' The tendency of acetylcoenzyme A to undergo condensation reactions was earlier discussed in terms of the possibility of reaching, in the transition-state, a resonance-stabilised electronic configuration of keten.[17]

This was suggested [20] to exclude a carbanion route and support a concerted mechanism in which formation of the new C–C bond is coupled with cleavage of the malonyl carboxy function. However, it is possible to write a stepwise mechanism for this reaction, using a buried base, which cannot equilibrate with solvent freely [equation (6)]. In the glyoxalase I reaction, the lack of protic exchange of solvent with substrate was, for many years, interpreted in terms of a hydride transfer mechanism. Study at higher temperatures (35 °C) showed that significant exchange did indeed occur.[20] The problems of buried bases are great in biochemistry. A keten route, if operating for the early stages of fatty acid biosynthesis, is possible in the previous biosynthetic step, formation of malonyl–acyl carrier protein, under the influence of malonyl transacetylase. Again a buried base route would allow the operation of this pathway without solvent–substrate-product proton exchange.

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