An Unusual Elimination–Addition (Keten-mediated) Aminolysis Pathway for Malonic *S*-Thioesters, including *S*-Malonyl Coenzyme A

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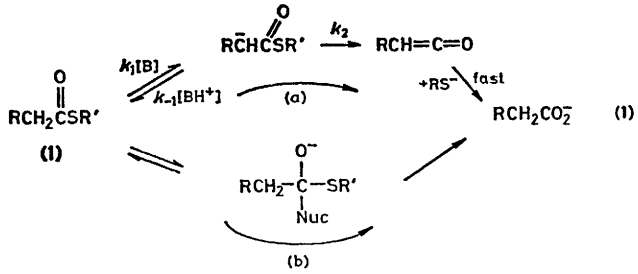
S-Monoesters of malonic acid, including *S*-malonyl coenzyme A itself, undergo aminolysis *via* a novel proton-assisted (keten-mediated) *E*1cB pathway.

Elimination–addition hydrolysis [equation (1a)] has been shown for *S*-acetoacetyl coenzyme A[1] (**1**; R = CH_3CO, R′ = CoA) but does not occur for *S*-acetyl coenzyme A[2] (**1**; R = H, R′ = CoA) which follows a bimolecular associative route [equation (1b)]. In view of the role of *S*-malonyl coenzyme A (**1**; R = CO_2^-, R′ = CoA) as a donor of 2-carbon units in *de novo* fatty acid biosynthesis and other processes,[3] we have considered the likelihood of a keten-mediated route [*e.g.* equation (1a)] for it. This is a possibility,[4] especially in view of Kirby and Lloyd's recent demonstration of a keten pathway for 4-nitrophenyl hydrogen malonate[5] whereas 4-nitrophenyl acetate follows a bimolecular mechanism.

A series of hydrogen malonate *S*-thioesters (**1**; R = HO_2C) was prepared by the method of Howard *et al.*[6] and had satisfactory elemental analyses (C, H, S). Their aminolyses were studied in aqueous solution at 1.0 M ionic strength using degassed buffers with 10^{-4} M EDTA present to minimize metal-ion catalysed oxidation problems. Reactions were followed either directly at an appropriate wavelength or, for



(1)

less reactive members, for which elevated temperatures were necessary, by titration of liberated thiol as a function of time using Ellman's reagent. Morpholinolysis was investigated in some detail and the observed pseudo-first order rate constants (k_{obs}) in morpholine buffer of constant pH (morpholine in an excess over ester) showed a saturation dependence, as reported[5] for 4-nitrophenyl hydrogen malonate. The kinetics could be described by equation (2), for the *S*-4-chlorophenyl,

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

S-phenyl, and *S*-4-methylphenyl thioesters as well as the *S*-benzyl ester and *S*-malonyl coenzyme A itself at elevated temperatures (50—60 °C). Other amines and buffers also showed such curved concentration dependencies. Trapping experiments with aniline were carried out to detect the (putative keten) intermediate, as this amine had been shown[5] not to react directly with 4-nitrophenyl hydrogen malonate. In Figure 1 is recorded the amount of monoanilide (based on t.l.c. and u.v. spectral identification/determination) formed for *S*-4-chlorophenyl hydrogen thiomalonate as a function of the concentration of $PhNH_2$ present in phosphate buffer (80% free base form). At low levels of $PhNH_2$ the hydrolysis rate is unaffected by the concentration of $PhNH_2$ although the yield of monoanilide is markedly changed in the same region. Thus, rate- and product-determining steps are not identical; the most reasonable interpretation is intermediacy of a keten such as in equation (1a), especially in view of the *O*-ester analogue precedents.[5] For route (1a), the steady-state rate equation is equation (3) or $k_{obs} = ([B]k_1k_2K_a/k_{-1}[H^+])/(k_2K_ak_{-1}[H^+] + [B])$ *i.e.* $K = k_2K_a/k_{-1}[H^+]$ and in a given

$$k_{obs} = k_1k_2[B]/(k_2 + k_{-1}[BH^+]) \quad (3)$$

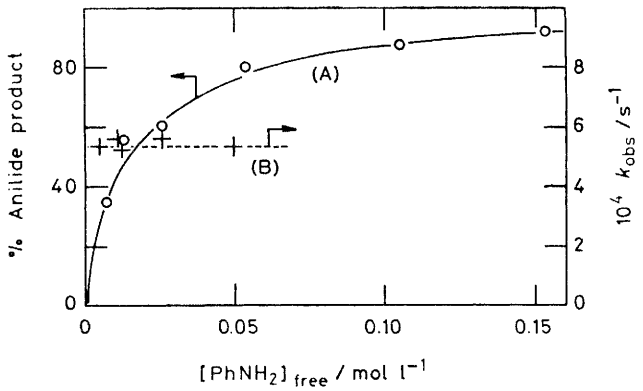


Figure 1. Percentage anilide produced (A) from *S*-4-chlorophenyl hydrogen thiomalonate as a function of increasing aniline concentration in 0.04 M phosphate buffers (pH 7.2, 25 °C); points are experimental, line is notional to assist visualisation. Also shown are rates of hydrolysis of *S*-4-chlorophenyl monothioester (B) in the presence of increasing concentrations of aniline under similar conditions to those used for product analysis: points are experimental, line is the mean of the points.

buffer at a given pH, $K_a/[H^+]$ is constant. The effect of leaving-group variation on the K term can be assessed through plots of log K *vs.* $pK_{L.G.}$ (the pK_a of conjugate acid of the appropriate leaving-group), whose slope is $\beta_{L.G.}(K)$. Now $\beta_{L.G.}(K) = \beta_{L.G.}(k_2) - \beta_{L.G.}(k_{-1})$. Analysis of the data for this sytem indicates that, as $\beta_{L.G.}(K) = +0.15$, either the reprotonation step (k_{-1}) is *extremely* sensitive to 'leaving-group' structure or the elimination step (k_2) is unusually insensitive to leaving-group variation, possibly because of some form of proton-transfer, perhaps solvent-mediated, to the nucleofuge in the transition-state, as has been suggested for phosphate, benzoylphosphate, and carbamylphosphate mono-anions and sulphate monoesters in acid.[7]

Thus, *S*-monothioesters of malonic acid, including *S*-malonyl coenzyme A itself, undergo malonyl transfers to N-species[13] *via* an elimination–addition (ketenoid) pathway but one which probably involves a degree of leaving-group protonation in the transition-state.

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