Barton and colleagues have made the interesting observation that octahedral complexes of phenanthroline, particularly of the 4,7-diphenyl derivative, demonstrate enantiomeric specificity.[41] The Δ isomer binds preferentially to B DNA; both the Λ and Δ isomer bind to Z DNA. Therefore the Λ is potentially a Z-specific probe. In the presence of light, the Λ isomer of the cobalt complex cleaves pBR 322 at a limited number of sites which may correspond to DNA regions in a left-handed Z format. Major groove binding with intercalation of at least one OP into the DNA has been proposed for the binding of the Δ isomer to DNA. Possibly, the 4,7-diphenyl-1,10-phenanthroline–cuprous complex, which exhibits anomalous reactivity, also interacts with the major groove.

Concluding Remarks

The artificial nuclease activity of $(OP)_2Cu^+$ cleaves DNA by oxidatively degrading the deoxyribose from its binding site within the minor groove of DNA. Since the coordination complex is small and structurally constrained, it detects local structural variability of this feature of the DNA helix. In experiments with the lac operon control elements, the reagent has revealed that the conformation of the promoter-conserved sequence is altered by single base changes which increase promoter strength. The coordination complex can also reveal functionally important conformational changes induced by protein binding and be used as a "footprinting" reagent to visualize ligand–DNA interactions.

Further work is proceeding in two additional directions. $(OP)_2Cu^+$ is active in the Ames mutagenesis test and therefore cleaves DNA intracellularly. Since methods are now available to analyze nucleolytic cuts indirectly without prior labeling of the substrate DNA,[42] structural features of the nucleic acids can be compared both in vivo and in vitro. For complex structures such as nucleosomes, this should prove advantageous. Targeting the reaction to specific nucleic acid sequences, viruses,[43] or specific cell types might have useful practical implications.

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(41) (a) Kumar, C. V.; Barton, J. K.; Turro, N. J. *J. Am. Chem. Soc.* **1985**, *107*, 5518–5523. (b) Barton, J. K.; Raphael, A. L. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 6460–6464, and references cited therein.

(42) (a) Gralla, J. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 3078–3081. (b) Jackson, P. D.; Felsenfeld, G. *Ibid.* **1985**, *82*, 2296–2300. (c) Ephrussi, A.; Church. G. M.; Tonegawa, S.; Gilbert, W. *Science* **1985**, *227*, 134–140.

(43) Lembach, K. J.; Meider, P. J.; Smith-McCollum, R. M. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **1985**, *44*, 1072.

Elimination–Addition Pathways for Thiol Esters

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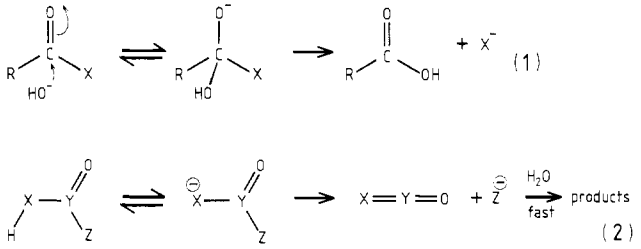
A central feature of the acyl-transfer mechanism is the common, associative route involving a tetracoordinate intermediate, eq 1[1,2]. During the late sixties and the seventies, awareness increased of a poor relation, the elimination–addition pathway for acyl transfer at carbon, phosphorus-, and sulfur-based acyl sites,[3,4] eq 2.



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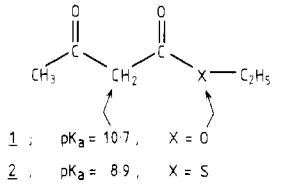
Equally established by that time was the acidity at the α-carbon of thiol esters, a feature used to rationalize much of their behavior in organic synthesis, as well as in biochemical mechanisms.[5] The acidifying effect of a thioalkyl substituent is substantial. Compare the pK_a of the bridging methylene group in ethyl acetoacetate, 1, with that in *S*-ethyl thioacetoacetate 2. For an



equilibrium, such as acid dissociation, $\Delta G°_{eq} = -RT \ln K_a$. Thus, the difference in free energy ($\Delta\Delta G°_{eq}$) is given by $\Delta\Delta G° = 2.303\,RT\,(\Delta pK_a)$, where ΔpK_a is the difference in pK_a values of the two species. For the

(1) Bender, M. L. *Chem. Rev.* **1960**, *60*, 53.
(2) Johnston, S. L. *Adv. Phys. Org. Chem.* **1967**, *5*, 237.
(3) Douglas, K. T. *Prog. Bioorg. Chem.* **1976**, *4*, 193.
(4) Williams, A.; Douglas, K. T. *Chem. Rev.* **1975**, *75*, 627.
(5) Bruice, T. C.; Benkovic, S. J. *Bioorganic Mechanisms*; Benjamin: New York, 1966; Vol. I, pp 259.
(6) Douglas, K. T.; Yaggi, N. F. *J. Chem. Soc., Perkin Trans. 2*, **1980**, 1037.

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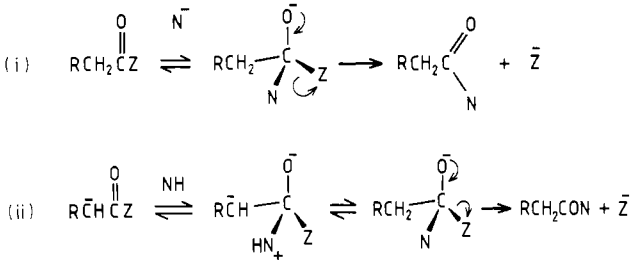
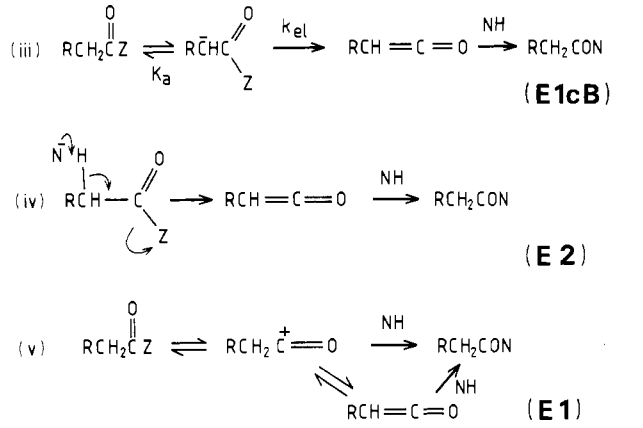
Scheme I

Addition-Elimination Pathways



Elimination-Addition Pathways



acetoacetate example above the free energy change ($\Delta\Delta G°$) is −2.5 kcal mol^{-1} (i.e., 1.36 × ΔpK_a). The change from X = O to X = S, on the face of it relatively minor, has far reaching chemical consequences. Whether on the basis of α-proton acidities, sensitivity to borohydride reduction, acyl transfer potential,[7] or infrared/Raman spectroscopy,[8] it is clear that a thiol ester is closer in nature to a ketone than to an oxygen-containing ester.

With the tendency towards elimination of the anions (formed by α-site deprotonation) of esters and the acidity of thiol esters, a question resurfaced. Do thiol esters undergo acyl transfer via associative or dissociative pathways? Further, if they do follow elimination routes in model systems, what occurs within the confines of enzyme-active sites?

This Account discusses the elimination–addition route for thiol esters, predominantly in model systems but with frequent reference to appropriate enzymology.

General Types of Mechanism for Acyl Transfer

Whereas esters such as phenyl benzoate hydrolyze only by means of a bimolecular attack of hydroxide ion on the neutral ester in basic solution, the presence of an acidic hydrogen on the atom alpha to the acyl group (e.g. RCH_2COX) leads to the possibilityof additional routes;[3,4] see Scheme I.

Mechanism i, Scheme I, is the $B_{AC}2$ pathway with its tetracoordinate intermediate formed by nucleophilic addition of N^- to the carbonyl carbon atom, followed by subsequent elimination of the leaving group. An alternative, kinetically equivalent, addition–elimination

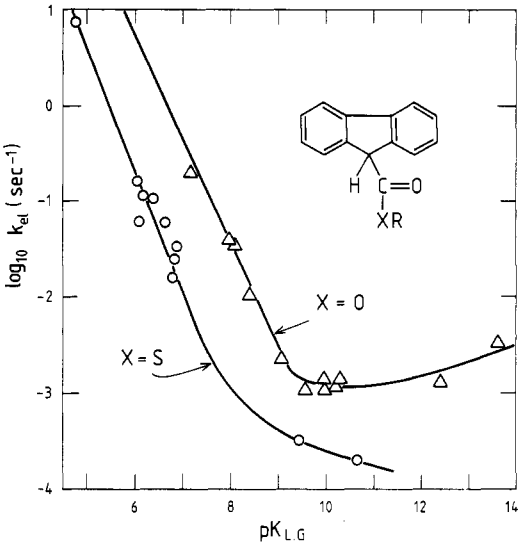


Figure 1. Brønsted leaving group plots for the pH-independent terms in strongly basic media for fluorene-9-carboxylate esters (Δ-Δ-Δ) and thiolesters (-O-O-). Data were taken from references 10 and 34, respectively, with additional unpublished data of F. M. Said and K. T. Douglas for the thiol esters.

route involves attack of NH on the conjugate base of the substrate (Scheme I, ii). Also shown are some examples of elimination–addition (EA) pathways (Scheme I, iii–v). At one extreme (of bond reorganizational timing) is the E1cB pathway iii which involves a unimolecular elimination from the conjugate base of the acyl substrate. In contrast to this route with its anionic intermediate, is the true El path (route v), with its acylium ion formed by leaving group (nucleofuge) expulsion. Between these lies a rainbow of concerted pathways, which includes the E2 process wherein formation of the ketene (RCH=C=O) involves approximately "simultaneous" deprotonation of the α-carbon position and nucleofuge expulsion.

What Molecular Features Lead to EA Paths?

Ready elimination requires both a reasonably low ester pK_a and an active leaving group. For the E1cB pathway we can write eq 3. Values of $\beta_{L.G.}$ (the slope

$$H\text{-}X\text{-}Z \underset{K_a}{\rightleftharpoons} {}^{-}X\text{-}Z \xrightarrow{k_{el}} X + Z^{-} \qquad (3)$$

of a Brønsted leaving group plot of $\log_{10}$ (rate constant) vs. the pK_a of the conjugate acid of the nucleofuge) are typically much more negative for E1cB processes than for associative acyl transfers. Such tremendous sensitivity to nucleofuge is characteristic of E1cB transition states.[3,4] With this extreme response of rate to the nature of the nucleofuge, the E1cB route often yields to alternative mechanisms.

For poorly activated nucleofuges, with inadequate power behind the elimination, a change to a bimolecular route frequently occurs,[9,10] and is manifest as a break in a linear free energy relationship (see Figure 1). For esters of fluorene-9-carboxylic acid with stronger leaving groups, an E1cB mechanism is followed, but for alkyl esters mechanism changes abruptly at $pK_{L.G.}$ ~9.5 to $B_{AC}2$ (Figure 1). As the pK_a of the nucleofuge increases beyond ~9.5 in these cases, the nucleofuge is no longer

(7) Hall, C. M.; Wemple, J. *J. Org. Chem.* **1977**, *42*, 2118.
(8) El-Aasar, A. M. M.; Nash, C. P.; Ingraham, L. L. *Biochem.* **1982**, *21*, 1972.
(9) Pratt, R. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1970**, *92*, 5956.
(10) Alborz, M.; Douglas, K. T. *J. Chem. Soc., Perkin Trans. 2* **1982**, 331.

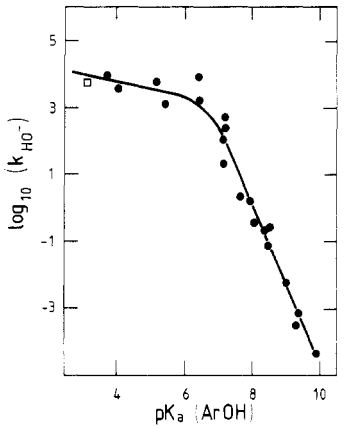


Figure 2. Plot of leaving-group dependence ($\log_{10} k_{HO}$, the logarithm of the second-order rate constant for hydroxide ion attack vs. pK_a (ArOH), the pK_a of the conjugate acid of the leaving group) for the $PhCH_2SO_2Ar$ series. Data used were from ref 11.

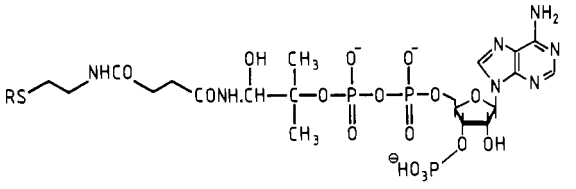
stable enough as the anion to be expelled in the elimination step. Consequently, the $B_{AC}2$ route takes over for weaker leaving groups.

In contrast to this mechanistic change is the behaviour of aryl phenylmethanesulfonates ($PhCH_2SO_2OAr$), for which mechanism changes from E1cB to E2 (see Figure 2) as the leaving group becomes more active.[11,12] This changeover is dictated by the lifetimes of the (predicted) conjugate bases of very activated sulfonyl derivatives (with $pK_{L.G.} < 6$) becoming too short ($< 10^{-14}$ sec) for them to exist as discrete intermediates, i.e., their predicted rates of breakdown in the E1 step would exceed the "vibration limit" approximately set by the vibration time of an S–OAr bond.

In view of the acidity of thiol esters and the reputations of thiolate ions as powerful nucleofuges in organic chemistry, we looked at the EA reactivity of some biologically important thiol esters. The biochemical importance of *S*-acyl coenzyme A and a host of other thiol esters provided one spur for these studies. The other came from the potential[9,6] of this pathway for providing quantitative information on nucleofugalities.[13,14]

Reactivity of Thiol Esters: Biological Aspects

The bulk of biological thiol ester reactions involves coenzyme A (**3a**), often as the acetate **3b**, malonate half ester **3c**, or acetoacetate, **3d**. Closely related are the acyl carrier protein derivatives **4**, especially involved in malonyl transfer. *S*-Acetoacetyl coenzyme A (**3d**) is very acidic (bridging methylene $pK \sim 8.5$),[6,15] and although *S*-malonyl (**3c**) and *S*-acetyl (**3b**) analogues are less acidic, the possibility[16] of an EA (ketene) path exists, in principle, for all of them. *S*-Acetoacetyl coenzyme A is central to many metabolic processes and *S*-malonyl coenzyme A is a donor of 2-carbon units in fatty acid synthesis etc., making use of *S*-malonyl acyl carrier protein (**4b**). *S*-Acetyl coenzyme A undergoes innumerable condensation reactions (at least formally implicating its carbon acidity) and acetyl transfer re-

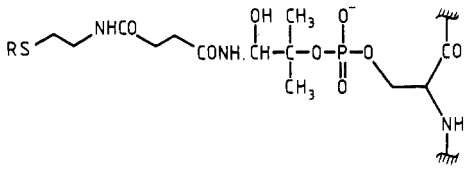


3a, R = H : coenzyme A

3b, $R = COCH_3$: S-acetylcoenzyme A

3c, $R = COCH_2CO_2^-$: S-malonylcoenzyme A

3d, $R = COCH_2COCH_3$: S-acetoacetylcoenzyme A



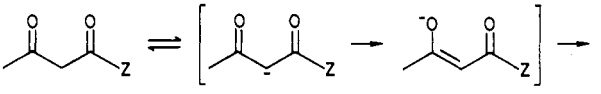
4a, R = H : acyl carrier protein (ACP)

4b, $R = COCH_2CO_2^-$: S-malonyl ACP

actions in vivo. Thiol esters crop up in many other biochemical situations, e.g., for cysteine proteases,[17] aldehyde dehydrogenases,[17] glyoxalase II,[8] the generation of receptor sites in human α_2– macroglobulin.[19] Frequently there is an α-H which might be expected to ionize.

Ketene Routes for Coenzyme A Derivatives

Acetoacetates. Aryl acetoacetates with powerful leaving groups and acidic α-hydrogen atoms undergo E1cB acyl transfer and basic hydrolysis[9] (eq 4 (Z = OAryl)). The most powerful evidence for this is amine



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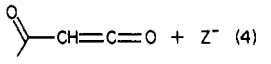


(4)

6

trapping of a reactive intermediate (presumably **6**) and the sensitivity to leaving group change.[9] At base concentrations well above the pK_a of the ester concerned, the substrate is totally in its conjugate base form (**5**), and the rate-determining step is nucleofuge expulsion (k_{el}). In agreement with mechanism 4, $\beta_{L.G.}$ values for k_{el} are −1.29 and −1.13 for aryl acetoacetates[9] (Z = OAr) and thioacetoacetates[6] (Z = SR), respectively.

The E1cB rate advantage for *S*-acetoacetyl coenzyme A over the corresponding bimolecular path is only 10–100-fold. Even this appears to be lost[6] in *S*-acetoacetylpantatheine and coenzyme A reactions[20] for ace-

(11) Davy, M. B.; Douglas, K. T.; Loran, J. S.; Steltner, A.; Williams, A. *J. Am. Chem. Soc.* **1977**, *99*, 1196.
(12) King, J. F.; Beatson, R. P. *Tetrahedron Lett.* **1975**, 973.
(13) Marshall, D. R.; Thomas, P. J.; Stirling, C. J. M. *J. Chem. Soc., Perkin Trans. 2* **1977**, 1898.
(14) Stirling, C. J. M. *Acc. Chem. Res.* **1979**, *12*, 198.
(15) Lynen, F. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1953**, *12*, 685.
(16) Cornforth, J. N. *J. Lipid Res.* **1959**, *1*, 1.
(17) Walsh, C. *Enzymatic Reaction Mechanisms*; Freeman: San Fransico, 1979.
(18) Ball, J. C.; Vander Jagt, D. L. *Biochem.* **1981**, *20*, 899.
(19) Van Leuven, F.; Marynen, P.; Cassiman, J-J.; Van den Berghe, H. *Biochem. J.* **1982**, *203*, 405.
(20) Drummond, G. I.; Stern, J. R. *Arch. Biochem. Biophys.* **1961**, *95*, 323.

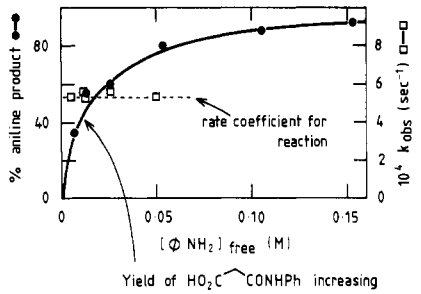
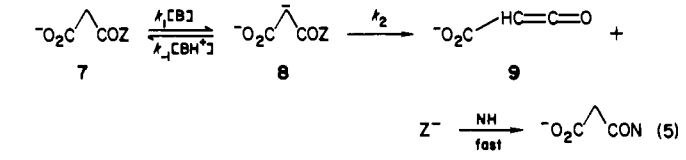


Figure 3. Percentage anilide (-●-●-) produced from *S*-p-chlorophenyl hydrogen malonate ($pClC_6H_4SCOCO_2^-$) as a function of added aniline concentration in 0.04M phosphate buffer at pH 7.2, 25 °C. Also shown is the aniline independence of the rates of hydrolysis of this thiol ester (-□-□-) in the same medium and under the same conditions. Data redrawn with permission from: Douglas, K. T.; Alborz, M.; Rullo, G. R.; Yaggi, N. F. *J. Chem. Soc., Chem. Commun.* **1982**, 245. Copyright 1982, The Chemical Society, London.

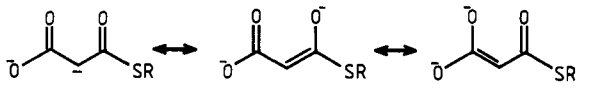
toacetyl transfer to glutathione. The powerful thiolate nucleophile (glutathione) may have forced a mechanistic change to a bimolecular route or the rate-determining step may have become general base catalyzed proton abstraction (carbanion formation) by thiol as the reaction shows first-order dependence on the acceptor (glutathione) concentration.[20] EA routes usually have rates independent[3,4] of the concentration of the final acceptor molecule which attacks *after* the rate-determining step (NH in Scheme I, iii–v).

Malonates. The low E1cB rate advantage for *S*-acetoacetyl coenzyme A might arise from its stable delocalized anion. In accord with this, 4-nitrophenolate ion is eliminated 10^3-fold faster from an acetoacetate[9] than from a fluorene-9-carboxylate[10] of comparable ester pK_a but whose carbanion is aromatic. A carbanion from *S*-malonyl coenzyme A should be less stable (higher pK_a) than from *S*-acetoacetyl coenzyme A leading to the question of whether malonyl coenzyme A follows an EA pathway, as established for 4-nitrophenyl hydrogen malonate[21] (eq 5).



Malonate thiol esters (7, Z = SR), including *S*-malonyl coenzyme A, show saturation kinetics in aqueous malonyl transfers,[22,23] a kinetic feature typical of E1cB-active carbon-acidic esters[9,21,24] and often indicating a change in the rate-determining step. The now classical technique of aniline trapping of the putative carboxyketene intermediate was successful for the *S*-malonates[22] and *O*-malonates;[21] see Figure 3. Reaction of *S*-4-chlorophenyl hydrogen thiomalonate in phosphate buffer in the presence of aniline gave malonic acid monoanilide. In an aniline concentration region where the yield of monoanilide depended critically on the concentration of free aniline, there was no catalysis by aniline detectable. Such a clear demonstration that rate- and product-determining steps differ is in accord with nucleophilic trapping (by $PhNH_2$) of a reactive intermediate, presumably **9**.

The proton transfer step of mechanism 5 holds an apparent paradox. The C–H deprotonation of 4-chlorophenyl hydrogen thiomalonate is general base catalyzed, with $\beta = 0.59$ for a series of nitrogen bases.[23] Surprisingly, variation of "leaving group" (SR) in the $-O_2CCH_2COSR$ series affects the C–H deprotonation rate tremendously. The β values for SR variation in the k_1 (C–H cleavage, equation 5) step is −1.04 for morpholine. Remember that the –SR group does not depart in this step, but leaves in the k_2 process. Such a large value of β for k_1 appears to indicate considerable negative charge development on the malonate skeleton, localized largely on the ester carbonyl (i.e. a species analogous to **11**) in the transition state for C–H fission.



10 **11** **12**

Such extensive charge localization on the malonate component seems to imply almost complete proton transfer to the base in the transition state. However, β for deprotonation is only 0.59!

Malonyl Transfer to Thiols

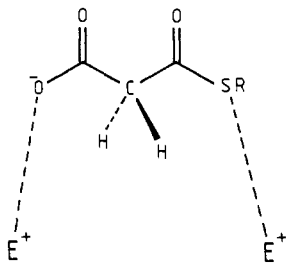
The saturation kinetics in thiol buffers[23] for thiomalonates appear to conflict with the findings of Sedgwick et al.,[27] who found that the thiolysis product of 4-nitrophenyl malonate (chiral at the methylene site) retained the chirality of the starting material, excluding a free ketene pathway. If saturation is caused by the E1cB pathway it is difficult to see why the change from a thiol ester to an oxy ester leads to a mechanistic change for a thiol nucleophile. One possibility[23] is formation of an intimate carbanion:acceptor complex which preserves α-methylene chirality throughout its subsequent aqueous reactions.

Biological Implications

In enzyme-catalyzed deprotonations of *S*-malonyl derivatives we may expect charge development to be onto the ester carbonyl bond as in **12**, unless some electrophilic stabilisation of the $-CO_2^-$ moiety occurred. Such electrophilic binding to $-CO_2^-$ would deactivate the molecule to decarboxylation, to ketene elimination pathways, and to nucleophilic activity 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 thiolic moiety and would be markedly assisted if protonation, for example, of this group occurred in an active site because of the markedly negative value of "$\beta_{L.G.}$" (~1.04) for this process.[23] Electrophilic interaction at the –SR group sulfur would also assist CO–S cleavages, see Chart I.

(21) Kirby, A. J.; Lloyd, G. J. *J. Chem. Soc., Perkin Trans. 2* **1976**, 1762.
(22) Douglas, K. T.; Alborz, M.; Rullo, G. R.; Yaggi, N. F. *J. Chem. Soc., Perkin Trans. 2* **1982**, 1681.
(23) Alborz, M.; Douglas, K. T.; Rullo, G. R.; Yaggi, N. F. *J. Chem. Soc., Perkin Trans. 2* **1982**, 1681.
(24) Holmquist, B.; Bruice, T. C. *J. Am. Chem. Soc.* **1969**, *91*, 1993, 3003.
(25) Kirby, A. J.; Varvoglis, A. G. *J. Am. Chem. Soc.* **1967**, *89*, 415. DiSabato, G.; Jencks, W. P. *Ibid.* **1961**, *83*, 440. Allen, C. M., Jr.; Jamieson, J. *Ibid.* **1971** *93*, 1434. Fendler, E. J.; Fendler, J. H. *J. Org. Chem.* **1968**, *33*, 3852.
(26) Hupe, D. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1977**, *99*, 451.
(27) Sedgwick, B.; Cornforth, J. W.; French, S. J.; Gray, R. T.; Kelstrup, E.; Willatson, P. *Eur. J. Biochem.* **1977**, *75*, 481.

Chart I



- blocks CO_2 loss
- deactivates CH_2 as nucleophile
- may aid C—H fission

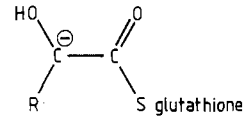
- aids C—S cleavage
- strongly (β ≈ -1.04 for SR change) aids C—H fission

Why Does *S*-Acetyl Coenzyme A Not Use a Ketene Route?

S-Acetyl coenzyme A hydrolyzes via an associative ($B_{AC}2$) route:[28] presumably the high pK_a of *S*-acetyl coenzyme A (estimated at ~26) prevents a kinetically useful concentration of carbanion, thus blocking the E1cB pathway. For a simple E1cB reaction of an acidic ester $(k_{HO^-})^{app}$ is equal to $k_{el}K_a/K_w$. For *S*-acetyl coenzyme A, k_{HO^-} is 0.123M^{-1} sec^{-1} and K_a is 10^{-26}. Thus, the calculated value of k_{el} would be ~10^{11} sec^{-1}, close to the vibration limit. If the pK_a were >28 (not impossible in view of the mode of estimation) the E1cB route would be prevented by the (hypothetical) lifetime of the ester carbanion being less than the vibration time of its component bonds. It could not exist free and proton transfer would have to be coupled with some other molecular process to avoid it. If free carbanions do exist for *S*-acetyl coenzyme A, part of the function of the enzyme(s) involved will be to ensure that the pK_a is reasonably low. Alternatively, one may anticipate largely concerted processes involving *S*-acetyl coenzyme A proton transfer from carbon. If the carbanion of *S*-acetyl coenzyme A ever did form, from a lowered pK_a etc., it would be expected to break down extremely rapidly to ketene. Using vibration limit arguments, it has been suggested[31] that the pK_a of $CH_3COSCoA$ *bound to* citrate synthase must be ≤18. These authors suggested that enzymes use routes either to avoid or to stabilise free carbanions and that the latter might be achieved by compressing the reacting groups together for the proton transfer.

Thiol Ester Eliminations in Enzymology?

Although ketene formation from thiol ester α-carbanions appears possible for a number of enzymic situations[16] there is no evidence of it as yet. The possibility has been discussed for enzyme-catalysed (*S*)-malate synthesis from glyoxal and *S*-acetyl coenzyme A[29,23] and for the condensation reaction of malonyl acyl carrier protein under the influence of β-ketoacetyl acyl carrier protein (condensing enzyme)[23,30]. Glyoxalase II catalyzed hydrolysis of *S*-(α-deuteriomandeloyl)glutathione in H_2O showed no exchange of the α-deuteron with solvent arguing against an E1cB pathway involving a hydroxyketene,[18] although the anion of this thiol ester (**13**) is produced by the action of another enzyme glyoxalase I.[32] The carbanion formed during the action of propionyl–CoA carboxylase on β-fluoropropionyl–CoA (**14**) loses F^- but, does not eliminate $CoAS^-$ to give a ketene.[33] Similarly, in spite of α-proton abstraction by β-hydroxydecanoyl thiol ester dehydrase from the suicide substrate, *S*-3-decynoyl-*N*-acetylcysteamine (**15**) elimination to the ynylketene does not occur (an allene is produced).[33]



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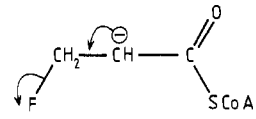

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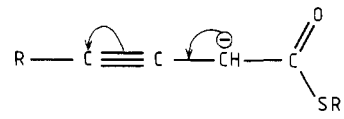


15

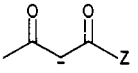
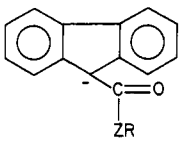
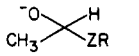
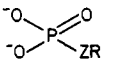
Casting back to the acetoacetyl thiol esters, the E1 process for the ester carbanions was *only slightly favoured in free solution* for thiolate ion nucleofuges of the power of coenzyme A. It needed only a strong nucleophile (a thiol) to tip the balance in favor of an associative mechanism. In the enzymic thiol ester cases listed above, the proton abstraction occurs in an enzyme-bound substrate, whose reactivity is directed by other forces such as reactive neighboring functions (e.g. C–F bonds, –C≡C–) or by the active site of the enzyme itself, evolved for rearrangements, condensations etc. For thiol esters of *very* low pK_a (thioacetoacetates) the elimination step is not strongly favored for poorer nucleofuges: for less acidic esters (thioacetates) the proton abstraction is usually concerted with some other process, which overpowers the ketene-forming route.

A Spin-Off of the E1cB Mechanism: Quantitative Nucleofugalities

The k_{el} step in equation (3) represents a unimolecular process—the fragmentation of the ester anion into a heterocumulene (analogue), called the *residuum*,[34] and a nucleofuge[14] (Z^-). For a series HXZ, value of k_{el} measure *directly* the nucleofugalities (with constant X, solvent etc.) of a series of leaving groups, Z^-, simplifying the interpretation of rate data in terms of the factors contributing to the observed nucleofugalities.[13] Considerable use has been made of the *greater* leaving

(28) Douglas, K. T.; Yaggi, N. F.; Mervis, C. M. *J. Chem. Soc., Perkin Trans. 2* **1981**, 171.
(29) Cornforth, J. W.; Redmond, J. W.; Eggerer, H.; Buckel, W.; Gutschow, C. *Nature (London)* **1969**, *221*, 1212. Luthy, J.; Rêtey, J.; Arigoni, D. *Ibid.* **1969**, *221*, 1213.
(30) Arnstadt, K.-I.; Schindlbeck, G.; Lynen, F. *Eur. J. Biochem.* **1975**, *55*, 561.
(31) Thibblin, A.; and Jencks, W. P. *J. Am. Chem. Soc.*, (1979) *101* 4963.
(32) Sellin, S.; Rosevear, P. R.; Mannervik, B.; Mildvan, A. S.; *J. Biol. Chem.* **1982**, *257*, 10023.
(33) Bloch, K. *The Enzymes, 3rd ed.*; Academic: New York, 1971; Vol. 5, p 441.
(34) Alborz, M.; Douglas, K. T.; Said, F. M. *J. Chem. Soc., Perkin Trans. 2* **1982**, 1467.

Table I
Nucleofugality Ratios for Various Substrates[a]

R	ΔpK (= $pK_{ROH} - pK_{RSH}$)	$k_{el}(S/O)$ 16	17	18	19	20	21
					$PhSO_2^-CHCH_2ZR$		$HN^-.COZR$
$4NO_2C_6H_4$	2.42	1	29	–	–	34000	–
$4ClC_6H_4$	3.24	18	79	–	–	–	–
C_6H_5	3.38	32	178	–	0.6	–	225
$4CH_3C_6H_4$	3.37	29	145	–	–	–	–
C_2H_5	5.50	54000	128000	153	–	–	–

[a] Data from ref 35.

ability of RS^- than RO^-, but until recently a direct comparison of their nucleofugalities has been lacking. The E1cB reaction provided a potential[9] probe of this facet and detailed results are now available.[6,10,34] Some elimination rates are summarized in Table I as ratios of the rate of elimination of a given ^-ZR species with Z = S to the corresponding oxygen analogue with Z = O. This ratio, $k_{el}(S/O)$, is called[35] the *nucleofugality ratio* of the two species concerned. These nucleofugality ratios, reflect a number of experimental variables (temperature, solvent, etc.) as well as structural influences (most importantly the structure of the *residuum*).

Can We Compare Nucleofuges on a Structural Basis?

The table presents isostructural comparisons of nucleofuges, i.e., the data are expressed for various structural pairs of nucleofuges which differ only in their heteroatom (here O vs. S). Comparing nucleofugality ratios for phenyl leaving groups, the ratio varies from ~200 for –HNCOZR and fluorene-9-carboxylates, through 32 for acetoacetates, to near unity for the olefin-forming reaction. A number of significant differences exist in these systems. For example, we can contrast the heterocumulene intermediates formed for **16** and **17** with the olefin for **19**. The solvent systems for **16** and **17** were predominantly aqueous, in contrast to the ethanolic medium for **19**. At the 4-nitrophenyl level, in a fixed medium the low nucleofugality ratios for **16** and **17** contrast with the enormous value for **20** (cf. also **16** or **17** with **18**).

Depending on the *residuum* the effect of going from 4-nitrophenyl to phenyl can be marked (29-fold for **16**) or small (6-fold for **17**). This effect also shows in the slightly differing $\beta_{L.G.}$ values for thiol esters and the corresponding oxygen esters (Figure 1). The nucleofugality ratio depends on the region of the Brønsted leaving group plot chosen for the comparison. The arbitrary choice of, say PhZ^-, for nucleofugal comparisons is unreasonable. Clearly, isostructural comparisons of nucleofuges are unjustifiable.

Isobasic Comparisons

To help overcome some of these problems we introduced[35] the concept of an isobasic plot (see Figure 4). In this is plotted $\log_{10}$ (nucleofugality ratio) vs. $\Delta pK_{L.G.}$ (= $pK_{ROH} - pK_{RSH}$) for a given structure of R. At the isobasic point where $\Delta pK_{L.G.} = 0$, we have defined a hypothetical thiolate:phenolate pair for which, not only are the structures identical, saving the identity of the heteroatom, but also the basicities. At this point, any differences in rates of elimination of RO^- and RS^- must be ascribed to some distinction between RO^- and RS^- not reflected in their pK_a values and/or to the natures of the *residua*. At the isobasic point RO^- is a *better* nucleofuge than RS^- by 5×10^3 for acetoacetates and by 79 for fluorene esters. Presumably, extrapolation to $\Delta pK_{L.G.} = 0$ has allowed for differences in solvation between RS^- and RO^- and the differences at the isobasic point reflect an intrinsic difference between O and S in these systems.

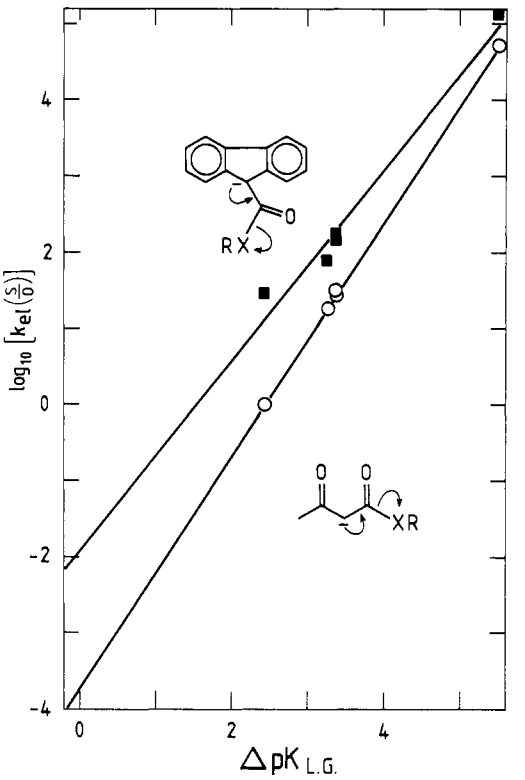


Figure 4. Isobasic plot of $\log_{10}$ (nucleofugality ratio) vs. $\Delta pK_{L.G.}$ (= $pK_{ROH} - pK_{RSH}$) for structurally identical, saving the nature of the heteroatom thiol:alcohol pairs. Redrawn with permission from ref 35. Copyright 1981, The Chemical Society, London.

Isobasic Analysis Exposes the Major Driving Force of the Elimination

This isobasic analysis *reverses* the apparent order of nucleofugality relative to that determined by direct rate

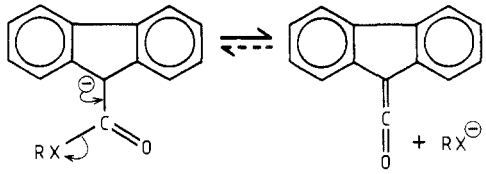
(35) Douglas, K. T.; Alborz, M. *J. Chem. Soc., Chem. Commun.* **1981**, 551.

(36) Said, F. M.; Douglas, K. T. In 50% acetonitrile:water using dual-wavelength stopped-flow, unpublished results.

measurement of unimolecular processes (i.e. compared to the observed data in the table). This reversal indicates that there are at least two separable features which determine the experimentally measured differences in rates of elimination. The major contribution is the difference in pK_a values (mostly a solvation effect) of isostructural pairs of thiols:phenols. Much of the driving force of the E1cB reaction in water is the solvation of the forming nucleofugal anion in the transition state. Underlying this are additional factors of intrinsic differences between S and O in these systems, whose explanation awaits clarification.

Other Nucleofuges

Using reaction 6 we have measured the rate of expulsion of $PhSe^-$ directly (k_{el} = 20 sec^{-1} at 25 °C). It



(6)

is 6.2 × 10^4 greater than that observed for PhO^- and 180-fold greater than that for PhS^- expulsion in this system. In Stirling's nucleofugality scale[14] $PhSe^-$ is highly ranked in an alkene-forming transition state, which is very reactant-like. In the ketene-forming, product-like transition state, where nucleofuge solvation is a major driving force for RO^- and RS^- expulsion, it is also highly ranked. Other nucleofuges remain to be assessed by this new probe.

Conclusion

In physical organic chemistry, thiol esters clearly do react via EA pathways. However, this course of reactivity does not appear to transfer to enzymology where, perforce, competing reaction pathways have been optimized by evolution. Nonetheless, the E1cB route has provided a direct probe of the quantitative basis of nucleofugality. At present the main systems studied have been carbon-based (sp^2 and sp^3) but other suitable reactions exist elsewhere in the periodic table (e.g., P(V), S(VI), N). The ability to isolate a single, chemical step offers an unusual chance of a very deep look into the chemistry of the participating atoms.

For the synthetic organic chemist knowledge that ketene intermediates occur for thiol esters (not just related acyl halides etc.) should allow better choice of reaction conditions. The EA route for thiol esters is, no doubt, extendable to P(V) and sulfur ($-SO_2-$ level, $-SO-$ level) chemistry also. The isobasic approach should be applicable not only to nucleofugality but also to the reverse reaction (eq 6, dotted arrow)–nucleophilicity.

At the very least, after a few years in a thiol chemist's laboratory

> ".....the air
> Nimbly and sweetly recommends itself
> Unto our gentle senses"
>
> Duncan to Banquo, *Macbeth*.

It is a pleasure to recognize the support and stimulation from by research colleagues and students in this area which made this account possible.