

Model Experiments pertaining to the Mechanism of Action of Vitamin B₁₂-dependent α -Methyleneglutarate Mutase

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Photolysis of di-*t*-butyl 1-bromomethylcyclopropane-1,2-dicarboxylate (**1a**) in cyclopropane containing triethylsilane and di-*t*-butyl peroxide gave the 4-methylenepent-2-yl-1,5-dioic acid radical (**3c**), which was also produced from treatment of (**1a**), its *cis*-isomer (**2b**), di-*t*-butyl-2-bromo-4-methyleneglutarate (**3a**), or di-*t*-butyl 2-bromo-methyl-3-methylenesuccinate (**4b**), with triphenyltin hydride; these experiments support a mechanism *via* protein-bound free radicals for the B₁₂-dependent enzyme α -methyleneglutarate mutase.

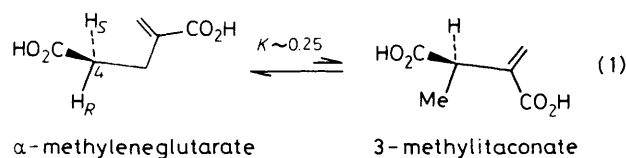
The B₁₂-dependent enzyme α -methyleneglutarate mutase catalyses the molecular rearrangement shown in equation (1), in which H_R is specifically removed from α -methyleneglutarate and the transfer of the acrylate moiety occurs with inversion of configuration at C-4 of α -methyleneglutarate.^{1,2} This is one of more than ten enzyme-catalysed rearrangements dependent on the B₁₂ coenzyme adenosylcobalamin (AdoCbl).³ Of the many mechanisms put forward for these reactions, that currently favoured postulates the intermediacy of protein-bound free radicals.⁴ For the reaction shown in equation (1) it is necessary to propose at least three intermediate radicals, as shown in Scheme 1.^{5,6} We now describe the attempted generation, characterisation, and interconversion of these radicals (as di-*t*-butyl esters) in model systems for the real enzymic reaction.

Reaction of *t*-butyl 2-bromomethylacrylate with *t*-butyl diazoacetate (each reactant 0.45 M in toluene, 80 °C/3 h) gave a 9 : 7 mixture of the bromides (**1a**) and (**2a**), easily separable by silica chromatography (elution with ether-petrol, 1 : 25). The structure of (**1a**) was proved by crystal structure analysis of the

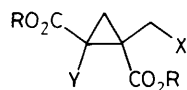
corresponding iodide⁷ and by its conversion (in formic acid, 4 h/room temp.) into the known^{6b} dicarboxylic acid (**1b**).

Treatment of *t*-butyl 2-bromomethylacrylate (0.75 M) in dimethylformamide with *t*-butyl bromoacetate containing NaH (1 equiv.) at 0 °C (2 h) $\rightarrow$ room temp. (30 min), gave di-*t*-butyl 2-bromo-4-methyleneglutarate (**3a**) [55% after silica chromatography, elution with dichloromethane-petrol, 1 : 4]. The carbanion from reaction of di-*t*-butyl citraconate (0.2 M) in tetrahydrofuran with lithium di-isopropylamide (1 equiv.) at -78 °C was trapped by methanal (an excess of gaseous CH₂O was bubbled into reaction mixture over 2 h) to give di-*t*-butyl 2-hydroxymethyl-3-methylenesuccinate (**4a**) [39% after silica chromatography, elution with ethanol-ethyl acetate-petrol, 0.5 : 1 : 10]. The alcohol (**4a**) was converted into the bromide (**4b**) by treatment of a 1.0 M solution of (**4a**) in dimethylformamide with triphenylphosphine (1 equiv.) and bromine (1 equiv.) [*n.b.* addition of alcohol (**4a**) to Ph₃P·Br₂, room temp./overnight]. The bromide (**4b**) was obtained in 41% yield after silica chromatography (elution with dichloromethane-petrol, 1 : 2).

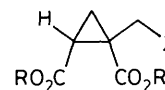
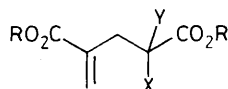
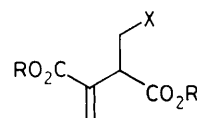
The bromides (2.3×10^{-3} M in benzene) containing a trace of azobisisobutyronitrile) were treated with Ph₃SnH[†] (10^{-2} M)



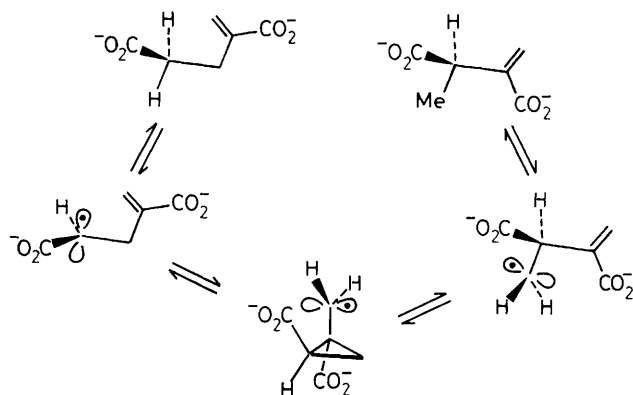
[†] The use of Ph₃SnH rather than Bu₃SnH (*cf.* Tada *et al.*⁸) was preferred in our studies because it enabled us to exclude more readily the formation of products derived from addition of the tin hydride to the double bond of (**3a**) or (**3b**).

(1a); R = C(Me)₃, Y = H, X = Br

(1b); R = Y = H, X = Br

(1c); R = C(Me)₃, Y = H, X = free electron(1d); R = C(Me)₃, Y = D, X = Br(2a); R = C(Me)₃, X = Br(2b); R = C(Me)₃, X = free electron(3a); R = C(Me)₃, Y = H, X = Br(3b); R = C(Me)₃, Y = X = H(3c); R = C(Me)₃, Y = H, X = free electron(3d); R = C(Me)₃, Y = D, X = free electron(3e); R = C(Me)₃, Y = H, X = Co(dmgh)₂py(4a); R = C(Me)₃, X = OH(4b); R = C(Me)₃, X = Br(4c); R = C(Me)₃, X = H(4d); R = C(Me)₃, X = free electron(4e); R = C(Me)₃, X = Co(dmgh)₂py

dmgh = dimethylglyoxime monoanion, py = pyridine.



Scheme 1. Pathway for α -methyleneglutarate mutase *via* free radicals [*n.b.* the abstraction of a hydrogen atom from a substrate molecule is achieved by the adenosyl radical derived from homolysis of the Co–C σ -bond of AdoCbl (see refs. 3 and 4)].

for 80–200 min at 50 °C.⁸ Compounds (1a), (2a), and (3a) gave di-*t*-butyl α -methyleneglutarate (3b) as the exclusive product. However, compound (4b) gave a 2 : 1 mixture of (3b) and (4c) [the latter was not detected ($\leq 2\%$) in the reaction mixtures derived from (1a), (2a), and (3a)]. We suggest that reaction of (4b) with Ph₃SnH gives radical (4d), which is either quenched by Ph₃SnH or rearranges *via* (1c) or (2b) into radical (3c), which is quenched by Ph₃SnH. Obviously, starting from (1a) gives (1c) directly, which ring opens to (3c) faster than it can be quenched by Ph₃SnH. Similarly, (2a) $\rightarrow$ (2b) $\rightarrow$ (3c), whilst (3a) yields (3c) directly.

Photolysis at -112°C of bromide (1a) in cyclopropane containing triethylsilane and di-*t*-butyl peroxide, in the cavity of an e.s.r. spectrometer,⁵ generated an e.s.r. spectrum consistent with the formation of radical (3c): g 2.0032(5),

$a(2\text{H})$ 21.37 G, $a(1\text{H})$ 20.37 G, $a(1\text{H})$ 1.6 G, $a(1\text{H})$ 0.75 G ($G = 10^{-4}$ T). Confirmation of this assignment was obtained by analogous photolysis of the deuteriobromide (1d) [prepared from *t*-butyl 2-deuteriodiazoacetate and *t*-butyl 2-bromomethylacrylate in a similar manner to (1a)], which led to radical (3d): g 2.0034(9), $a(2\text{H})$ 21.5 G, $a(\text{D})$ 3.125 G, $a(1\text{H})$ 1.125 G, $a(1\text{H})$ 0.375 G (note the reduction of one of the coupling constants by a factor of 6.51). Curiously, similar photolytic experiments with bromides (3a) and (4b) did not generate radical (3c) [or (4d) from (4b)]. The nature of the radicals formed in these trials is subject to further investigation.

The experiments described have spectroscopically characterised radical (3c) and have shown its derivation from radical (4d) *via* (1c) or (2b). They provide a model system to support the postulate that the enzymic reaction of equation 1 proceeds *via* protein-bound free radicals (as in Scheme 1). We believe that alternative mechanisms *via* organocobalt intermediates are less plausible, especially as the alkylcobaloximes (3e) and (4e) derived from bromides (3a) and (4b), respectively, are relatively stable.⁹ For the enzymic reaction it can be inferred that the cyclopropylcarbinyl radical shown (Scheme 1) is the intermediate connecting the two but-3-enyl radicals. This is because the *trans*-isomer, but not the *cis*-isomer, of 1-methylcyclopropane-1,2-dicarboxylic acid is a competitive inhibitor of α -methyleneglutarate mutase.¹ From the evidence presented above it is not possible to determine the relative merits of (1c) and (2b) as intermediates between (3c) and (4d). Furthermore, the equilibrium and rate constants involving the protein-bound but-3-enyl radicals (Scheme 1) are such as to enable thermodynamic equilibrium to be attained between α -methyleneglutarate and 3-methylitaconate, equation (1). However, in the model experiments described, the equilibrium and rate constants are such as to preclude detection of radical (4d) as a product of the ring opening of either (1c) or

(2b), and it is not formed in significant amount when bromide (3a) is reacted with Ph_3SnH ; no (4c) was seen.

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