Investigation of a Coenzyme B_{12} Model Reaction by ^{13}C NMR Spectroscopy†

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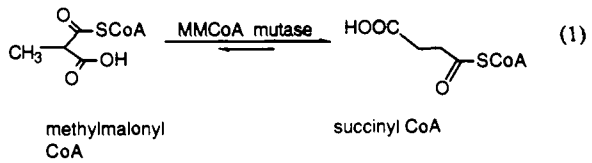
In continuation of our interest in the carbon–carbon rearrangements catalyzed by the coenzyme B_{12} containing enzymes,[1a] we have investigated a model system of methylmalonyl CoA mutase (MMCoA mutase), which catalyzes the rearrangement of methylmalonyl CoA to succinyl CoA,[1b] a process essential to many metabolic pathways (eq 1). Both anionic and radical



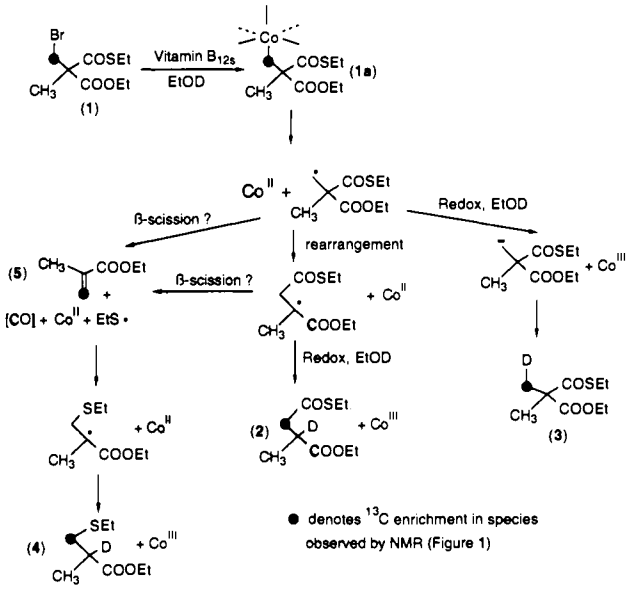
(1)

processes have been proposed for this reaction, which involves the 1,2-migration of a thioester group.

Model studies have shown that both anionic[2] and radical[3] pathways are competent mechanisms for this rearrangement. The radical pathway was shown to have a rate constant for rearrangement of 2.5 s^{-1} at 25 °C.[3] A radical mechanism for the enzymatic mechanism, which is believed to consist of hydrogen abstraction and rearrangement steps, is supported by ESR studies.[4] Recently Dowd and co-workers reported the incorporation of both of these features in a model system.[5] By incorporating a ^{13}C label in the bromomethyl group of the diethyl ester of 2-bromomethyl-2-methylmalonylmonothiolate (**1**), a model previously developed in this laboratory,[1a] we hoped to observe the intermediacy of a Co–C bond and to follow the fate of this carbon (●, Figure 1) by dynamic NMR spectroscopy, thereby observing features which might shed further light on the mechanism. The ^{13}C labeled substrate was synthesized according to published procedures,[6a] using labeled methylene bromide. Vitamin B_{12s} was generated from hydroxocobalamin and sodium borohydride in deuterated ethanol and filtered into an ethanolic solution of the bromomethylmalonate (**1**) in an NMR tube.

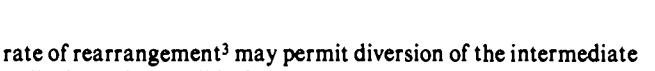
A typical time course (over 14 hours at room temperature) for the reaction is shown in Figure 1. The onset of the reaction is

Scheme 1



indicated by the decrease of the signal corresponding to the bromide (**1**, $\delta = 36$) and the appearance of a new component (**1a**) at $\delta = 37$, the chemical shift being consistent with a carbon–cobalt bond.[7] This intermediate then leads to three products with chemical shifts of $\delta = 125.4$, 47.0, and 22.8. The peaks at 22.8 (not shown in Figure 1) and 47.0 ppm are readily ascribed to the dimethylmalonate (**3**) and methylsuccinate (**2**) esters, arising from reductive and rearrangement pathways, respectively.[6b] The component with $\delta = 125.4$ subsequently disappears, and a new peak at $\delta = 35.1$ arises. The latter, hitherto undetected species was isolated by preparative GC, characterized by ^1H NMR, ^{13}C NMR, and GC–MS, and shown to possess the structure **4**.[6c] Premature cessation of the reaction followed by GC–MS analysis allowed characterization of the transient intermediate ($\delta = 125.4$) as the methacrylate ester **5**. Each of the products **2**, **3**, and **4** was found to contain one deuterium atom ($(m+1)/z$) when the reaction was conducted in CD_3CD_2OD and CH_3CH_2OD and no deuterium when the reaction was carried out in CD_3CD_2OH, results which can be explained by quenching of the appropriate anion by solvent.

A plausible mode of formation of **5** is β-cleavage of the radical intermediate as shown in Scheme 1, a reaction reminiscent of the β-cleavage of β-ketoalkyl radicals (eq 2).[8a,b] The relatively slow



(2)

rate of rearrangement[3] may permit diversion of the intermediate radical via the possible fragmentation pathways shown, if high concentrations of quenching agents are not present. Addition of the 2,2,6,6-tetramethyl-1-piperidinyloxy radical (TEMPO)[9] to the reaction mixture produced several NMR signals in the $\delta = 78$–80 range, consistent with O-alkylated TEMPO derivatives, the structures of which are now under investigation. However the incorporation of deuterium from EtOD into the products

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(1) (a) Scott, A. I.; Kang, J.; Dalton, D.; Chung, S. K. *J. Am. Chem. Soc.* **1978**, *100*, 3603. Scott, A. I.; Hansen, J. B.; Chung, S. K. *J. Chem. Soc.* **1980**, 388. (b) Barker, H. A. *Annu. Rev. Biochem.* **1972**, *41*, 91.

(2) Grate, J. H.; Grate, J. W.; Schrauzer, G. N. *J. Am. Chem. Soc.* **1982**, *104*, 1588. Choi, G., Choi, S.-C.; Galan, A.; Wilk, B.; Dowd, P. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3174.

(3) Wollowitz, S.; Halpern, J. *J. Am. Chem. Soc.* **1988**, *110*, 3112.

(4) Zhao, Y.; Such, P.; Rétey, J. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 215.

(5) Dowd, P.; Wilk, B.; Wilk, B. K. *J. Am. Chem. Soc.* **1992**, *114*, 7949.

(6) (a) The malonate ester **1** was prepared by the method of Keese *et al.* using $^{13}CH_2Br_2$. Stamm, E.; Keese, R. *Synthesis* **1981**, 231. (b) Vitamin B_{12s} was prepared by reduction of vitamin B_{12a} with excess $NaBH_4$ and filtered into a solution of the bromomethylmalonate **1** in a 5-mm NMR tube under an inert atmosphere according to published procedures. Scott, A. I.; Kang, J.; Dowd, P.; Trivedi, B. K. *Bioorg. Chem.* **1980**, *9*, 227. Spectra were recorded on a Bruker AM-500 spectrometer using a 45° pulse and a delay time of 1 s. Data were acquired using 16*K* data points. (c) The thioether **4** is formed in 5–10% yield, whereas the methylsuccinate **2** and methylmalonate **3** esters are formed in approximately equal proportions.

(7) Branchaud, B.; Meier, M. S.; Malekzadeh, M. N. *J. Org. Chem.* **1987**, *52*, 212.

(8) (a) Bertini, F.; Caronna, T.; Grossi, L.; Minisci, M. *Gazz. Chim. Ital.* **1974**, *104*, 471. (b) A control experiment was performed to ensure that the methylacrylate ester **5** was not formed when the bromomethylmalonate **1** was treated with sodium borohydride in the absence of B_{12}.

(9) The TEMPO free radical has also been used to trap the adenosyl radical ($AdCH_2$•) formed during the thermolysis of coenzyme B_{12}. Finke, R. G.; Smith, B. L.; Mayer, B. J.; Molinero, A. A. *Inorg. Chem.* **1983**, *22*, 3677.

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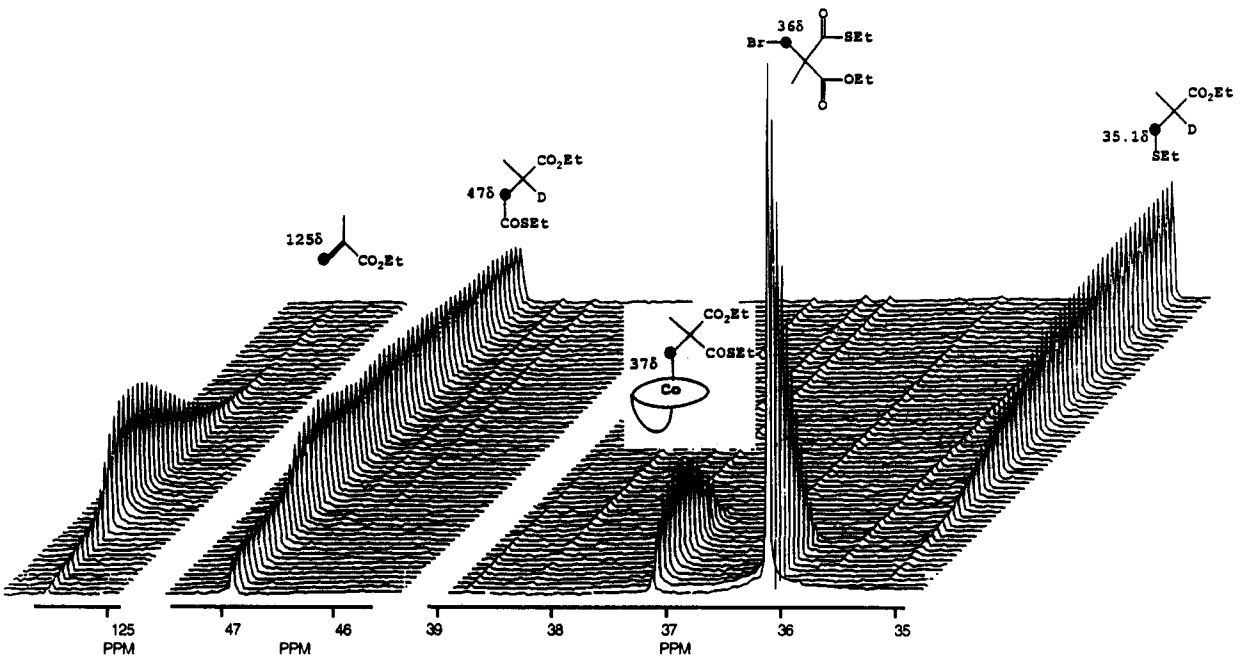

Figure 1. ^{13}C NMR time course profile for the reaction of vitamin B_{12s} and compound **1**. See ref 6 for NMR conditions.

indicates that there is a definite carbanionic character to the reaction, the carbanion presumably being generated by electron transfer between Co(II) and the radical species.[10]

To test for intermolecularity in the reaction, the diethyl ester of 2-bromomethyl-2-methylmalonylmonothiolate was also synthesized with a ^{13}C label on the carbonyl group of the thioester, and an equimolar mixture of this material and the ^{13}C bromomethyl species (**1**) was reacted with vitamin B_{12s}. The lack of any coupling in the ^{13}C NMR signals of the carbonyl and methylene groups in the product **2** confirmed the intramolecular nature of the reaction.[3] Studies aimed at trapping the radical intermediate in the enzymatic reaction are ongoing.

(10) A similar pattern of deuterium incorporation from the solvent was observed in studies carried out by Dowd and co-workers, ref 5.