Mechanism of Action of Vitamin B_{12}. Ultrafast Radical Clocks Provide No Evidence for Radical Intermediates in Cyclopropane Models for the Methylmalonyl-CoA to Succinyl-CoA Carbon Skeleton Rearrangement

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Abstract: To probe for free radical intermediates in the model studies for the coenzyme B_{12}-dependent, methylmalonyl-CoA to succinyl-CoA carbon-skeleton rearrangement, new models incorporating cyclopropane rings (unsubstituted **23** and 2-phenyl-substituted **28**) at the 2-position were developed. The reaction of **23** or **28** with vitamin B_{12s} gives only rearranged succinate **24** or **29**, respectively, with the cyclopropyl group intact. When this reaction was carried out in EtOD/D_2O, a monodeuterided product, **24-*d*$_1$** or **29-*d*$_1$**, was obtained and the deuterium was incorporated at the 2-position. Control reactions of the 2-phenylselenylsuccinate with tri-*n*-butyltin hydride yielded the ring-opened 2-propanylidenesuccinate via a free radical pathway. The results suggest that the skeletal rearrangement step in the B_{12}-catalyzed isomerization of methylmalonyl-CoA to succinyl-CoA occurs not by a radical pathway but by an anionic or organocobalt pathway.

Introduction

Coenzyme B_{12} is an obligatory cofactor in a remarkable series of twelve enzyme-catalyzed rearrangement reactions.[1] Among these, there are three carbon-skeleton rearrangements that have been particularly intriguing because of the lack of precedent for such transformations among organic reactions. The B_{12}-catalyzed interconversion of methylmalonyl-CoA **1** and succinyl-CoA **2** has been extensively studied by experiments in both enzyme and solution model systems, and the sequence of steps involved in this interconversion is often formulated as shown in Figure 1.[2] Homolysis of **3** to give adenosyl radical **4** is followed by hydrogen transfer to give methylmalonyl radical **5**. Skeletal rearrangement to **6** and back hydrogen transfer complete the sequence.

The intermediacy of radicals **4**, **5**, and **6** is now widely accepted, although the means by which the enzyme can effect the needed hydrogen transfer reactions are still not well understood. The defining feature of the sequence is the mechanism by which the skeletal rearrangement (**5** → **6**) itself occurs. Given the overwhelming evidence for radical intermediates, it is perhaps natural that a radical hypothesis has gained favor.[3] In this hypothesis, radicals **5** and **6** interconvert by migration of the thioester group either directly (a formal 1,2-shift) or through the intermediacy of a very short-lived alkoxyl cyclopropyl radical (3-*exo* closure/opening). 1,2-Shifts of sp^2-hybridized functional groups to radicals are well-known reactions.[4a−d] Because thioesters are among the poorest of the

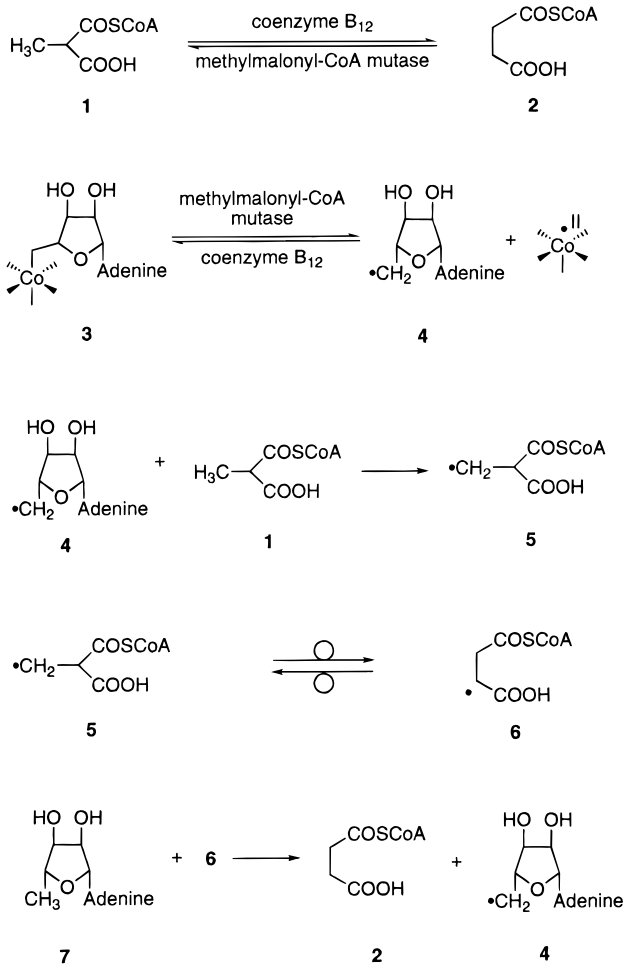

Figure 1. The B_{12} catalyzed rearrangement of methylmalonyl-C_oA.

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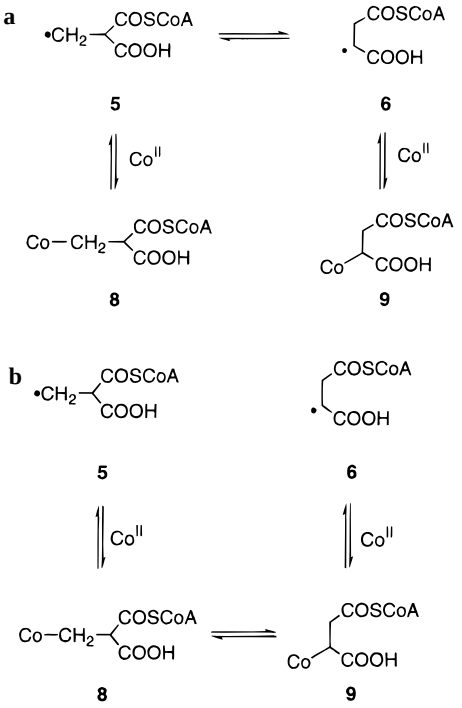

Figure 2. Skeleton rearrangement of radicals (a) and organocobalts (b).

known migrating groups,[5] it is usually postulated that the enzyme plays a role either in facilitating the interconversion of **5** and **6** or blocking otherwise competing reactions.

In 1975, we reported the first working model for a B_{12} carbon skeletal rearrangement, and provided direct evidence implicating a carbon−cobalt substrate bond.[6] This evidence is readily accommodated in the mechanism by postulating reactions of the radicals **5** and **6** with the neighboring Co^{II}. Anionic 1,2-shifts of sp^2-hybridized groups are also well-known.[4e] This then raises crucial mechanistic questions: are cobalt species such as **8** and **9** formed in the enzyme catalyzed reaction and if so are they temporary resting places for the radicals (as shown in Figure 2a), or are they competent intermediates for migration (as shown in Figure 2b)? And if the organocobalt species are competent intermediates for the migration, how can their intermediacy be distinguished from the intermediacy of radicals?

We report herein the closing chapter of detailed studies on the model (nonenzymatic) rearrangement of substituted halomethyl malonates to succinates[1] with both Vitamin B_{12} and tributyltin hydride. Labeling studies confirm that the B_{12} mediated reactions occur through anionic (or organocobalt) intermediates. Comparison of the B_{12} and tin hydride models with ultrafast, cyclopropane-based radical probes[7,8] reveals major differences. These differences establish unambiguously that the model rearrangement in the presence of B_{12} does not occur through a radical mechanism but instead proceeds directly through anionic (or organocobalt) intermediates. These model results further strengthen the case that the enzymic migrations also occur through cobalt not radical intermediates.

Results and Discussion

The basic design of the model systems is shown in Figure 3. The precursor **10** bears an R group that contains some sort of radical clock that is designed to report on the existence of a radical either before (**11**) or after (**12**) the migration. The behavior of authentic radicals is probed by reduction of **10** with tributyltin hydride, and then these results are compared to the behavior in the now standard cobalt model reactions. In the cobalt reactions, labeling experiments with ROD report on whether the final products derive from radical or anionic (organocobalt, **15**) intermediates.

Experiments to probe the existence of radicals such as **11** have already been reported, and two key observations are summarized in Figure 4.[9] In a control experiment, reduction of **17** with tributyltin hydride provides **18**, **19-*E***, and **20** in the indicated yields. As expected from rate constants in related systems,[5] the radical 1,2-shift of the thioester group cannot compete with 6-*exo* cyclization and 1,5-hydrogen transfer. Treatment of the same substrate **17** with vitamin B_{12s} provided small amounts of radical-derived products **19-*Z*** and **22**, but the major product was 1,2-shift product **21**. In labeling experiments, products **21**, **19-*Z***, and **20** all contained deuterium, thereby showing that they ultimately arose from organocobalt intermediates. These crucial results demonstrate: (1) that radical **11** is generated at least to some extent under the cobalt conditions (as evidenced by formation of **22** and **19-*Z***) and (2) that the radical **11** is not an intermediate on the way to rearranged product **21** (because the radical 1,2 migration is precluded by faster reactions of **11**).

A more sophisticated model system yielded results consistent with these in addition to providing some information about the hydrogen transfer step.[10] These model studies also provide limited information about the potential intermediacy of the rearranged radical **12** (Figure 3, R = $CH_2CH_2CH_2CH{=}CH_2$). The option for 5-*exo* cyclization was not exercised, so if this intermediate is involved, its lifetime must be much shorter than the rate of cyclization. However, couplings of radicals with Co^{II} species have very high bimolecular rate constants[11] and the 5-*exo* cyclization of radicals such as **12** is a rather slow unimolecular process. We therefore need a faster reporter on the intermediacy of radical **12**. For this we choose the cyclopropyl and 2-phenylcyclopropyl substituents.[12]

We initially selected the model compound **23** bearing an unsubstituted cyclopropyl group. The results with this model have been reported in a preliminary communication,[13] and full details for its synthesis are contained in the Supporting Information. Likewise, the syntheses of new precursors and authentic products are also detailed in the Supporting Information. The model compound **23** reacted with vitamin B_{12s}, which

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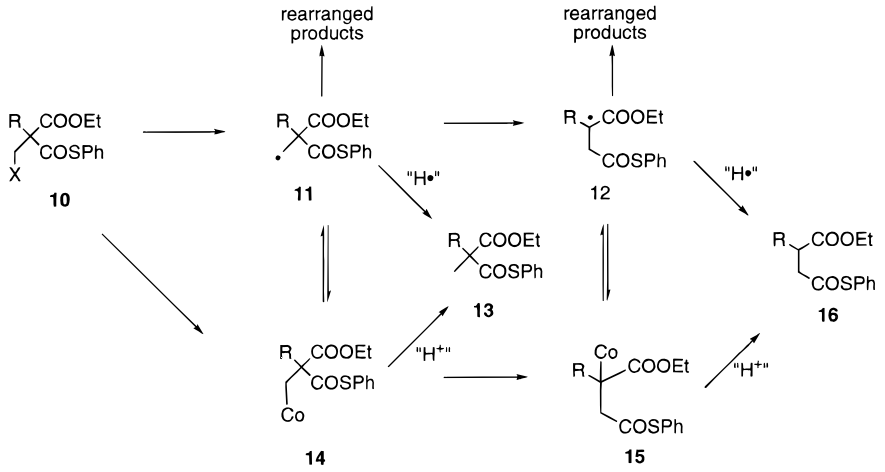


Figure 3. Reactions of model 10.

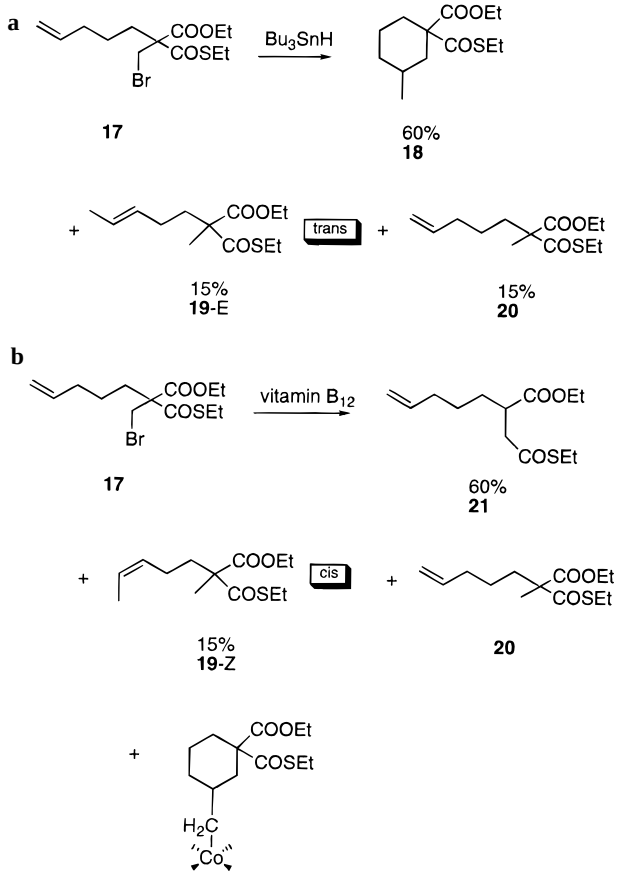
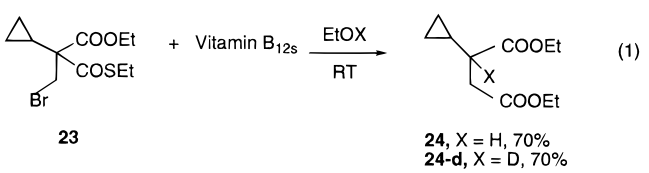


Figure 4. (a) Control: radical 1,2-shift cannot compete with other radical rearrangement. (b) Model: anionic 1,2-shift is favored over radical rearrangement.

was prepared by reduction of vitamin B_{12a} with sodium borohydride in ethanol at room temperature under argon and in the dark. The rearranged succinate **24** was obtained in 70% yield, *with the cyclopropane ring intact* (eq 1). The product

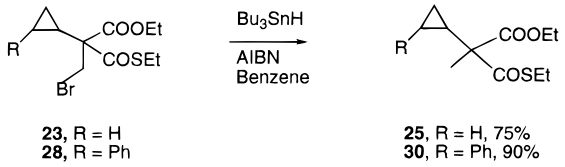


(1)

24 is a diethyl ester, reasonably presumed to arise from exchange of the thioester with solvent ethanol following the rearrangement.[9] In a related reaction conducted in water (see Supporting Information), hydrolysis did not occur and the rearranged thioester was isolated. An authentic sample of the ring-opened product was prepared (see Supporting Information) for comparison with the crude product obtained from reaction of **23**. The ^{1}H NMR spectra of the total crude product showed no peaks of the ring-opened product, and the structure of **24** was established spectroscopically and by preparation of authentic sample. The GC-MS spectrum exhibited only one peak corresponding to product **24.**

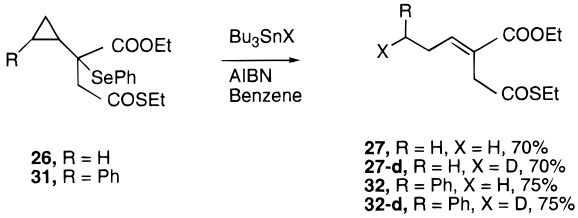
When the rearrangement of **23** was carried out with B_{12s} in EtOD, deuterium was incorporated into the product at the α-position to the ester group yielding **24-*d***. This suggests that the final intermediate leading to **24** is a carbanion or organocobalt species which could be formed as a primary product of rearrangement or as a product of electron transfer from B_{12s} or B_{12r} to a radical intermediate.[2,10]

In an initial control reaction, the bromide **23** was treated with tributyltin hydride and only direct reduction product **25** (75%) was produced; no succinate **24** was observed (eq 2). The structure of **25** was established by spectroscopic means and by independent synthesis.



(2)

If there were a radical pathway for the rearrangement, the possible radical intermediate could be stabilized by the ester group, and thus might have a lifetime long enough to abstract a hydrogen before ring-opening occurs. So a second control reaction in which a free radical is produced in this series was essential. We chose the reaction of phenylselenocyclopropylsucinate **26** with tributyltin hydride. The reaction of **26** was initially carried out in benzene (eq 3). After heating at 70 °C

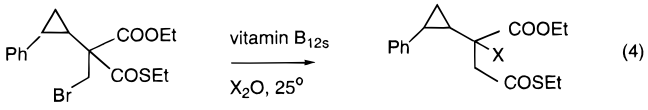


(3)

for 5 h, the ring-opened propylideneyl succinate **27** was obtained (70%). The configuration of **27** was proved to be *E* by NOE and H−H 2D NMR studies. In ethanol, which is the solvent of B_{12} reactions, the product **27** was obtained in almost the same yield (70%) upon tin hydride reduction. When **26** was treated with tributyltin deuteride, the expected monodeuteride **27-*d*** was obtained in 70% yield. These results demonstrate that the intermediate ester-substituted cyclopropylcarbinyl radical *does* undergo ring opening of the cyclopropyl group.

Recent literature data on radical rate constants for both hydrogen transfer and cyclopropylcarbinyl radical opening now allow us to make good estimates for the lifetime of the radical derived from **26**.[14,15] In our preliminary communication, we estimated, based on indirect measurements of Beckwith and Bowry,[14] that this radical would have a lifetime <100 ns. However, more recent direct measurements of closely related radicals by Newcomb and co-workers suggest that this was an overestimate.[16] Indeed, the lifetime of this radical can now conservatively be estimated as 10 ns or less. Thus, the model **23** actually contains a faster (that is, more sensitive) probe than we had originally thought.

Newcomb and co-workers have shown that 2-phenyl-substituted cyclopropylcarbinyl radicals have ring-opening rate constants of $(3-5) \times 10^{11}$ s^{-1}.[7c] We synthesized the bromide **28** and allowed it to react with vitamin B_{12s} (eq 4). This will be a much faster model than the former one, and any radical intermediate generated in this series will cause opening of the cyclopropane ring due to the extremely fast reaction.



(4)

28

29, X = H, 97%
29-d, X = D, 90%

The reaction of **28** with vitamin B_{12s} in water, at room temperature, in the dark and under argon gave the rearranged succinate **29** in 97% yield, *with the cyclopropane ring intact* (eq 4). GC-MS analysis of the total crude product showed a very clean chromatogram that consisted of only one peak identified as product **29**. Furthermore, no vinyl proton was observed in the ^1H NMR spectrum. The structure of the product **29** was confirmed spectroscopically and by independent synthesis of an authentic sample. In D_2O, the same reaction gave the product **29-*d*** with deuterium incorporated into the product at the α-position to the ester group. The ^2H NMR spectrum of **29-*d*** showed a single peak at δ 2.40.

When the bromide **28** was treated with tributyltin hydride (eq 2), only direct reduction product **30** (90%) was detected; no succinate **29** was observed. The structure of **30** was established by spectroscopic means and independent preparation of authentic sample.

A control reaction of 2-phenylselenyl-2-*trans*-2′-phenylcyclopropylsuccinate **31** with tributyltin hydride (0.003 M) was conducted in benzene under argon (eq 3). After 9 h of reflux, the ring-opened product 3-phenylpropylidenyl succinate **32** was obtained in 75% yield. A similar reduction in neat tributyltin hydride gave the same result. The product **32** was identical with an authentic sample. The configuration of **32** was proved to be *E* by NOESY spectroscopy: there is no coupling between H−C_3 and H−C_2. The reaction of **31** with Bu_3SnD in benzene gave the expected monodeuterated product **32-*d*** (75%), as shown in eq 3. ^2H NMR spectroscopy showed a single peak at δ 2.80.

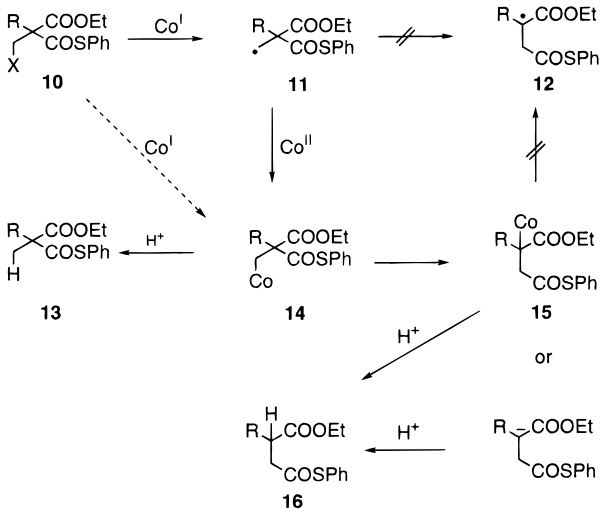


Figure 5. Reactions of the B_{12} model.

The inability of hydrogen transfer from tin hydride under any conditions to compete with ring opening of the cyclopropane ring is anticipated by results of Newcomb.[12,15,16] At 70 °C, tertiary ester-substituted radicals react with tin hydride with a rate constant of roughly 1×10^6 M^{-1} s^{-1}.[15] Assuming that neat tin hydride has a concentration of about 3.7 M and that 5% of a rearranged product could be detected (a very conservative estimate), then neat tin hydride can only intercept radicals with unimolecular rearrangement rate constants of 10^8 M^{-1} s^{-1} or less. However, from Newcomb's most recent results, the rate constant for opening of the radical derived from **31** can be conservatively estimated as 10^{10} s^{-1}.[16,17] Again by assuming that 5% rearranged product could have been detected, the lifetime of any radical formed in the cobalt model experiments in eq 4 is conservatively limited to 50 ps or less. *This effectively eliminates the intermediacy of a radical since no bimolecular reaction of any type can remove a radical on this time scale.*

Conclusions

These and the prior studies with radical probes now combine to provide a unified picture of the chemistry involved in the solution reactions of substituted halomethylene thiomalonates with vitamin B_{12s}. This picture is summarized in Figure 5. Vitamin B_{12s} reacts with halide **10** via dissociative electron transfer to provide vitamin B_{12r} and the radical **11**. This radical can undergo relatively rapid radical rearrangements or can couple with the B_{12r} to provide organocobalt intermediate **14**. Although the radical **11** is formed, its slow 1,2-thioester shift cannot compete with the other pathways. An alternative pathway is that part or all of the organocobalt intermediate is formed by direct S_N2 substitution of **10** with B_{12s} to give **14** in competition with dissociative electron transfer. Direct substitu-

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(16) (a) Newcomb, M.; Horner, J. H.; Emanuel, C. J. *J. Am. Chem. Soc*. **1997**, *119*, 7847−48. (b) A rate constant for opening of the radical derived from **29** can be estimated by taking the calculated rate constant at 70 °C (1.3×10^8 s^{-1}) of related radical **6**e in the above reference and dividing by 4 (to offset the effect of the "reported group" in **6**e).

(17) We arrive at this estimate by dividing the rate constant for opening on the parent phenylcyclopropyl carbinyl radical (ref 8) by a factor of 25 to account for the retarding effect of the ester and methyl substituents (ref 16a).

tion is the expected pathway for normal primary halides, but neopentyl halides such as **10** probably react through the electron-transfer route.[18]

Cobalt intermediate **14** undergoes the 1,2-migration of the thioester group to give **15** in competition with protonation by the solvent to give **13**. Our evidence precludes the intermediacy of radical **12**; it cannot be formed either as a result of the rearrangement of **11** or dissociation of a cobalt species such as **15**. Although these studies rule out the intermediacy of a free radical in this key step, they do not provide any detailed information about either the pathway that the rearrangement of **14** might take or any immediate product that may be formed. In essence, this can be viewed as a variant of an anionic 1,2-shift. Unlike cobalt species **14**, the hypothetical intermediate **15** has never been isolated or observed. Its existence is therefore open to question.

There is some evidence in the literature to suggest that organocobalt species **15** may not be the product of the 1,2-migration. Related cobalt intermediates with simpler ligands are proposed in cobalt-mediated additions of radicals to alkenes.[18] These intermediates tend to undergo competing protonation and disproportionation in ratios that are pH dependent. The protonation reactions are supposed to be slow, and a reversible homolytic cleavage reaction is thought to operate in the background.[18] This scenario cannot be occurring in the model studies herein because any dissociation would lead to instant cyclopropane opening. Thus, it seems most probable the rearrangement occurs through some kind of anion pair or electron transfer mechanism to provide a transient "carbanion", which is rapidly protonated. Alternatively, if **14** rearranges to cobalt species **15**, then protonation of **15** is fast with respect to homolytic dissociation.

As in any model system, there are important differences between the model transformations and the natural, enzyme-catalyzed transformations. Nonetheless, the multiple observations of enzyme-like migrations in cobalt-mediated model systems and the sustained absence of any evidence for a radical component in these migrations suggests that an analogous transformation in the enzymic system is likely. Thus, although radicals are certainly involved in the enzyme-catalyzed transformations and key intermediates are interchanged by radical hydrogen transfer reactions, the defining migration step does not involve radicals and derives directly from the fundamental chemistry of the B_{12} coenzyme.

Acknowledgment. M.H. dedicates this paper to the memory of Professor Paul Dowd, who died on November 21, 1996. We thank the National Institutes of Health for funding this work. Mu He thanks Professor Dennis P. Curran for his help in writing parts of the paper, and Mu He and Professor Dennis P. Curran thank Professor Martin Newcomb for helpful discussions and suggestions for revision of the paper.

Supporting Information Available: Full details for all the model and control experiments reported in the paper, as well as synthesis and characterization of precursors and authentic samples and products (25 pages). See any current masthead page for ordering and Internet access instructions.

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