Mechanism of Action of Vitamin B_{12}. A New Test for Free Radical Intermediates Using a Cyclopropane Model for the Methylmalonyl-CoA to Succinyl-CoA Carbon Skeleton Rearrangement

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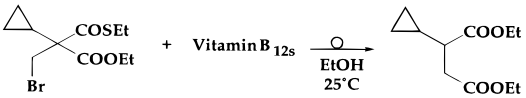
In recent years, evidence has emerged of the involvement of free radicals in the coenzyme B_{12}-dependent carbon skeleton rearrangement reactions.[1] However, the rearrangements promoted by coenzyme B_{12} are multistep sequences, and it continues to be worthwhile to ask whether nonradical steps accompany the radical reactions. We have shown for a model of the methylmalonyl-CoA carbon skeleton rearrangement that no radical intermediates are trapped by a pendant 4-pentenyl side chain.[2] Accordingly, the permissible lifetime of any radical intermediate associated with the model rearrangement must then be not greater than 10^{-5} s, corresponding to the rate of cyclization of the 5-hexenyl radical.

To develop probes that can be examined as models, as well as potential substrates for the enzyme, we have begun to explore the chemistry surrounding the cyclopropylmalonate **1** and others of its class.[3] Treatment of the bromide **1** in ethanol at 25 °C with vitamin B_{12s} yielded the rearranged succinate **2** in 62% yield, *with the cyclopropyl group intact* (eq 1).[4]



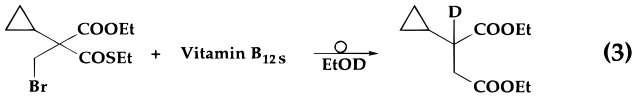
1 **2** 62% **(1)**

The structure of **2** was established by spectroscopic means and by independent synthesis of an authentic sample. That was accomplished by alkylation of **3** with ethyl bromoacetate (eq 2). Although we did not observe the UV−visible spectrum of

3 **2** 81% **(2)**

an intermediate carbon−cobalt bonded adduct that might have been formed from **1**, such species are often unstable and difficult to detect.[2,5,6]

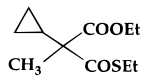
When the rearrangement of **1** was carried out in EtOD, deuterium was incorporated into the product, yielding **2**-*d* (eq 3). This suggests that the final intermediate leading to **2** is an



1 **2-d$_1$** 60% **(3)**

organometallic species that could be formed as a primary product of rearrangement or as a product of electron transfer.[2,5]

Treatment of the bromide **1** with tri-*n*-butyltin hydride yielded (97%) the direct reduction product **4**; no succinate **2** was detected. The structure of **4** was established spectroscopically and by preparation of an authentic sample.

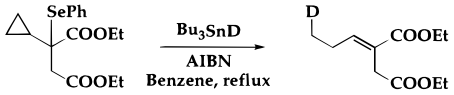


4

It was a surprise that the cyclopropyl group in the intermediate leading to **2** had not undergone ring opening as one might have expected for the intervention of a free radical intermediate. To establish that ring opening is the expected course for a free radical in this series, we prepared the phenylselenocyclopropylsuccinate **5** and reduced it with tri-*n*-butyltin hydride in ethanol at 70 °C (eq 4). This experiment yielded the allyl-

5 **6** 70% **(4)**

carbinyl derivative **6** and demonstrated that the intermediate radical does undergo ring opening of the cyclopropyl group to **6** upon tin hydride treatment. Treatment of **5** with Bu_3SnD in benzene yielded the expected monodeuteride **6**-*d* (eq 5). In this



5 **6-d** 70% **(5)**

series, there was no discernible difference between the yields and products of the tin hydride reactions (eq 5) in ethanol or benzene.

The advantage of the present experimental approach is that the permissible lifetime of any free radical intermediate arising during the B_{12s}-promoted rearrangement of **1** must now be placed[7] at $\sim 10^{-7}$ s or less.[8] This series of experiments provides another example of a coenzyme B_{12}-like carbon skeleton rearrangement that apparently does not require the intermediacy of free radical intermediates.

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(1) See: Zhao, Y.; Such, P.; Rétey, J. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 215−216.

(2) Choi, G.; Choi, S.-C.; Galan, A.; Wilk, B.; Dowd, P. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3174−3176.

(3) For examples, see: Griller, D.; Ingold, K. U. *Acc. Chem. Res.* **1980**, *13*, 317−323. Bowry, V. W.; Lusztyk, J.; Ingold, K. U. *J. Am. Chem. Soc.* **1989**, *111*, 1927−1928. Bowry, V. W.; Lusztyk, J.; Ingold, K. U. *J. Am. Chem. Soc.* **1991**, *113*, 5687−5698. Newcomb, M.; Johnson, C. C.; Manek, M. B.; Varick, T. R. *J. Am. Chem. Soc.* **1992**, *114*, 10915−10921. Newcomb, M. *Tetrahedron* **1993**, *49*, 1151−1176. Ortiz de Montellano, P. R.; Stearns, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 3415−3420. Bowry, V. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1991**, *113*, 5699−5707. Liu, K. E.; Johnson, C. C.; Newcomb, M.; Lippard, S. J. *J. Am. Chem. Soc.* **1993**, *115*, 939−947. Inchley, P.; Lindsay Smith, J. R.; Lower, R. J. *Nouv. J. Chim.* **1989**, *132*, 669−676. Atkinson, J. K.; Ingold, K. U. *Biochemistry* **1993**, *32*, 9209−9214.

(4) The product isolated was the diethyl ester, reasonably presumed to arise from exchange of the thioester with solvent following rearrangement.

(5) Cf.: Scott, A. I.; Kang, K. *J. Am. Chem. Soc.* **1977**, *99*, 1997−1999. Scott, A. I.; Kang, J.; Dalton, D.; Chung, S. K. *J. Am. Chem. Soc.* **1978**, *100*, 3603−3604. Scott, A. I.; Kang, J.; Dowd, P.; Trivedi, B. K. *Bioorg. Chem.* **1980**, *9*, 227−230.

(6) Alternatively, the reaction of **1** → **2** may proceed through an electron transfer sequence.

(7) Beckwith, A. L. J.; Bowry, V. W. *J. Am. Chem. Soc.* **1994**, *116*, 2710−2716.

(8) See footnote 30 in the following: Newcomb, M.; Horner, J. H.; Filipkowski, M. A.; Ha, C.; Park, S.-U. *J. Am. Chem. Soc.* **1995**, *117*, 3674−3684.

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