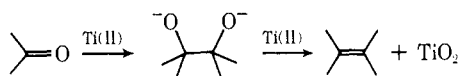
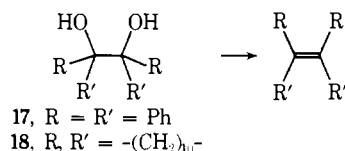


complex followed by concerted loss of  $\text{TiO}_2$  or by stepwise loss of the oxygens then gives the product olefin.



Evidence in support of this hypothesis comes from observation of a black color characteristic of Ti(II) species<sup>17</sup> and from the fact that, when the reaction is allowed to proceed only to partial completion, pinacols can be isolated as by-products in many cases. van Tamelen has already shown that the 1,2-diol, *meso*-hydrobenzoin, is reduced to *trans*-stilbene by a  $\text{TiCl}_3\text{-CH}_3\text{Li}$  reagent [presumably *via* Ti(II)],<sup>18</sup> and we have further demonstrated that treatment of either benzpinacol (17) or the dilithium salt of cyclododecanone pinacol (18) with the  $\text{LiAlH}_4\text{-TiCl}_3$  reagent also yields



the corresponding olefins. Thus the reagent affords a method for the reduction of 1,2-diols to olefins and competes favorably with other methods of doing the transformation.<sup>19</sup>

One further observation which should be made is that if  $\text{LiAlH}_4$  is serving merely to reduce Ti(III) to Ti(II), then other reagent systems producing Ti(II) might also effect the coupling reaction. There have in fact been two quite recent reports on the use of  $\text{Zn-TiCl}_4$  and  $\text{Mg-TiCl}_4$  reagents to couple aromatic ketones.<sup>8</sup> We ourselves have also shown that a variety of reducing agents including  $\text{LiBH}_4$ ,  $\text{CaH}_2$ , and  $\text{LiH}$  will reduce either  $\text{TiCl}_3$  or  $\text{TiCl}_4$  to a reagent which will couple aromatic ketones. In our experience, however, the  $\text{LiAlH}_4\text{-TiCl}_3$  system has given superior yields, particularly in the case of aliphatic ketones.

Our study is continuing, but it seems clear already that low-valent titanium reagents have considerable potential in organic synthesis.

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(18) K. B. Sharpless, R. P. Hanzlik, and E. E. van Tamelen, *J. Amer. Chem. Soc.*, **90**, 209 (1968).

(19) See K. B. Sharpless and T. C. Flood, *J. Chem. Soc., Chem. Commun.*, 370 (1972), and references therein.

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Received January 28, 1974

### Electron Spin Resonance Studies on Diol Dehydrase.<sup>1</sup> III. Rapid Kinetic Studies on the Rate of Formation of Radicals in the Reaction with Propanediol

Sir:

Recent electron spin resonance studies with diol dehydrase,<sup>2,3</sup> ethanolamine ammonia-lyase,<sup>4,5</sup> and glyc-

(1) This work was supported by research grants from the National Institutes of Health (GM-12633 [R.H.A.] and Training Grant GM-212 [J.E.V.]) and the Public Health Service (GM-1869 [J.A.F.]). This is publication No. 957 from the Graduate Department of Biochemistry, Brandeis University, Waltham, Mass.

erol dehydrase<sup>6</sup> have provided evidence that the coenzyme  $\text{B}_{12}$  dependent rearrangements catalyzed by these enzymes proceed through radical intermediates. Mechanisms which have been proposed to account for these observations have postulated that the carbon-cobalt bond of the coenzyme is cleaved homolytically in the presence of substrates to give  $\text{B}_{12}(\text{r})$  (a paramagnetic low-spin cobalt(II) species) and organic radicals. Substrates and substrate analogs, when incubated with holo-diol dehydrase, convert 40–100% of the available coenzyme to these intermediates.<sup>2</sup> Although the substantial conversion of coenzyme  $\text{B}_{12}$  to  $\text{B}_{12}(\text{r})$  strongly implicates  $\text{B}_{12}(\text{r})$  in the catalytic process, it has so far not been demonstrated that this rate of formation is rapid enough to function as an intermediate. We have now employed rapid freeze-quench electron spin resonance techniques to determine the rate of formation of  $\text{B}_{12}(\text{r})$  and the organic radical in the reaction catalyzed by diol dehydrase with its normal substrate.

Rapid freeze quenching of reactions for esr studies was accomplished with the use of an apparatus designed according to Ballou.<sup>7,8</sup> The total dead time including mixing and freezing was 5–7 msec; other pertinent technical information and the procedures employed have been described.<sup>8</sup> All operations were carried out in the dark to avoid photolysis of the coenzyme. Diol dehydrase (free of propanediol) and a fivefold excess of coenzyme  $\text{B}_{12}$  were deoxygenated by three cycles of evacuation and flushing with  $\text{O}_2$ -free nitrogen in a vessel which served as a reservoir for the holoenzyme during a series of rapid freeze experiments. Anaerobic conditions were necessitated since oxygen inactivation of the holoenzyme in the absence of substrate would normally occur during the course of one series of experiments (3–4 hr). In the rapid freeze experiments reported here, 0.2–0.4 ml of the holoenzyme was rapidly mixed, at 5–7°, with an equal volume of 0.2 M propanediol. The samples were frozen after reaction times of 3–200 msec in isopentane at  $-140^\circ$  and esr spectra of the packed samples were obtained in a Varian E-9 spectrometer with the sample temperature maintained in a stream of nitrogen at  $-130^\circ$  to  $-140^\circ$ . Manual mixing experiments were conducted by incubating 55 units of dioldehydrase and a fivefold excess of coenzyme  $\text{B}_{12}$  aerobically, at room temperature for 4 min to form the holoenzyme followed by the addition of propanediol to a concentration of 0.1 M in a final volume of 0.27 ml. After 15 sec of incubation at 5°, the samples were frozen in isopentane at  $-140^\circ$ .

Figure 1A shows that after 3 msec of reaction time, the esr spectrum obtained from the reaction of 1,2-propanediol with the holoenzyme was qualitatively the same as that produced in a manual mixing experiment (15 sec) (Figure 1B), with the exception of an addi-

(2) T. H. Finlay, J. Valinsky, A. S. Mildvan, and R. H. Abeles, *J. Biol. Chem.*, **248**, 1285 (1973).

(3) J. E. Valinsky, R. H. Abeles, and A. S. Mildvan, *J. Biol. Chem.*, **249**, 2751 (1974).

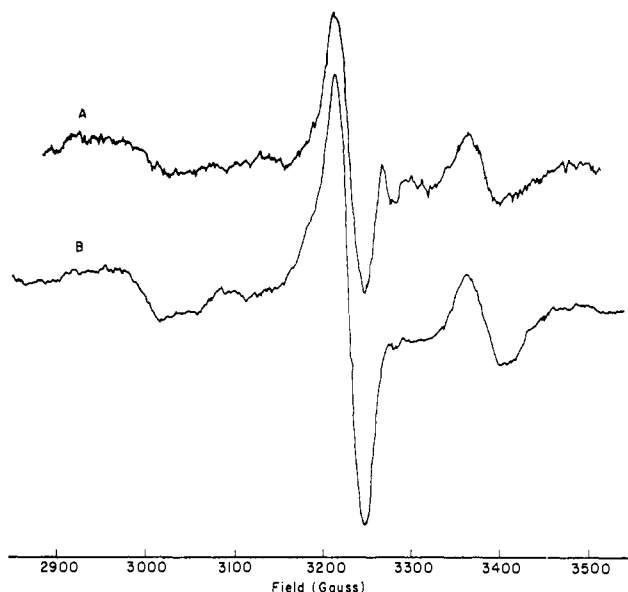
(4) B. Babor and D. Gould, *Biochem. Biophys. Res. Commun.*, **34**, 441 (1969).

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(6) S. A. Cockle, H. A. O. Hill, R. J. P. Williams, S. P. Davies, and M. A. Foster, *J. Amer. Chem. Soc.*, **94**, 275 (1972).

(7) We gratefully acknowledge the advice and assistance of Dr. Graham Palmer and Dr. David Ballou in the initial rapid freeze experiments and the assistance of Robert A. Hannula in performing the experiments.

(8) D. P. Ballou, Ph.D. Thesis, University of Michigan, 1968.



**Figure 1.** Electron spin resonance spectra of propanediol and the diol dehydrase- $B_{12}$  coenzyme complex. For the rapid freeze experiment (1A), an aliquot of preformed holoenzyme (58 units) was mixed in 3 msec with an equal volume of 0.2 *M* propanediol to give a total volume of 0.4 ml. The frozen material was packed into a quartz esr tube. Conditions for obtaining the spectrum were: scan length, 1000 G; scan time, 4 min; time constant, 0.3 msec; modulation amplitude, 10 G; modulation frequency, 100 kHz; microwave frequency, 9.232 GHz; microwave power, 20 mW; relative gain,  $4 \times 10^3$ ; temperature,  $-130^\circ$ . For the manually mixed reaction (1B), an aliquot of preformed holoenzyme (55 units) was treated with propanediol for 15 sec prior to freezing in isopentane at  $-140^\circ$ . The final propanediol concentration was 0.1 *M*. Conditions for obtaining the esr spectrum were identical with Figure 1A except that the gain setting was  $2.5 \times 10^3$ .

**Table I.** Summary of Data from Rapid Freeze and Manual Mixing of ESR Experiments<sup>a</sup>

Sample	Signal (G)	<i>g</i> value	(Total spins)/(mole enzyme)	% conversion to radical
[1,1- <sup>2</sup> H <sub>2</sub> ]DL-1,2-Propanediol (3 msec) (rapid freeze)	3000	2.22	1.23	61.5 <sup>b</sup>
	3230	2.05		
	3387	1.96		
[1,1- <sup>2</sup> H <sub>2</sub> ]DL-1,2-Propanediol (15 sec) (manual mix)	3000	2.22	1.40	70.5
	3230	2.05		
	3385	1.96		

<sup>a</sup> Data were calculated from spectra described in Figure 1. (Total spins)/(mole of enzyme) was calculated from double integration of the over-all signals in the field range 2850–3500 G, using  $Cu^{II}SO_4$ -EDTA ( $10^{-5}$  *M*) as a reference standard. "Conversion to radical" was determined using 2.0 as the maximum value. The copper standard was prepared by manually freezing the sample in isopentane at  $-140^\circ$ . Differences in sample packing between manually and rapidly frozen samples are included in the calculations.<sup>8</sup> <sup>b</sup> Conversion of DBCC to radical species at other times in rapid freeze experiments was: 14.7 msec, 65.5%; 36.5 msec, 54.5%; 74.5 msec, 55.1%; 202 msec, 52.5%.

tional resonance at 3275 G, which appears in the rapid freeze experiment.<sup>9</sup> This resonance also appears in the spectra (not shown) of reactions of intermediate times (15–200 msec). Table I shows that the integrated intensities of these two spectra are identical within the error of the double integration method. This result

(9) This peak is probably an artifact since temperature studies show that it disappears after warming to  $-80^\circ$  and recooling to  $-130^\circ$ .

implies that the steady state concentration of radical intermediates is attained in less than 10 msec (3 msec reaction time + 7 msec maximum dead time) implying a  $t_{1/2} < 3$  msec. At  $37^\circ$  the first-order rate constant for propionaldehyde formation is 250–300  $sec^{-1}$ . Using the Arrhenius plots for this reaction,<sup>10</sup> one may estimate that, at  $5^\circ$ , the rate should be decreased by a factor of 5–6. This rate corresponds to a  $t_{1/2}$  of 11–14 msec. The result of this experiment is that the rate of radical formation, at least, is two to three times faster than the overall rate.

In order to confirm the calculation of spins per mole of enzyme, the optical spectrum of the enzyme-coenzyme complex was examined in the presence of propanediol. The coenzyme spectrum was immediately converted to that of  $B_{12}(r)$ . Calculations indicate that about 50% conversion to  $B_{12}(r)$  occurred, in accord with the results in Table I.

In conclusion, the evidence presented in this study indicates that radical formation by diol dehydrase, in the conversion of 1,2-propanediol to propionaldehyde, is kinetically competent and provides strong evidence for a radical mechanism in  $B_{12}$ -catalyzed rearrangements of this type.<sup>11,12</sup> A more detailed description of the spectra will follow in a later communication.

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(11) Babior and coworkers have recently demonstrated that 2-amino-1-propanol, a poor substrate for ethanolamine deaminase, produces radical species with this enzyme at a kinetically significant rate. Information concerning this observation was kindly provided by Dr. Babior prior to publication.

(12) While this manuscript was in preparation, R. L. Blakely, *J. Biol. Chem.*, **249**, 2338 (1974), reported that  $B_{12}(r)$  appears at a kinetically competent rate with ribonucleotide reductase, a  $B_{12}$ -dependent enzyme which does not catalyze a rearrangement reaction.

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Received April 20, 1974

### Electron Transfer Quenching of the Luminescent Excited State of Tris(2,2'-bipyridine)ruthenium(II). A Flash Photolysis Relaxation Technique for Measuring the Rates of Very Rapid Electron Transfer Reactions

Sir:

Quenching processes involving the luminescent excited states of tris(2,2'-bipyridine)ruthenium(II),  $Ru(bipy)_3^{2+*$ , and related metal complexes are of considerable current interest. For example, it has been demonstrated that  $Ru(bipy)_3^{2+*$  can be quenched in electronic energy transfer processes by both organic and transition metal ion quenchers.<sup>1–3</sup> It has also been suggested that quenching of  $Ru(bipy)_3^{2+*$  can occur by electron transfer quenching.<sup>4,5</sup> Gafney and Adam-

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(2) N. Sabbatini and V. Balzani, *J. Amer. Chem. Soc.*, **94**, 7587 (1972).

(3) M. Wrighton and J. Markham, *J. Phys. Chem.*, **77**, 3042 (1973).

(4) H. Gafney and A. W. Adamson, *J. Amer. Chem. Soc.*, **94**, 8238 (1972).

(5) J. N. Demas and A. W. Adamson, *J. Amer. Chem. Soc.*, **95**, 5159 (1973).