

**Table I.** Temperature and Volume Dependencies of  $a^N$  for Di-*tert*-butyl Nitroxide

| solvent           | measd quantities                                     |  |  | calcd  |
|-------------------|--|--|--|--|
|                   | A  | B  | C  | A - BC   |
|                   | $((\partial a^N/\partial T)_P)$<br>G/K $\times 10^4$ | $((\partial a^N/\partial V)_T)$<br>G/L $\times 10$ | $((\partial V/\partial T)_P)$<br>L/K $\times 10^3$ | $((\partial a^N/\partial T)_V)$<br>G/K $\times 10^4$ |
| cyclo-<br>pentane | $-7.7 \pm 1.1$                                       | $-5.1 \pm 0.4$                                     | 1.17   | $-1.7 \pm 1.5$                                       |
| acetone           | $-15.2 \pm 1.5$                                      | $-6.8 \pm 0.8$                                     | 1.44   | $-5.4 \pm 2.3$                                       |
| ethanol           | $-6.5 \pm 0.8$                                       | $-7.3 \pm 1.0$                                     | 1.04   | $1.1 \pm 1.8$  |

hyperfine splitting seems to be a sensitive probe of local environment produced by solvent molecules around the solute (the cybotactic region<sup>15</sup>). This is deduced<sup>10,12,13,16</sup> from the fact that the magnitude of  $a^N$  in different solvents is best correlated with solvent polarity parameters such as the Kosower  $Z$  value<sup>17</sup> and the Dimroth-Reichardt  $E_T$  value<sup>18</sup> which are based upon model chemical reactions. By contrast, only poor correlations are obtained<sup>13</sup> with bulk parameters such as dielectric constant.

We can describe  $a^N$  for di-*tert*-butyl nitroxide as a function of the pressure, volume, and temperature of the solvent. Since a knowledge of any two of  $P$ ,  $V$ , and  $T$  is sufficient to define the third, then the following equation holds:<sup>19</sup>

$$(\partial a^N/\partial T)_P = (\partial a^N/\partial T)_V + (\partial a^N/\partial V)_T(\partial V/\partial T)_P$$

Measurements<sup>20</sup> were carried out for di-*tert*-butyl nitroxide ( $10^{-3}$  M) in cyclopentane, acetone, and ethanol, these being regarded as typical of nonpolar, polar, and hydrogen-bonding solvents. For each solvent  $(\partial a^N/\partial T)_P$  was measured in a standard variable-temperature experiment at 1 bar over the temperature range of  $\sim 40$  to  $-80$  °C. Similarly,  $(\partial a^N/\partial V)_T$  was obtained by measuring the change in  $a^N$  with pressure (range, 1–1400 bar) at 23 °C, which was then converted to a volume change using the known compressions of the solvents.<sup>22</sup> For acetone and ethanol  $(\partial V/\partial T)_P$  at 1 bar was obtained from literature data<sup>23</sup> and for cyclopentane it was measured directly. Graphs of  $a^N$  vs. volume or temperature were straight lines to within experimental error. The results are summarized in Table I and are available in detailed form as supplementary material. The error limits represent one standard deviation derived from least-squares fitting of the data. The various slopes refer to a point where  $P = 1$  bar,  $V = 1$  L, and  $T = 23$  °C.

As stated previously, the temperature dependence of hyperfine splitting is generally studied at constant pressure. It is assumed that the systems behave as though they had a constant volume; i.e., it is assumed that  $(\partial a^N/\partial T)_P = (\partial a^N/\partial T)_V$ . This assumption is incorrect. For di-*tert*-butyl nitroxide in cyclopentane and ethanol all of the change in hyperfine with temperature is due to change in the volume of the solvent; there is no intrinsic change with temperature at constant volume. Only for acetone does the system reveal a genuine temperature dependence of the hyperfine. However, this temperature dependence is so small that we prefer not to speculate as to its origin at the present time.

The interpretation of small changes in hyperfine splittings with temperature is a currently popular form of EPR spectroscopy. It is recommended that this activity should not be attempted without careful consideration of radical-solvent interactions.<sup>24,26</sup>

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**Supplementary Material Available:** Listings of  $a^N$  and  $g$  for di-*tert*-butyl nitroxide as a function of temperature and volume in acetone, cyclopentane, and ethanol solvents (9 pages). Ordering information is given on any current masthead page.

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D. Griller

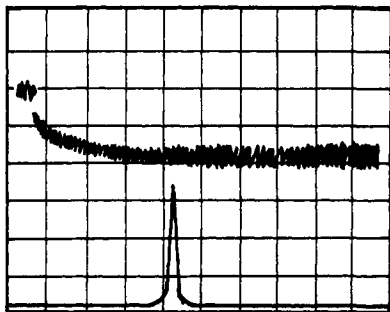
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Received April 17, 1978

## Rate of Primary Photoproduct Formation for Aqueous Tris(ethylenediamine)chromium(III)

Sir:

We report here what we believe to be the first direct measurement of the rate of primary photoproduct formation for a Cr(III) coordination compound. The experiment is one of monitoring the rate of absorbance change, following excitation at 530 nm by a high intensity, 20-ns laser pulse.<sup>1</sup>

Aqueous Cr(en)<sub>3</sub><sup>3+</sup> (at pH 2) was chosen as the initial system because of the wealth of other information available. The photochemistry has been studied in detail;<sup>2</sup> aquation occurs with quantum yield  $\phi = 0.37$  around 25 °C, the primary product being Cr(en)<sub>2</sub>(enH)(H<sub>2</sub>O)<sup>4+</sup>.<sup>3</sup> Emission from the first hexi doublet state,  $D_1^0$ , has been observed<sup>4</sup> and its lifetime



**Figure 1.** Tracing of oscilloscope photograph showing photoproduct formation for 0.2 M  $[\text{Cr}(\text{en})_3](\text{ClO}_4)_3$  in  $1 \times 10^{-2}$  M  $\text{HClO}_4$ . The upper trace (10 mV/division, 2  $\mu\text{s}$ /division) shows the transmitted monitoring beam intensity (each division representing 8% transmitted light). The lower trace (100 ns/division) shows the photodiode trace of the laser pulse, which was  $\sim 1$  J.

measured in room temperature solution.<sup>5</sup> In addition, emission quenching studies have shown that 50–60% of  $\phi$  is quenched on extensive quenching of emission (with 1.5 M  $\text{Co}^{2+}$  or  $\text{Fe}^{2+}$  ions<sup>6</sup> or 0.1 M  $\text{OH}^-$  ions<sup>7</sup>). The general mechanistic picture is that of Figure 4-3.<sup>2</sup> Excitation produces a Franck-Condon manifold,  $Q_{\text{FC}}$ , which thermally equilibrates rapidly to the first thi quartet state,  $Q_1^\circ$ . Intersystem crossing to  $D_1^\circ$  appears to be prompt, that is, to occur before complete thermal equilibration.<sup>8</sup>

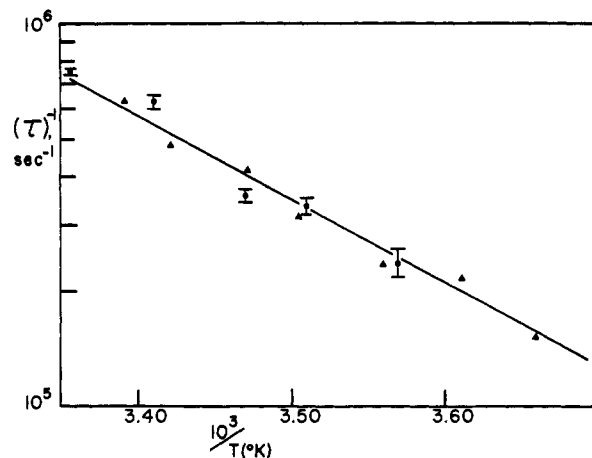
The most direct interpretation of the emission quenching results is certainly that some 50–60% of photoreaction occurs via  $D_1^\circ$ , with the rest from  $Q_1^\circ$ . However, the required quencher concentrations are sufficiently high that simple mass action kinetics may not be valid, and it is difficult absolutely to rule out the possibility that the effect includes some static or even dynamic  $Q_1^\circ$  quenching. The question has been complicated by not knowing whether intersystem crossing is prompt or only through  $Q_1^\circ$ , and by not knowing the lifetime of  $Q_1^\circ$ .

The present line of investigation was undertaken as a means of determining lifetimes of reactive excited states directly, that is, through the rate at which they produce primary photoproducts. As a minimum, in the present case, we expected to gain information on the unknown  $Q_1^\circ$  lifetime; we might also be able to test or add to inferences from the emission results.

Our typical result is shown in Figure 1. The 20-ns excitation pulse, shown by one of the sweeps of the dual beam oscilloscope, calibrates the pulse energy for that experiment. The second sweep, usually with another time base, follows the monitoring beam intensity. When monitoring at 580 nm, there is a prompt increase in absorbance (which follows the excitation pulse in rate of appearance), followed by a slower increase of  $\sim 1$   $\mu\text{s}$  grow-in time; no further change in absorbance occurs out to at least 200  $\mu\text{s}$ . We identify the absorbance change as due to primary photoproduct formation. The total absorbance increase is within experimental error of that calculated for the expected amount of photolysis. In addition, the absorbance changes at various wavelengths were just those expected from the separately determined reactant and product absorption spectra, including wavelengths at which decreased rather than increased absorption should occur.

Excited-state absorption would complicate our interpretation. The ratio  $R$  (see eq 1 below) was constant, however, at several wavelengths over the region from 575 to 600 nm. Excited-state absorption, if important, should vary in effect with wavelength, so that  $R$  should vary, contrary to observation. We conclude that in our monitoring region this potential complication was not important.<sup>9</sup>

The slower or delayed photoproduct appearance is definitely coupled with  $D_1^\circ$ . The grow-in is exponential, and, as shown



**Figure 2.** Rate constant for delayed product grow-in, ●, and  $\tau^{-1}$  for  $D_1^\circ$  emission, ▲. Apparent activation energy is 10 kcal/mol.

in Figure 2, the rate constants agree with those for  $D_1^\circ$  emission (determined in parallel experiments to the monitored ones). We assign this component of reaction to  $D_1^\circ$ .

The prompt absorbance increase must therefore be due to reaction from  $Q_1^\circ$ . We can thus conclude that the lifetime of  $Q_1^\circ$  is  $< 20$  ns. The ratio,  $R$ , of the delayed to the prompt increase in optical density (at 580 nm) is 2.35 (essentially independent of temperature). Analysis gives

$$R = \frac{\phi_D(\gamma - 1) + f_{\text{isc}}}{\phi_Q(\gamma - 1) - f_{\text{isc}}} \quad (1)$$

where  $\phi_D$  and  $\phi_Q$  are the partial quantum yields associated with  $D_1^\circ$  and  $Q_1^\circ$  ( $\phi_D + \phi_Q = \phi$ ),  $\gamma$  is the product to reactant extinction coefficient ratio, and  $f_{\text{isc}}$  is the intersystem crossing efficiency. The presence of the  $f_{\text{isc}}$  terms is a consequence of allowing for ground-state bleaching. We find the value of  $\gamma$  is  $\sim 40$  at 580 nm; the  $f_{\text{isc}}$  terms are therefore almost negligible and eq 1 gives  $\phi_D/\phi = 66$ –69%. We conclude that about two thirds of photoproduct formation occurs from or through  $D_1^\circ$ .

The above figure is somewhat, but perhaps not seriously, higher than the estimates from quantum yield quenching studies.<sup>6,7</sup> It was, of course, desirable to determine the effect of known quenchers on our prompt and delayed absorbance changes. At the higher quencher concentrations needed, however, the monitoring transients became quite complex—we suspect additional chemistry to simple quenching, and are pursuing both this aspect and the behavior of other Cr(III) amines. The present results provide direct evidence, however, that  $D_1^\circ$  is indeed the rate-controlling precursor to a majority of the photoreaction, and set a maximum lifetime for  $Q_1^\circ$ , the other producer of photoproduct.

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## References and Notes

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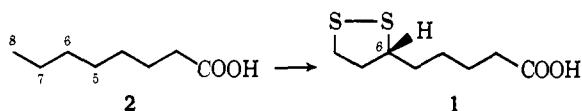
IBM Corporation, San Jose, California, 95193

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## Biosynthesis of Lipoic Acid. 2. Stereochemistry of Sulfur Introduction at C-6 of Octanoic Acid

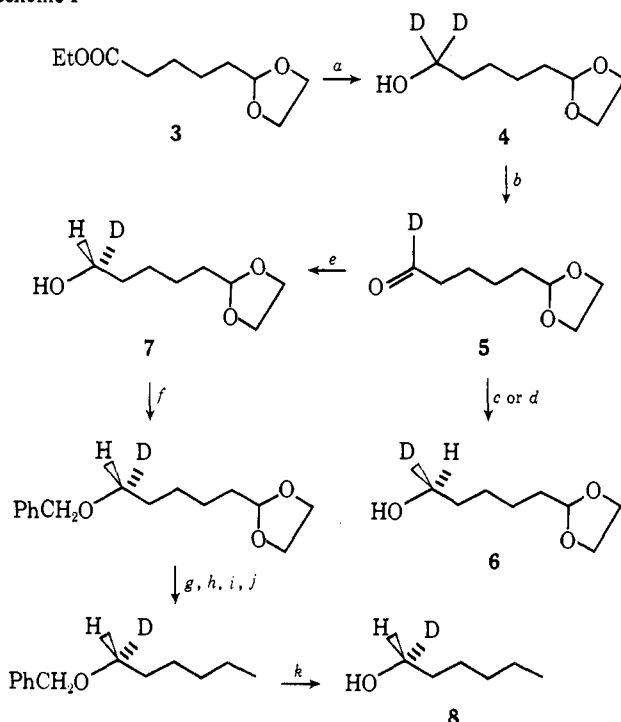
Sir:

$\alpha$ -(+)-Lipoic acid (**1**) is an essential coenzyme for all systems of  $\alpha$ -keto acid dehydrogenase complexes that have been investigated.<sup>1</sup> We recently reported experiments which establish that the biosynthesis of **1** in *Escherichia coli* proceeds



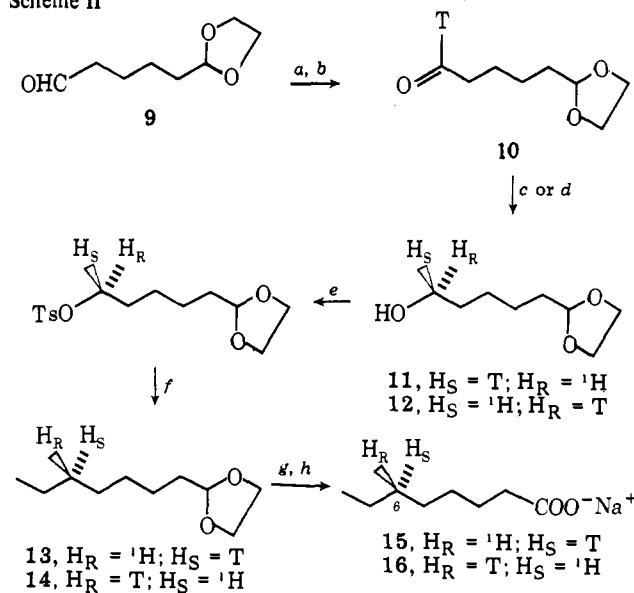
from octanoic acid (**2**) via the introduction of sulfur at C-6 and C-8 of **2** without apparent involvement of C-5 and C-7.<sup>2</sup> Earlier investigations demonstrated the operation of similar processes in the conversion of (+)-dethiobiotin to (+)-biotin.<sup>3</sup> Since the nature of reactions involved in the introduction of sulfur at saturated carbon atoms is currently unknown, we decided to investigate the stereochemistry of the sulfur introduction process. We now report the results of experiments which elucidate the stereochemistry of the introduction of sulfur at C-6 of octanoic acid.

Scheme I



<sup>a</sup>  $\text{LiAlD}_4$ . <sup>b</sup>  $\text{C}_2\text{H}_5\text{NH}^+\text{CrO}_3\text{Cl}^-$ . <sup>c</sup> Horse-liver alcohol dehydrogenase, NADH, cyclohexanol. <sup>d</sup> (+)- $\alpha$ -Pinene-9-BBN. <sup>e</sup> (-)- $\alpha$ -Pinene-9-BBN. <sup>f</sup> NaH,  $\text{PhCH}_2\text{Br}$ ,  $\text{H}_3\text{O}^+$ . <sup>g</sup>  $\text{LiAlH}_4$ . <sup>h</sup>  $\text{Ph}_3\text{P}$ ,  $\text{CBr}_4$ . <sup>i</sup>  $\text{LiBEt}_3\text{H}$ . <sup>k</sup>  $\text{H}_2$ , Pd/C.

Scheme II



<sup>a</sup> [<sup>3</sup>H]- $\text{KBH}_4$ . <sup>b</sup>  $\text{C}_2\text{H}_5\text{NH}^+\text{CrO}_3\text{Cl}^-$ . <sup>c</sup> (+)- $\alpha$ -Pinene-9-BBN. <sup>d</sup> (-)- $\alpha$ -Pinene-9-BBN. <sup>e</sup>  $\text{C}_2\text{H}_5\text{SO}_2\text{Cl}$ ,  $\text{C}_5\text{H}_5\text{N}$ . <sup>f</sup>  $\text{Et}_2\text{CuLi}$ . <sup>g</sup>  $\text{O}_3$ ,  $\text{CH}_3\text{OH}$ . <sup>h</sup> NaOH.

The elucidation of the stereochemistry of sulfur introduction was accomplished by means of precursor incorporation experiments with sodium [(6*S*)-6-<sup>3</sup>H]- and [(6*R*)-6-<sup>3</sup>H]octanoate. The synthesis of the chirally tritiated forms of octanoic acid was achieved as follows. The acetal ester **3**<sup>4</sup> (Scheme I) was reduced with lithium aluminum deuteride to the deuterated acetal alcohol **4** (93%). Oxidation of **4** with pyridinium chlorochromate<sup>5</sup> yielded the deuterated aldehyde **5** (78%). Reduction of the deuterated aldehyde with horse-liver alcohol dehydrogenase, NADH, and cyclohexanol<sup>6</sup> yielded the chirally deuterated alcohol **6**. On the basis of the stereochemistry observed when a wide variety of aldehydes are reduced by liver alcohol dehydrogenase,<sup>7</sup> it was expected that the alcohol **6** would possess the *S* configuration. Derivatization of **6** with (-)-camphanoyl chloride and examination of the NMR spectrum of the camphanate ester<sup>8</sup> in the presence of  $\text{Eu}(\text{dpm})_3$  supported this prediction: the diastereotopic hydrogen atom at C-1 of the camphanate ester of **6** resonated at higher field, as anticipated.<sup>9</sup> However, derivatization of **6** with *p*-bromophenyl isocyanate and mass spectral analysis of the derivative revealed that the chirally deuterated alcohol contained  $\sim 20\%$  of dideuterio alcohol with no detectable quantity of undeuterated alcohol being present. The dideuterated alcohol presumably arises via a dismutation reaction which is known to be catalyzed by horse-liver alcohol dehydrogenase.<sup>10</sup> A more suitable preparation of the *S* alcohol **6** proved to be the reduction of the deuterated aldehyde **5** to **6** (28%) with the adduct of (+)- $\alpha$ -pinene (81% optical purity) and 9-BBN.<sup>11</sup> NMR analysis of the camphanate ester of alcohol **6** obtained from the (+)- $\alpha$ -pinene-9-BBN reduction indicated that the reaction had proceeded to give the *S* alcohol with an optical purity of  $\sim 80\%$ . Similarly, reduction of aldehyde **5** with the adduct of (-)- $\alpha$ -pinene (74% optical purity) and 9-BBN yielded (28%) the *R* alcohol **7** ( $\sim 72\%$  optical purity). As an additional check of the chirality assigned to **7**, this alcohol was degraded in the manner shown (Scheme I) to [(1*R*)-1-<sup>2</sup>H<sub>1</sub>]hexanol (**8**) (28% yield from **7**) whose chirality was verified using the camphanate method.

The chirally tritiated alcohols **11** and **12** were then prepared using the same technique (Scheme II). Reduction of the aldehyde **9** with potassium borotritide (94%) and oxidation of the resulting labeled alcohol with pyridinium chlorochromate gave (81%) the tritiated aldehyde **10**. Reduction of **10** with the