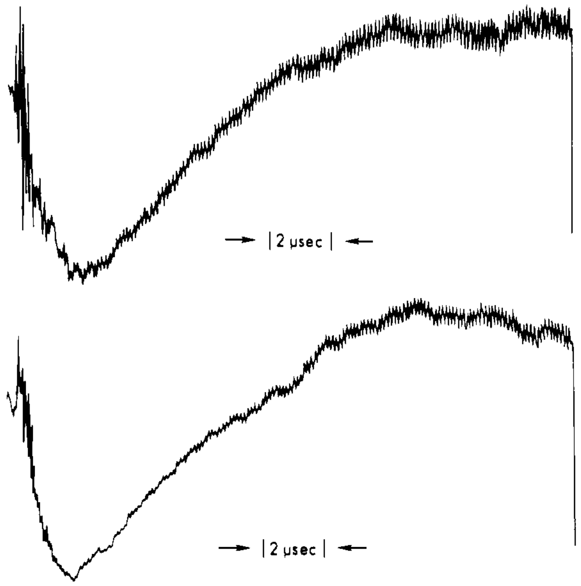

Figure 1. Transient response on formation of **1**. (a) 500 pulses at high-field XY resonance minus 500 pulses off resonance. The low-field transient has the same kinetics but is inverted. (b) 1000 pulses at high-field XY resonance minus 1000 pulses at low-field XY resonance. The signal to noise ratio is enhanced by this procedure. Subtraction removes the field-independent transients which result from the perturbation of the cavity of the laser flash.

The photolyses with plane-polarized light were carried out only in methyltetrahydrofuran glass owing to its superior optical qualities. The optical absorption of the product biradical is dichroic following short photolysis with plane-polarized light in the region 330–380 nm. The peak at 510 nm ($T_0 \leftrightarrow T_1$) is polarized parallel to the polarization of the photolyzing beam, and the peak at 496 nm ($T_0 \leftrightarrow T_2$) is polarized perpendicular to it.[7]

In the observations of the magnetic anisotropy following a short period of photolysis with plane-polarized light, the EPR spectra were recorded with the direction of the photolyzing electric vector, now permanently imprinted in the rigid sample, either parallel to or perpendicular to the static magnetic field. The change is accomplished by rotating the sample tube. The procedure is superior to separate photolyses with the two different polarizations, since the number of biradicals in the cavity is unchanged as the sample is rotated. When the direction of the electric vector of the photolyzing beam is parallel to the static magnetic field, the Z peaks are of low intensity; rotation of the sample by 90° leads to increased intensity of the Z peaks and decreased intensity of the XY peaks (Figure 2).

The experiments on the anisotropies reveal the following relations between steric properties of the parent compound **2** and the biradical **1**: (1) The transition moment of the near-ultraviolet absorption of **2** is parallel to the transition moment of $T_0 \leftrightarrow T_1$ of **1** and perpendicular to the transition moment of $T_0 \leftrightarrow T_2$.[7] (2) The principal axis of the axially summetric spin–spin interaction in **1** is perpendicular to the direction of the near-ultraviolet transition moment of **2**.

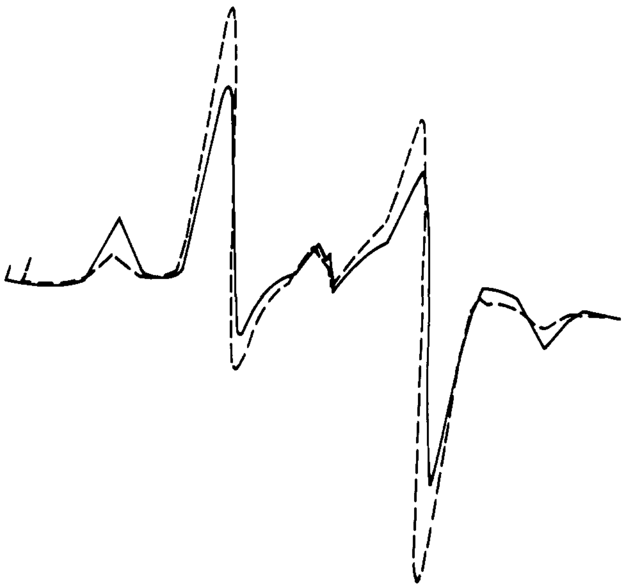

Figure 2. CW EPR spectra of **1** following photolysis by plane-polarized light. (—) Static magnetic field perpendicular to electric vector of light. (---) Static magnetic field parallel to electric vector of light.

Although the results described above establish relations between orientations of characteristic vectors of **1** and **2**, they do not fix them in the molecular axis system. The reasonable assumption that the principal axis of the dipole–dipole interaction of **1** is parallel to the axis joining its ·C–C· biradical centers removes part of the uncertainty; the transition moment in **2** is perpendicular to the ·C–C· direction.

Interpretation of the transient experiments reveals with certainty that the biradical is formed within $\sim 10^{-7}$ s of the illumination and that at 77 K it has an effective transverse relaxation time of about 5 μs. But to determine which eigenstates are populated following photolysis requires knowledge of the sign of the spin–spin splitting parameter D.

If the spins are predominantly localized on the positions indicated in structure **1**, D is negative, and the transient results require that the zero-field eigenstates T_x, T_y (the z axis being the interspin axis) are initially populated. It is likely then that the singlet state which is the immediate precursor of the crossing into the triplet manifold has nonvanishing projections on T_x and T_y.

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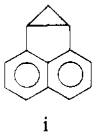
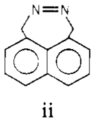
(6) Attempts to perform similar experiments on i and ii failed; the compounds are not photolyzed by light at 337 nm. Continuous irradiation of these materials is known to generate the corresponding 1,8-naphthoquinodimethanes. See: Muller, J.-F.; Muller, D.; Dewy, H.; Michl, J. *J. Am. Chem. Soc.* **1978**, *100*, 1629. Pagni, R. M.; Burnett, M. N.; Dodd, J. R. *Ibid.* **1977**, *99*, 1972. See also: Platz, M. *Ibid.* **1979**, *101*, 3398.



i



ii

(7) The latter statement also follows from the earlier observation (footnote 2) that the transition moments $T_0 \leftrightarrow T_1$ and $T_0 \leftrightarrow T_2$ are perpendicular to each other.

Enzyme-Catalyzed Organic Synthesis: NADH Regeneration by Using Formate Dehydrogenase[1]

Sir:

Existing methods for regeneration of NADH from NAD$^+$ for use in organic synthetic procedures based on NADH-dependent enzymes all have disadvantages.[2,3] Here we describe the use of

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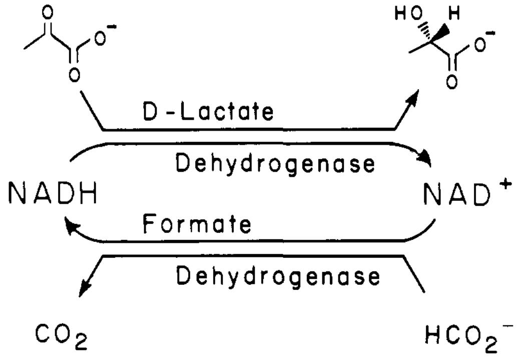
(1) Supported by the National Institutes of Health (Grant GM 26543).

Scheme I. Synthesis of D-Lactate from Pyruvate, Using Formate and Formate Dehydrogenase to Regenerate NADH



formate and formate dehydrogenase to regenerate NADH: we believe this regeneration system is superior to others presently available for many applications in synthetic enzymology. Details of the operation of the system are illustrated by the enantioselective synthesis of D-lactate from pyruvate (Scheme I).

A 3-L three-necked round-bottomed flask was equipped with a pH electrode connected to a pH controller, an argon line, and a magnetic stirring bar. The flask was charged with 1 L of degassed distilled water. Sodium formate (99.9%, 34 g, 500 mmol), sodium pyruvate (99+%, 55.0 g, 500 mmol), dithiothreitol (DTT, 10 mmol), and EDTA (10 mmol) were added. The solution was adjusted to pH 7.6. Formate dehydrogenase (FDH, EC 1.2.1.2, Boehringer-Mannheim, from *Candida boidinii*)[4] and D-lactate dehydrogenase (D-LDH, EC 1.1.1.28, Sigma) were coimmobilized in PAN gel:[5] 240 mL of swollen gel contained 52 units[6] of FDH (47% yield on immobiliztion) and 310 units of D-LDH (51% yield). The gel particles were suspended in the reaction mixture, and NAD (0.39 mmol) was added.[7] The stirred reaction mixture was maintained at ambient temperature between pH 7.4 and 7.8 by addition of 2 N HCl, using an automatic pH controller. Argon was bubbled gently through the mixture. Reaction was complete in 15 days.[8] The gel was allowed to settle, and the supernatant was decanted under positive argon pressure by using a stainless-steel cannula. The gel was washed, and the washings were combined with the original reaction solution. Zinc lactate (47.9 g, 395 mmol of lactate, 79% based on pyruvate) was isolated in 95% purity by using a modification of the procedure of Brin;[9] it contained a 92% enantiomeric excess of D-lactate.

The activities of FDH and D-LDH in the recovered gel after two consecutive runs (30 days) were respectively 86% and 89% of the original immobilized activities. After each run, the residual activity of nicotinamide cofactors (NAD plus NADH) was approximately 55%.[10] The turnover number for NAD in the experiment described was 1020; in other experiments, it reached 1500.

The formate–formate dehydrogenase system for NADH regeneration has advantages and disadvantages. The advantages are the following: formate is inexpensive and a strong reducing agent;[11] CO$_2$ and formate are innocuous toward most enzymes; CO$_2$ can be removed from the reaction as it is formed, and does not complicate the workup of the reaction; the enzyme is commercially available, readily immobilized, and stable (if protected against autoxidation). The disadvantages are the following: the commercial enzyme is presently expensive (~$62/100 U)[12] and is unable to accept NADP as substrate.[4,13]

Acknowledgment. We thank our colleagues D. Walt and V. Rios-Mercadillo for gifts of NAD and C.-H. Wong for advice concerning the isolation of zinc lactate.

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(2) Jones, J. B.; Beck, J. F. *Tech. Chem. (N.Y.)* **1976**, *10A*, 107–401; *Ibid.* **1976**, *10B*, 495–505.

(3) The most common schemes for NADH regeneration are based on alcohol dehydrogenases. These use relatively high concentrations of organic reactants and products in solution, and generate aldehydes or ketones which are often deactivating toward enzymes. Other enzymatic methods involve expensive reactants and/or weak reducing agents. Most chemical and electrochemical methods show only modest selectivity for generation of NADH: Baricas, W. H.; Chambers, R. P.; Cohem, W. *Anal. Lett.* **1976**, *9*, 257–276. Mosbach, L.; Larsson, P. O.; Lowe, C. *Methods Enzymol.* **1976**, *44*, 859–887. Wang, S. S.; King, C.-K. *Adv. Biochem. Eng.* **1979**, *12*, 119–146.

(4) Schütte, H.; Flossdorf, J.; Sahm, H.; Kula, M.-R. *Eur. J. Biochem.* **1976**, *62*, 151–160. Michaelis constants for NAD and formate are 0.09 mM and 13 mM, respectively. Activities were assayed according to Makave, A. B.; McMartin, K. E.; Palese, M.; Tephly, T. R. *Biochem. Med.* **1975**, *13*, 117–126.

(5) Pollak, A.; Blumenfeld, M.; Wax, M.; Baughn, R. L.; Whitesides, G. M. *J. Am. Chem. Soc.*, in press.

(6) I U = 1 μmol product produced per min.

(7) NAD was synthesized in this laboratory from AMP, and used in crude (~30% purity) form; Walt, D.; Rios-Mercadillo, V., unpublished data. Indistinguishable results were obtained with commercial NAD (Sigma).

(8) The reduction of NAD to NADH was the rate-limiting step in the catalytic cycle. When the reaction was carried out at 40 °C (again at 500-mmol scale), conversion of pyruvate to lactate was complete in 7 days. Both enzymes retained ~100% of their activity over the course of this experiment. The aggregate activity of the nicotinamide cofactors (NAD + NADH) at the conclusion of the reaction was 34% of that at its start.

(9) Brin, M. *Biochem. Prep.* **1953**, *3*, 61–6. The supernatant (1100 mL) was concentrated under reduced pressure at 45 °C to a volume of 500 mL. The pH of this solution was adjusted to 2.5 with concentrated HCl. Absolute ethanol (100 mL) was added and suspended solids removed by suction filtration. The solution was neutralized to pH 6.5 by addition of a slight excess of zinc carbonate and filtered. The solution was heated to 65 °C, and cold absolute ethanol was added to the solution until turbidity appeared. Crystallization was allowed to proceed for 24–48 h at 4 °C. The crystals were collected by filtration and washed twice with cold ethanol and twice with ethyl ether. Assays of chemical and enantiomeric purities were based on enzymatic methods: Bergmeyer, H. U. "Methods of Enzymatic Analysis", Verlag Chemie: Weinheim; Academic Press: New York, 1974; Vol. 3, pp 1446 and 1492.

(10) Pig heart L-lactate dehydrogenase catalyzes the conversion of NAD to an inhibitory complex of pyruvate and NAD: Wilton, D. C. *Biochem. J.* **1979**, *177*, 951–7. The same reaction may occur with the yeast D-LDH used here.

(11) The equilibrium constant for the reaction HCO$_2^-$ + NAD$^+$ $\rightleftharpoons$ CO$_2$ + NADH is log K = 5.79 (based on $\Delta G = -7.95$; "Biochemist's Hand Book"; Long, C., Ed.; Spon.: London, 1961; p 90).

(12) The effective use of the formate dehydrogenase system for regeneration of a NAD modified to improve retention in an ultrafiltration membrane reactor is described by Wichmann, R.; Wandry, C.; Bückmann, A. F.; Kula, M.-R. Abstracts, VIth International Fermentation Symposium, July 1980 London, Ontario; National Research Council, Ottawa, Canada; Abstr. F-12.1.24 (P), p 125. This group has also described an exceptionally useful new isolation procedure for formate dehydrogenase: Kula, M.-R.; Bückmann, A. F.; Hustedt, H.; Kroner, K. H.; Morr, M. *Ibid.*; Abstr. F-13.25 (P), p 139.

(13) After storage for 3 months under nitrogen at 4 °C, the coimmobilized FDH and D-LDH retained >95% of their activity.

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Unexpected Features in the Chemistry of Six- and Seven-Coordinate Alkyl Isocyanide Complexes of Chromium

Sir:

There exists within those *homoleptic* isocyanide complexes of the group 6 elements which are isoelectronic with their carbonyl congeners M(CO)$_6$ an intriguing dichotomy. While aryl isocyanides (ArNC) form the neutral six-coordinate monomers M(CNAr)$_6$,[1] which in the case of M = Cr may be oxidized both chemically and electrochemically to the mono- or dications M-

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(1) See, for example: Mann, K. R.; Cimolino, M.; Geoffroy, G. L; Hammond, G. S.; Orio, A. A.; Albertin, G.; Gray, H. B. *Inorg. Chim. Acta* **1976**, *16*, 97.

0002-7863/80/1502-7105$01.00/0