Acknowledgment. G.W.G. thanks the NIH (GM 29150), and W.R. Grace; H.D.D. and L.E. thank the Army Research Office (DAAG-29-80-G-0015), the Petroleum Research Fund (10362-G6), administered by the American Chemical Society, The National Science Foundation (CHE-7915201), and the National Institutes of Health (RR-8102) for support of this work. H.D.D. and L.E. thank the National Science Foundation for funds used to purchase the JEOL-FX-90Q NMR spectrometer (CHE-791462). H.D.D. and G.W.G. also thank the Degussa Corp. for their generous gift of chemicals in support of this project.

Registry No. 2, 76719-75-0; **3**, 76719-76-1; 15-crown-5, 33100-27-5; sodium, 7440-23-5.

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Received April 16, 1982

Mechanistic Probes of the Hydride-Transfer Process in the Reduced Nicotinamide Adenine Dinucleotide Dependent Alcohol Dehydrogenase Reactions

Summary: NADH-dependent alcohol dehydrogenase reductions of several chemically based radical-probe molecules proceed without any indication of the radical anion intermediates.

Sir: Despite the large amount of the structural and kinetic information available on the NADH-dependent alcohol dehydrogenase reactions, a complete understanding of the chemical mechanism of the crucial hydrogen-transfer step and of the transition-state structure has not yet been achieved.[1,2] The key mechanistic question is whether the hydrogen transfer between the coenzyme and the substrate carbonyl occurs in a single step as hydride or in two steps as electron and hydrogen atom. The experimental data obtained from the enzymic studies and the nonenzymic models have been variously interpreted in support of either of the two mechanistic possibilities. The in vitro models have shown that a one-electron redox process is possible between dihydropyridines and some suitable substrates.[2]

We have examined the mechanism of the hydrogen-transfer process in NADH-dependent horse liver alcohol dehydrogenase (HLADH) reactions by means of several chemically based radical probes,[3] and we herein report some of these results. Since cyclopropyl methyl radicals and cyclopropyl ketyl radical anions are known to undergo a rapid ring-opening reaction,[4] the possible ring opening was first examined in the HLADH reduction of nortricyclanone (**1**).[5] Ketone **1** (42 mg, 0.389 mmol, at least 96% pure by GC analysis on 5 ft 10% FFAP) was incubated with NADH (400 mg, 0.51 mmol) and HLADH (4 mg; Sigma) in phosphate buffer (0.1 M, pH 7, 50 mL) at room temperature under Ar in the dark for 11 h. The reduction was virtually complete over this period, and the organic product was obtained by an extractive workup (ether). GC and ^{1}H NMR analyses clearly showed that nortricyclanol (**2**) was the exclusive product and that there was no trace of the possible ring-opened products such as norcamphor or *endo*-norborneol[6] (Scheme I).

Scheme I

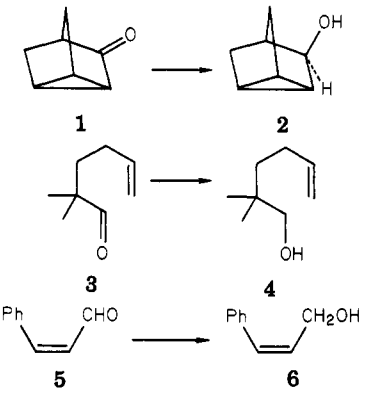

Table I

incubation time, min	cinnamaldehyde, % cis (δ 9.98)	cinnamaldehyde, % trans (δ 9.73)	cinnamyl alcohol, % cis (δ 4.48)	cinnamyl alcohol, % trans (δ 4.33)
0	97	3		
10	84		13	3
30	77		20	3
60	65		32	3
150	14		83	3

Shono et al. reported that electroreduction of nonconjugated olefinic ketones such as 6-hepten-2-one gave the cyclized alcohol products in high yields, thus demonstrating a facile cyclization of the 5-hexenyl ketyl radical anions.[7] Therefore, we next examined the possible ring closure in the HLADH reduction of 2,2-dimethyl-5-hexenal (**3**).[8] When the substrate **3** (50 mg, 0.397 mmol, ca. 90% isomeric purity) was incubated with NADH (310 mg, 0.397 mmol) and HLADH (5 mg; Sigma) in phosphate buffer (50 mL), the reduction was over within 1 h. The exclusive product was identified (GC, TLC, and ^{1}H NMR) to be 2,2-dimethyl-5-hexen-1-ol (**4**).

In view of the successful application of the stereochemical isomerization of enones as a radical anion probe in the reactions involving dialkylcuprates and Grignard reagents,[9] we have also studied the stereochemistry of the HLADH reduction of *cis*- and *trans*-cinnamaldehyde. The trans aldehyde was smoothly reduced by NADH and HLADH to *trans*-cinnamyl alcohol, as reported in the literature.[10] The cis substrate (**5**;[11] 50 mg, 3% (max) contamination by

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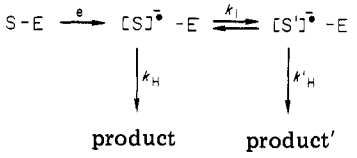
Scheme II



trans isomer) was incubated with NADH (287 mg) and HLADH (4 mg) in phosphate buffer (pH 7.0) at 5 °C under Ar in the dark. Progress of the reduction could be most conveniently monitored by analyzing the aliquots by ^{1}H NMR (Varian XL-200, FT spectrometer). The representative results of such experiments are summarized in Table I. These data together with results of similar incubations of the substrate with varying cis/trans ratios have unequivocally shown that the substrate geometry is retained during the enzymic reduction. The high ratio of *trans*- to *cis*-cinnamyl alcohol observed in the initial aliquots turned out to be due to the higher initial reduction rate of the trans isomer.

In a recent report,[12] Suckling et al. reported that (α-hydroxyalkyl)cyclopropanes were oxidized by NAD$^+$-dependent HLADH without the ring cleavage, and they have concluded that the radical intermediates are improbable in these reactions. Their data are essentially in accord with our observations. Although the most straightforward explanation for these results may well be noninvolvement of such radical anion intermediates, we point out that these probes do not take into consideration the potentially unique topology and the specific kinetic processes of the enzyme–substrate complex. Even if the ketyl intermediates are involved, the formation of the ring-opened or the cyclized product may be unfavorable or impossible, if $k_H \gg k'_H$—a likely condition because of the optimal vs. the unfavorable geometries for k_H and k'_H, respectively. Even for the stereochemical probe for which $k_H \simeq k'_H$, the topological restraints imposed by the E–S binding may not allow enough motion of the probe molecule necessary for the isomerization (Scheme II). Testing of these possibilities by computergraphic analysis and design of the better probes free of these limitations are currently under investigation.

Acknowledgment. We thank the National Institutes of Health and the Robert A. Welch Foundation for financial support of this work.

Registry No. 1, 695-05-6; 2, 695-04-5; 3, 52278-99-6; 4, 56068-50-9; 5, 57194-69-1; 6, 4510-34-3; HLADH, 9031-72-5; *trans*-cinnamaldehyde, 14371-10-9; *trans*-cinnamyl alcohol, 4407-36-7.

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