clear. Benkeser has proposed that in the n-Pr_3N–$HSiCl_3$ system, the trichlorosilyl anion ($SiCl_3^-$) is the reactive intermediate and reported nmr evidence in support of this.[10] On the other hand, Mislow suggested that n-Pr_3N–$HSiCl_3$ is an extremely complicated system and that perchloropolysilane is formed and is likely to be the reactive agent.[11] In the absence of definitive work we offer the following observations. The reaction does not occur without the addition of the tertiary amine. Thus deoxygenation of the S=O group by trichlorosilane alone, similar to the reduction of sulfoxides to sulfides,[6] does not take place. Secondly, a 1:1 adduct between sulfinate esters and trichlorosilane occurs almost instantaneously after mixing the two reagents. For example, the adduct (II:$HSiCl_3$) has quite different spectroscopic properties from either of the starting materials but decomposed on distillation. The sulfinate ester II could, however, be recovered quantitatively on hydrolysis of the adduct. It seems reasonable to suggest that it is the adduct which reacts with the amine on the pathway to product.

We are in the process of exploring the generality and mechanism of these reactions.

Acknowledgments. We thank the National Research Council and the Defense Research Board of Canada for financial support of this work. Acknowledgment is also made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial financial support.

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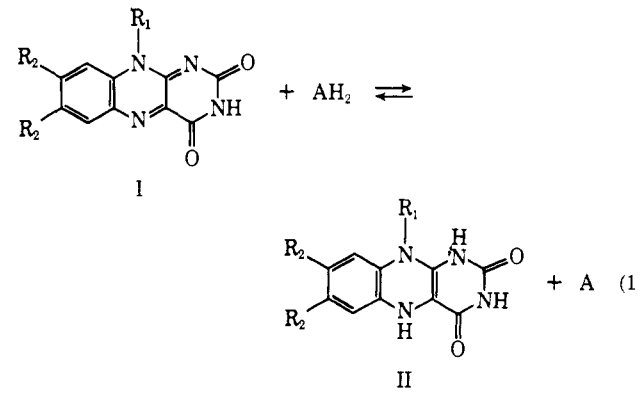
Some Model Reactions and a General Mechanism for Flavoenzyme-Catalyzed Dehydrogenations[1]

Sir:

Although the flavin coenzymes participate in many enzymic dehydrogenation reactions,[2] the mechanism of the dehydrogenation step (eq 1) has remained unclear. Several classes of such reactions, with enzymic examples in parentheses, are: (1) alcohol dehydrogenation (glucose oxidase, lactate dehydrogenase), (2) amine dehydrogenation (amino acid oxidases), (3) dehydrogenation α,β to a carbonyl



I + AH_2 ⇌ II + A (1)

(succinate dehydrogenase, acyl-CoA dehydrogenases), (4) dihydronicotinamide dehydrogenation (NADH dehydrogenases), (5) dithiol dehydrogenation (lipoamide dehydrogenase). Nonenzymic reactions related to classes 3–5 have been studied.[3-10] In this communication we report the first successful duplication of the reactions in classes 1 and 2 in a nonenzymic system in the dark. Also, a general mechanism for flavoenzyme-catalyzed reactions is suggested.

When 10-phenylisoalloxazine (I, R_1 = phenyl; R_2 = H) is allowed to react under anaerobic basic conditions with either methyl mandelate ($C_6H_5CHOHCO_2CH_3$, III) or methyl phenylglycine ($C_6H_5CH(NH_2)CO_2CH_3$, IV) in anhydrous dimethylformamide–*tert*-butyl alcohol the spectrum (solid line) shown in Figure 1 is obtained. This spectrum, especially the low absorption at 437 nm, is characteristic of the fully reduced flavin II;[11] the shoulders at 475 and 355 nm are attributable to the presence of small amounts of flavin radical anion.[12] Upon addition of dry air the reaction solution immediately turns yellow and a spectrum (Figure 1) characteristic of oxidized flavin (I) is obtained; the observed optical density at 437 nm indicates that I is regenerated in greater than 90% yield.

No spectral change is observed in the absence of *tert*-butoxide. In the absence of III and IV, *tert*-butoxide causes a shift of the 437-nm peak of I to a broad peak centered at 400 nm; this shift is reversed by acid but not by O_2. Therefore, under the reaction conditions *tert*-butoxide alone does not effect the reduction of I to II.

The expected dehydrogenation products of III and IV are $C_6H_5COCO_2CH_3$ (V) and $C_6H_5C(=NH)CO_2CH_3$ (VI), respectively. At pH 9, V is hydrolyzed to $C_6H_5COCOOH$ (VII) and the same is expected for VI. Following treatment of anaerobic reaction mixtures, which initially contained either III or IV, with aqueous

(1) This research was supported in part by a research grant (AM 13448) from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, and in part by a Predoctoral Fellowship (1967–1970) to L. E. B. from the National Institute of General Medical Sciences (GM 37,741). Presented at the 160th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1970, Abstract No. BIOL 50.
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(12) A. Ehrenberg, F. Muller, and P. Hemmerich, *Eur. J. Biochem.*, **2**, 286 (1967).

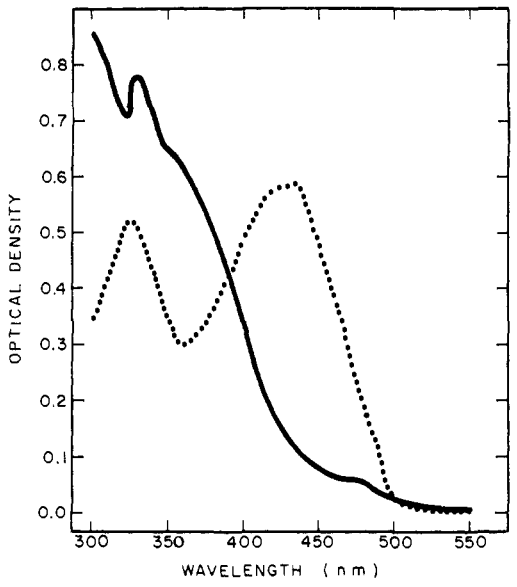
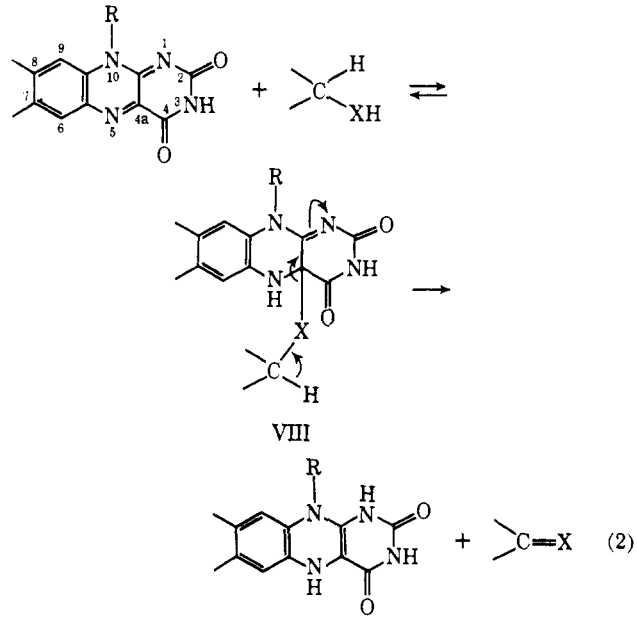


Figure 1. Spectra observed in flavin model reactions. Solid line is the spectrum observed for an anhydrous DMF–*tert*-BuOH (3.5:1, v/v) solution of I (6.0×10^{-5} *M*), III (5.8×10^{-4} *M*), and potassium *tert*-butoxide (6×10^{-4} *M*) 1 min after mixing the reagents at room temperature under anaerobic conditions in the absence of light. The spectrum changes little, if any, after an additional 10 min. The dotted line spectrum is observed upon addition of dry air to this reaction solution. Essentially identical spectral changes are observed if IV is substituted for III.

base (pH 9), VII was isolated and shown to be identical with authentic VII by comparison of uv spectra, R_f values (tlc) in glacial acetic acid and a 4:4:1 acetic acid–*n*-butyl alcohol–water solution, and uv spectra and R_f values (tlc) in glacial acetic acid, *n*-butyl alcohol, and chloroform of the 3-phenyl-2-quinoxalinol derivatives.[13] The yield of VII is 60–100% based on the initial amount of I.

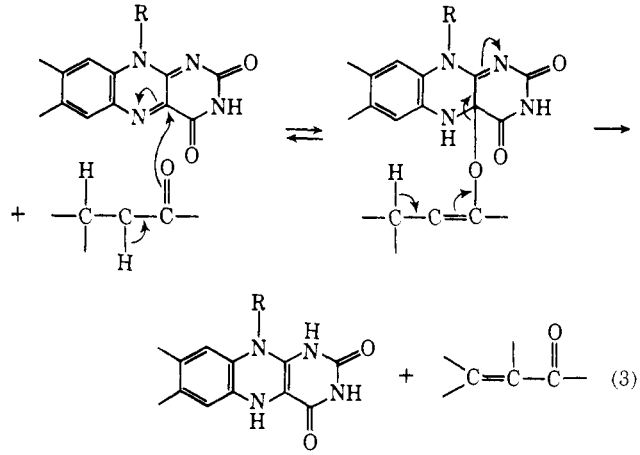
Other substrates which give spectral evidence for an O_2-reversed reduction of I under the usual conditions are the diethyl ester of aminomalonate and 9-hydroxyfluorene. However, no spectral evidence for O_2-reversible reduction of I could be obtained using any of the following as the reducing agent under the usual conditions: $C_6H_5CH_2CO_2CH_3$; $C_6H_5CH(OCH_3)CO_2CH_3$; $C_6H_5CH(NH_2)CONH_2$; $C_6H_5CHOHCOOH$; $(C_6H_5)_2CHOH$; $HCONHCH(CO_2C_2H_5)_2$; $NH_2CH_2CO_2C_2H_5$; fluorene. In each of these cases the observed spectral changes are the same as those in the absence of any substrate.

The results on substrate specificity make unlikely a mechanism for the transhydrogenation involving formation of the α carbanion of the substrate followed by a one-electron transfer to the flavin.[14] Also, the results (especially the fact that related alcohols and amines react at comparable rates) are inconsistent with a mechanism involving a hydride ion transfer. A mechanism consistent with all our results with the model system is shown in eq 2 (X = O or NH). The first step involves the addition of the substrate to the 4a position of the flavin ring system to give VIII. This should occur readily because it is similar to the



addition of nucleophiles to a Schiff base; there is evidence that such a reaction is very favorable if N-5 of flavin has a positive charge.[15] In the second step, it is proposed that the hydrogen on the α carbon is removed as a proton, electrons are transferred through I as shown, and N-1 picks up a proton. Results with the model system which point particularly to this mechanism are: substrates must have a nucleophilic group which can add to the flavin to give VIII, strong base is necessary to remove the proton from the α carbon of VIII, and it is not surprising that this hydrogen must be somewhat acidic as observed.

We suggest, and the evidence indicates, that all flavoenzyme-catalyzed dehydrogenations proceed by a similar mechanism. For enzymic alcohol and amine dehydrogenations the mechanism would be the same as shown in eq 2. A related mechanism for enzymic dehydrogenation α,β to a carbonyl is shown in eq 3;



similar mechanisms for reduced nicotinamide and dithiol dehydrogenations can be written. Evidence pointing to such mechanisms for the enzymic reactions includes the following: (1) Hellerman and his coworkers[16] found that the reaction catalyzed by D-amino

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acid oxidase proceeds by a two-step mechanism and the α hydrogen is lost as a proton; (2) Porter and Bright[17] observed that V_{max} for L-amino acid oxidase showed both a solvent deuterium isotope effect (expected if protonation of N-1 of flavin occurs in the rate-determining step) and an isotope effect in the cleavage of the α carbon–hydrogen bond; (3) for the reactions catalyzed by succinate dehydrogenase and acyl-CoA dehydrogenases, a kinetic deuterium isotope effect of differing magnitudes has been found for the removal of each of the hydrogens from the substrate, and one hydrogen exchanges more rapidly than the others with the medium;[18] (4) there is no evidence for free-radical intermediates in the dehydrogenation step of any flavoenzyme-catalyzed reaction[2] or in any model flavin reaction;[3-10] (5) hydride transfer from many typical substrates of flavoenzymes is without chemical analogy.

The distinctive features of the general mechanism proposed here for flavoenzyme-catalyzed dehydrogenations are: (1) both hydrogens are transferred as protons and (2) the substrate forms a covalent compound with the flavin ring system; the formation and breakdown of this intermediate provide a mechanism for electron transfer. Thus, the mechanism is closely related to that of most nonredox enzymic reactions. As in such cases, suitably placed acid and base groups on the enzyme surface would be expected to catalyze the flavoenzyme dehydrogenations, and may be largely responsible for the rapidity of the enzymic reactions compared to the model systems. This general type of mechanism is believed to occur widely in other enzymic redox reactions as well.[19-21]

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Theory of Chemically Induced Dynamic Nuclear Spin Polarization. VI. Polarization in Radical Transfer and Trapping Products and the Dependence on Nuclear Relaxation Times[1]

Sir:

Recently a theory has been developed capable of explaining chemically induced dynamic nuclear spin polarization (CIDNP) in radical combination and disproportionation reactions.[2-8] We now wish to show that free-radical transfer and trapping reactions can be treated with the same model when nuclear relaxation processes are included.[9] Radical transfer has also been discussed in ref 4 and 8.

We consider the formation of a geminate radical pair (RP) by a sudden reaction of the precursor molecule ^{m}M with electron spin multiplicity m. Because of singlet–triplet mixing *via* the isotropic hyperfine coupling, the time evolution of the electron spin wave function depends on the nuclear spin states of RP.[2,4,5] Consequently, the probability of cage collapse to combination or disproportionation products is a function of the nuclear spin states. Concurrent with radical pair collapse, diffusion-controlled separation into free radicals occurs with probability w_d. This competition results in enrichment of certain nuclear spin states in the cage products and depletion of the same states in the free radicals.[10] The degree of sorting can be calculated from the steady-state concentration of RP which for the nuclear spin state i is given by

$$[\mathrm{RP}]_i = [^m\mathrm{M}]k/(w_i + w_{er} + w_d)$$

where k is the rate constant for the formation of RP, w_i is the nuclear spin state dependent probability of cage collapse to be calculated from eq 9, ref 5 or eq 1, ref 6 for $m = 1$ or 3, respectively, and w_{er} covers all cage product formation resulting from nuclear spin independent singlet–triplet mixing in RP. The enhancement factor, P_{ij},[5] of an nmr transition between states i and j in the cage product is then given by (1), where $\langle I_{ij}\rangle_0$ is the nuclear spin expectation value at thermoequilibrium.

$$(P_{ij})_{cage} = \frac{w_d(w_i - w_j)}{[w_d(w_i + w_j + 2w_{er}) + 2w_{er}(w_i + w_j + w_{er}) + 2w_iw_j]\langle I_{ij}\rangle_0} \quad (1)$$

At the instant of their escape from the cage, the free radicals carry a nuclear polarization corresponding to

$$(P_{ij})_{rad} = \frac{(w_j - w_i)}{(w_i + w_j + 2w_{er} + 2w_d)\langle I_{ij}\rangle_0} \quad (2)$$

For small fractions of cage product eq 1 reduces to eq 2 of ref 6, giving the relationship between cage and free radical polarization as

$$(P_{ij})_{rad} = -(P_{ij})_{cage}/[1 + 2w_d/(w_i + w_j + 2w_{er})] \quad (3)$$

Since nuclear relaxation times in free radicals, $(T_1)_r$, are of similar magnitude as radical lifetimes, the enhancement factor in the diamagnetic product obtained in a radical-transfer reaction depends on the rate of the trapping step, k_{tr}[SH], as shown in

$$(P_{ij})_{tr} = (P_{ij})_{rad}k_{tr}[\mathrm{SH}]/(k_{tr}[\mathrm{SH}] + 1/(T_1)_r) \quad (4)$$

These considerations are supported by experiments centered around the benzyl–benzhydryl radical pair

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