are "sociation" rather than "association" constants and the limitations brought forth by Scott[8] should be kept in mind when evaluating enthalpies of adduct formation by this procedure. Nevertheless, for systems having enthalpies of adduct formation greater than about 1.5 kcal/mol, these[8] difficulties should be minimal. The incorrect spectroscopic constants will be obtained only when $\gamma_{DA}/\gamma_D\gamma_A$ is varying or is a constant very much different than unity. To detect the former complication, we strongly advocate treating the data by a Rose–Drago procedure[4,5,11] and looking at the intersections as a function of concentration.

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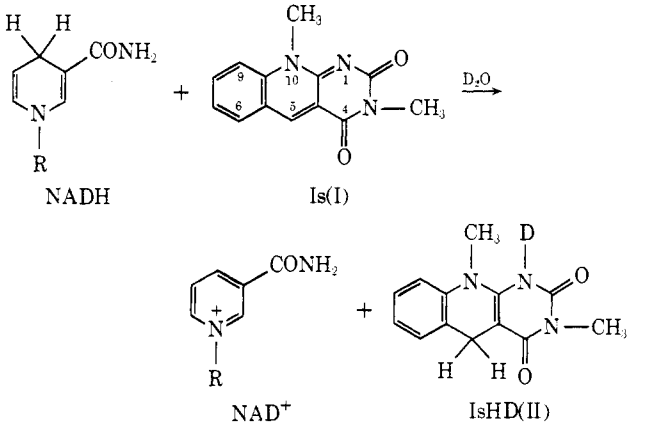
Demonstration of a Direct Hydrogen Transfer between NADH and a Deazaflavin

Sir:

Reductions by NADH have invariably been found to occur by direct transfer of a proton plus two electrons to the substrate molecule.[1] The mechanism of the biochemically important reduction of flavins (7,8-dimethyl-10-alkylisoalloxazines) by NADH is unknown though popular mechanisms reject direct hydride transfer and invoke covalent bond formation between the dihydronicotinamide and the flavin.[2] The difficulty in determining whether a direct hydride transfer occurs between NADH and flavin is undoubtedly due to the fact that the protons of the ultimate product (1,5-dihydroflavin) are bound to weakly basic nitrogens and are, therefore, exchangeable. Since the 5 nitrogen has been shown, *via* theoretical calculations,[3] to be the most electrophilic position of the flavin molecule and, therefore, the most likely recipient of a transferred hydride ion, we have investigated a compound where this nitrogen has been replaced by a carbon. The reaction of 3,10-dimethyl-5-deazaisoalloxazine (I)[4] with NADH has been examined in D_2O [80 mg of I suspended in 5 ml of D_2O containing 720 mg of the disodium salt of NADH was stirred for 3 days in the dark (argon atomsphere, 30°); the product (70 mg, 87%) was collected and washed with 2 ml of D_2O]. Except for the deuteron at position 1 the compound obtained was indistinguishable by nmr from that obtained on reduction of I with dithionite in H_2O, the nmr spectrum[5] establishing conclusively that the reduction product was IsHD(II) of the equation.



We have previously presented evidence that reduction of flavins by NADH takes place through a preequilibrium complex in which the dihdyronicotinamide does not occupy the area adjacent to the 1, 9, and 10 positions of the flavin.[6] If the present results are interpretable as evidence for transfer of two electrons from NADH to the flavin and a proton from the 4 position of the NADH to the 5 position of the flavin, then the geometry of the transition state becomes relatively defined. Evidence has been presented for direct hydrogen transfer from NADH to substrate *via* enzyme bound flavin.[7]

In passing it is of interest to note the general similarities of flavins and I. The second-order rate constants for the reactions of NADH and NPrNH with I [$k_{2,NADH}$ = 1.89 M^{-1} min^{-1} at pH 7.62, $k_{2,NPrNH}$ = 3.68 × 10^2 M^{-1} min^{-1} at pH 7.69 (30°, phosphate buffer containing 5 vol % DMF, μ = 0.19)] are not too dissimilar from the corresponding rate constants obtained with 3,10-dimethylisoalloxazine [$k_{2,NADH}$ = 53 M^{-1} min^{-1}, $k_{2,NPrNH}$ = 5.25 × 10^3 M^{-1} min^{-1}].[6] I reacts with the SO_3^{2-} component of sulfite buffer to provide the 5 adduct,[8] as previously shown for flavins.[9] Upon acidification of a sulfite–adduct solution in D_2O with DCl, pure I is generated as proven by the nmr spectrum. IsH_2 regenerates I on reaction with O_2,[10] as do 1,5-dihydroflavins,[11] and is oxidized by $(CH_3S)_2$ to yield I. The oxidation of mercaptans by flavins is well established[6,12,13] and the reduction of a disulfide by IsH_2 is the retrograde of this reaction. As in the case of flavins, I forms nonfluorescent complexes with tryptophan and β-resorcylic acid. The 1:1 complexing constants with tryptophan and β-resorcylic acid, determined by

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(4) *Anal.* Calcd for $C_{13}H_{11}N_3O_2$: C, 64.72; H, 4.60; N, 17.42. Found: C, 64.53; H, 4.55; N, 17.26.

(5) Nmr spectrum of I in $CDCl_3$: δ 8.95 [1, s, C(5)–H], 8.2–7.2 [4, m, Ar–H], 4.19 [3, s, N(10)–CH_3], 3.47 ppm [3, s, N(3)–CH_3]. Nmr spectrum of II in $CDCl_3$ (DMSO-d_6): δ 7.6–6.8 (7.6–6.8) [4, m, Ar–H], 3.83 (3.63) [2, s, C(5)–H_2], 3.51 (3.30) [3, s, N(10)–CH_3], 3.38 ppm (3.16) [3, s, N(3)–CH_3]. The proton at position 1 of IsH_2 shows a singlet at δ 4.49 ppm in $CDCl_3$.

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(8) 3,10-Dimethyl-5-sulfonate-5-deaza-1,5-dihydroisoalloxazine: λ_{max} 307 nm (ϵ 12,500 M^{-1} cm^{-1}) at pH 6.78 (10 vol % CH_3CN, μ = 0.9); K_{eq} = 3.88 × 10^2 M^{-1} at pH 6.93 (5 vol % DMF, μ = 0.19), 30°; k_f = 2.09 × 10^3 M^{-1} sec^{-1} at pH 6.89 (5 vol % DMF, μ = 0.1), 30°; nmr spectrum in D_2O δ 7.7–6.7 [4, m, Ar–H], 5.13 [1, s, C(5)–H], 3.33 ppm [6, s, N(3,10)–CH_3], the proton at position 1 shows a singlet at δ 5.4 ppm in H_2O.

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(12) I. M. Gascoigne and G. K. Radda, *Biochim. Biophys. Acta*, **131**, 498 (1967).

(13) M. J. Gibian and D. V. Winkelman, *Tetrahedron Lett.*, 3901 (1969).

fluorescence quenching, are 102 and 83 M^{-1}, respectively, for I and 86 and 57 M^{-1}, respectively, for 3,10-dimethylisoalloxazine at pH 7.85 (phosphate buffer containing 5 vol % DMF, $\mu = 1.95$).[13a]

Acknowledgment. This work was supported by a grant from the National Science Foundation.

(13a) NOTE ADDED IN PROOF. Professor P.-S. Song has extended his SDN, FOD, and π_{rr} calculations to I finding the 5 carbon the most electrophilic position. Since for both isoalloxazines and 3- and 5-deazaisoalloxazines the 5 position is the most electrophilic by these criteria, he concludes that the pattern of reactivity of the oxidized flavin should be independent of the atom at the 5 position (N *vs.* C) (private communication).

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Two Novel Reactions of Monomeric Formaldehyde with Trialkylboranes. A Remarkably Rapid Elimination Diverted by Oxygen to a Free-Radical Chain Addition

Sir:

Monomeric formaldehyde at 0° reacts rapidly with tri-*n*-butylborane to produce 1-butene and methyl di-*n*-butylborinate (eq 1). In the presence of air,

$$n\text{-Bu}_3\text{B} + \text{CH}_2\text{O} \longrightarrow n\text{-Bu}_2\text{BOCH}_3 + \text{CH}_3\text{CH}_2\text{CH}{=}\text{CH}_2 \quad (1)$$

however, the reaction takes another course, producing the one-carbon homologated ester (eq 2). The latter

$$n\text{-Bu}_3\text{B} + \text{CH}_2\text{O} \xrightarrow{\text{O}_2} n\text{-Bu}_2\text{BOCH}_2\text{Bu} \quad (2)$$

reaction proceeds through a free-radical chain mechanism involving the intermediates *n*-Bu· and *n*-BuCH$_2$O·. These two reactions appear common to other trialkylboranes.

Trialkylboranes undergo a facile 1,4 addition to α,β-unsaturated carbonyl systems.[1] The reaction involves free-radical intermediates.[2] No 1,2-addition product has been detected. Indeed, simple organoboranes do not add to the carbonyl group as do other organometallics.[3] When an attempt is made to force the addition at 100 to 150°, the reaction follows a different pathway, reductive dealkylation (eq 3).[4]

$$\text{R}_3\text{B} + \text{R}_2\text{CO} \xrightarrow{\Delta} \text{R}_2\text{BOCHR}_2 + \text{olefin} \quad (3)$$

Such reactions may involve thermal decomposition of the organoborane into R_2BH and olefin,[5] followed by reaction of the R_2BH with the aldehyde or ketone.

We now wish to report what appears to be the first direct addition of a simple trialkylborane to the carbonyl group. A 100-ml flask equipped with a magnetic stirring bar and septum inlet was charged with 20 ml of mineral oil and 3.0 g of paraformaldehyde (100 mmol). This flask was connected to a dry 100-ml reaction flask, equipped with a magnetic stirring bar and a septum inlet, by a connecting tube and distillation adapter. The vacuum takeoff of the distillation adapter was connected to a mercury bubbler and the system flushed with nitrogen. The reaction flask was cooled to 0° and then charged with 20 ml of tetrahydrofuran (THF) and 10 mmol of tri-*n*-butylborane. The mineral oil was heated to 120°, slowly raised to 140°, to generate monomeric formaldehyde.[6] After 90 min the borane (gc analysis) had disappeared and a new peak corresponding to methyl di-*n*-butylborinate had appeared. This compound was isolated and identified by comparison with an authentic sample. The reaction mixture was oxidized with 10 ml of 3 *N* sodium hydroxide and 10 ml of 30% hydrogen peroxide. Analysis by gc revealed 20.1 mmol of 1-butanol, 0.3 mmol of 1-pentanol, and 0.2 mmol of tetrahydrofurfuryl alcohol.

The small amount of 1-pentanol indicated that some addition of the tri-*n*-butylborane to the formaldehyde had occurred. The concurrent formation of the tetrahydrofurfuryl alcohol from the solvent suggested that the reaction might involve free-radical intermediates. Many free-radical reactions of organoboranes may be initiated by oxygen.[7] When the reaction was repeated and air added at 1–2 ml per min through a syringe needle directly above the THF solution, the reaction followed an entirely new path. Oxidation after 90 min produced 20.3 mmol of 1-butanol, 7.8 mmol of 1-pentanol, and 2.6 mmol of tetrahydrofurfuryl alcohol. The use of benzene avoided the formation of the side product.

The presence of iodine[8] (5 mol %) or copper *N*,*N*-diethyldithiocarbamate[9] completely inhibits the formation of the homologated alcohol without affecting the reductive dealkylation. Therefore, the homologation must proceed *via* a free-radical chain reaction while the dealkylation follows a nonradical process.

Oxygen reacts with trialkylboranes to produce alkyl radicals (eq 4). These can add to formaldehyde (eq 5),[10] producing alkoxy radicals capable of displacing alkyl radicals from the organoboranes[11] (eq 6).

$$\text{R}_3\text{B} + \text{O}_2 \longrightarrow \text{R}_2\text{BO}_2\cdot + \text{R}\cdot \quad (4)$$

$$\text{R}\cdot + \text{CH}_2\text{O} \longrightarrow \text{RCH}_2\text{O}\cdot \quad (5)$$

$$\text{RCH}_2\text{O}\cdot + \text{R}_3\text{B} \longrightarrow \text{R}_2\text{BOCH}_2\text{R} + \text{R}\cdot \quad (6)$$

The mild conditions of the reductive dealkylation suggest that it cannot involve prior dissociation of the organoborane. Instead, the reaction presumably proceeds through a six-member ring transition state similar to that proposed for the rapid dealkylation of organoboranes with *cis*-azobenzene[12] (eq 7). The precise reason why the reaction course is so different for formaldehyde and higher aldehydes is not yet clear.

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