CONTRIBUTIONS OF MOLECULAR ORBITAL TECHNIQUES TO THE STUDY OF DIHYDROPYRIDINES

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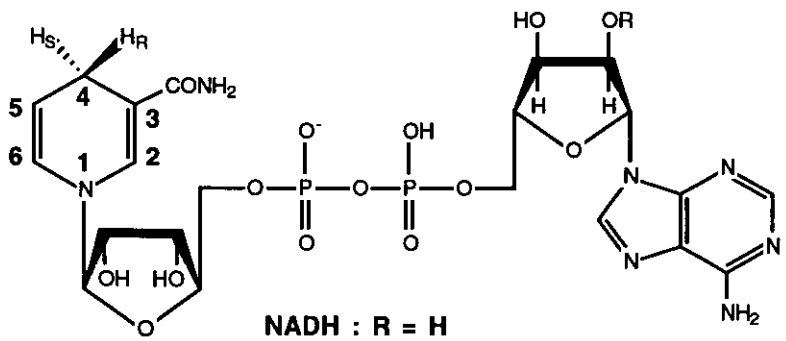
Abstract – The discovery that dihydropyridines constitute the operational subunits in important coenzymes such as NAD(P)H has directed major research efforts at understanding the redox chemistry of these heterocycles. Molecular orbital approaches have been extremely useful in this regard. This short review is designed to survey the application of semiempirical and *ab initio* techniques to the study of mechanisms of dihydropyridine oxidation, transition state structure and the mechanisms thought responsible for stereospecificity of enzymatic oxidoreductions which utilize NAD(P)H as a coenzyme.

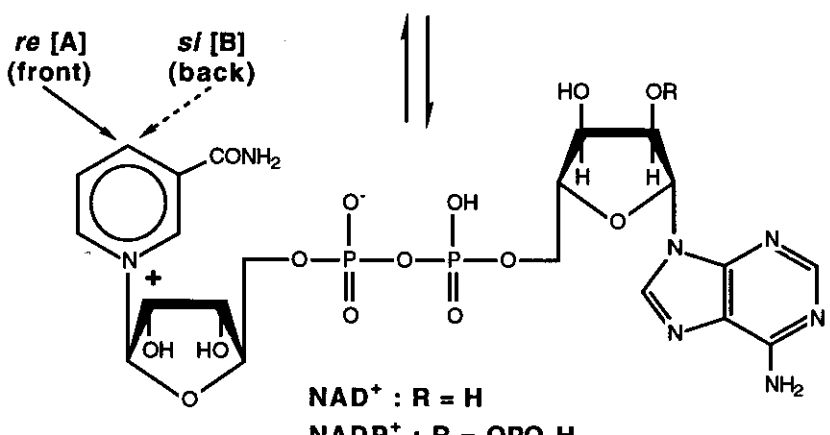
INTRODUCTION

Dihydropyridines have represented important chemical targets for research for some time and this trend is expected to continue well into the future. The interest in these compounds can be traced to several areas. *First*, a dihydropyridine substructure is contained within the reduced form of the biologically important coenzyme, nicotinamide adenine dinucleotide (NADH) and its phosphorylated analog (NADPH) constituting the biochemically active portion of the electron shuttle.[1,2] Numerous studies have therefore examined NAD(P)H as well simpler model compounds to understand the

mechanism of the redox chemistry of enzymatic trans- and dehydrogenation. In addition, the enzymes that utilize NAD(P)H as a coenzyme are highly stereoselective.[2,3] The two hydrogens at the C4 position of dihydronicotinates and dihydronicotinamides are enantiotopic and dehydrogenases are highly discriminating as to which of the two hydrogens is transferred with A-type enzyme transferring only the pro-R hydrogen and B-type enzymes, only the pro-S hydrogen.[2,3] When a substrate is oxidized, the migrating hydrogen is transferred to only one side of the pyridinium ring with A-type enzymes adding a hydrogen to the *re* (A) face of NAD^+ and B-type enzymes adding a hydrogen to the *si* (B) face. The stereospecificity of the enzymatically mediated reactions are indeed remarkable with only one mistake in 100,000,000 transfers.[4] Nonenzymatic oxidations usually lack this stereospecificity.



NADH : R = H
NADPH : R = OPO_3H



NAD^+ : R = H
$NADP^+$: R = OPO_3H

Secondly, highly substituted dihydropyridines have been found to be useful as calcium channel blockers, exerting potent hypotensive activity.[5-7] Since these compounds bind at a structurally specific receptor, the conformation of dihydropyridine-type antihypertensives is an important parameter in determining pharmacological potency.[5-7] Finally, a class of organ-targeting systems that use dihydronicotinate conjugates to enhance brain delivery of drugs has been recently described.[8-14] These chemical delivery systems rely on the conversion of dihydropyridines to pyridinium salts, biomimicking the NADH→NAD^+ oxidation, to achieve selective central nervous system sequestration.

While many questions on the chemistry and conformation of the above mentioned compounds can be evaluated experimentally, many are inaccessible to such treatment. As reviewed by Dewar,[15] reaction mechanisms cannot generally be examined by direct experimentation since they take place in such a short period of time. The duration of many reactions is less than 0.1 ps indicating that constraints on measurements are imposed by the Heisenberg uncertainty principle and not by the particular experimental techniques. *This places the study of reaction mechanisms squarely in the* purview of theory and in particular, molecular orbital (MO) theory. The purpose of this short review is to highlight recent contributions of various experimental and MO approaches to the study of the dihydropyridines with emphasis on (1) the mechanism of dihydropyridine oxidation, (2) the transition state structure for oxidation of dihydropyridines and (3) the basis for the stereospecificity demonstrated in NADH and similar compounds in dehydrogenations and other reactions. In the important review on dihydropyridines by Eisner and Kuthan in 1972,[16] fewer than a dozen papers on theoretical evaluations of simple systems were cited, all of which neglected σ-π electronic interaction. By contrast in 1993, Bruice described the binding of NADH and NAD^+ to several dehydrogenases using AM1 optimized NADH/NAD^+ fragments and molecular mechanics/dynamics simulation of the enzyme active site.[17]

METHODS

This review will examine studies of dihydropyridines in which three main classes of computational chemistry techniques have been applied, including molecular mechanics and dynamics, semiempirical molecular orbital techniques and *ab initio* methods. While anything close to an in-depth discussion of these methods is clearly outside of the scope of this compilation, a brief description of the methods may be helpful. Molecular mechanics/dynamics (MM2, CHARM, AMBER, DISCOVER) utilize a series of potential functions to represent the internal energy of a molecule.[18,19] The generated energy function treats atoms in the molecules as a collection of particles held together by an elastic (classical-mechanical) force. As an example, the AMBER (Assisted Model Building with Energy Refinement) force field takes the form:

$$E_{Total} = \sum_{Bond} k_r (r - r_0)^2 + \sum_{Angles} k_\theta (\theta - \theta_0)^2 + \sum_{Dihedral} \frac{k_\phi}{2}(1 + s \bullet \cos n\phi) +$$

$$\sum_{i<j} \left(\frac{a_{ij}}{r_{ij}^{12}} - \frac{b_{ij}}{r_{ij}^{6}} + \frac{q_i q_j}{D \bullet r_{ij}} \right) + \sum_{H\text{-bond}} \left(\frac{c_{ij}}{r_{ij}^{12}} - \frac{d_{ij}}{r_{ij}^{10}} \right)$$

where the first term examines bond stretching using a Hooke's law approximation, the second term, which is of the same form of the first, examines bond angle bending, the third term accounts for torsional or dihedral deformations, the fourth term contains both a Lennard-Jones term to estimate nonbonded (London) interaction as well as electrostatic terms derived from Coulomb's law and the last term estimates energetic contributions by hydrogen bonding.[18] Dynamics can be included by determining molecular trajectories from the forces on each atom. Clearly, these approaches do not explicitly treat electrons and therefore electronic properties of molecules cannot be predicted. In the context of this review, molecular mechanic/dynamics are used to model enzyme active sites and in some cases to examine NADH in the active site.

Both *ab initio* and semiempirical methods are based on the molecular orbital formalism with different philosophies.[18-22] *Ab initio* approaches select a model for a particular wavefunction and then perform the necessary calculations without further simplification. In such approaches, error is associated with

the selected basis set and the level of treatment of electronic correlation. Semiempirical techniques use experimental data to parameterize electronic models. This greatly improves their speed and "experimental" accuracy. While these methods do replace certain terms with parameters, they are based on the same molecular orbital formalism as *ab initio* methods.

Within the nonrelativistic approximation, a molecular system is described by the Schrödinger equation:

$$H\Psi = E\Psi$$

where H is the Hamiltonian operator, which is the sum of the quantum mechanical kinetic energy operator and the classical potential energy of a system of nuclei and electrons.[20] The eigenfunction, Ψ, is known as the wavefunction of the system, from which all molecular properties may be calculated and E is the total molecular energy. The Schrödinger equation is not separable and therefore cannot be solved exactly except for simple systems. Consequently, one is forced to make approximations, the first being the separation of electronic and nuclear motion (the Born-Oppenheimer approximation). Next, one usually makes the molecular orbital approximation, which assumes that Ψ can be written as a determinant of one-electron functions (orbitals). It is further assumed that these orbitals are linear combinations of a finite set of basis functions, which are usually similar to atomic orbitals. The coefficients are determined by minimizing the energy of this approximate wavefunction. This leads to the (matrix) Hartree-Fock equation:

$$\mathbf{FC} = \mathbf{SC}E$$

which must be iterated to self-consistency, since the Fock matrix **F** depends on the MO coefficients **C**. **S** is the overlap integral and E is a diagonal matrix of orbital energies. This method is called the self-consistent field (SCF) procedure. The time consuming steps in this method are the evaluation and manipulation of the m^4 two-electron integrals which are involved, where m is the size of the basis set.

A large number of basis sets have been derived and what follows is a superficial description of some of the more commonly used schemes. The simplest system contains a minimal basis set. This contains a basis function (χ) for the elementary valences of the atoms of interest. In methane, for example, these include the 1s, 2s and 2p orbitals for carbon and the 1s orbital for hydrogen. In the best case, Slater functions are used to represent the s- and p-orbitals. Unfortunately, integrals involving Slater functions are costly to evaluate and as a result, gaussian functions are used to mimic Slater orbitals.[19] Thus, in the STO-3G (Slater-type orbital) basis set a linear combination of three Cartesian gaussian functions is used to represent one Slater orbital (1s, 2s, 2p, etc.).[22] For the STO-5G minimal basis set, five gaussians are used to construct an orbital. Unfortunately, minimal basis sets cannot expand or contact valence molecular orbitals in response to environmental changes.[18]

Greater flexibility can be achieved by building valence orbitals out of two components, a compact core element and a diffuse outer element, both of which can be optimized during orbital construction. Thus, the N-31G (e.g. 4-31G) basis set is composed of N primitive gaussian functions that represent the core electrons and a composite of three and one gaussian functions to represent the inner and outer components, respectively, of valence orbitals. Polarization is included in *ab initio* techniques by allowing higher order orbital mixing. For example, by mixing d and p orbitals or p and s orbitals, the center of the orbital can be moved from the atom center.[18,22] Inclusion of d-orbitals to account for polarization is indicated by an asterisk so that the 6-31G* basis set augments the 6-31G set by a single set of six d-functions. The 6-31G** has both heavy atom polarization (d-orbitals) and hydrogen polarization (p-orbitals) included. SCF methods neglect the electron correlation energy which may be an important energetic contribution. The correlation energy may be estimated in a number of ways including second and third order Møller and Plesset perturbation theory (MP2 and MP3).

As the computationally intensive portion of *ab initio* SCF calculations is the evaluation and manipulation of the m^4 two-electron integrals, several attempts have been made to increase the speed of SCF calculations by reducing the number of two-electron integrals and by introducing parameters into the MO formalism. Pople used higher level *ab initio* calculations to derive parameters

for semiempirical MO at three levels of ZDO (zero differential overlap) approximations.[22] In the complete neglect of differential overlap (CNDO) framework, all exchange integrals are neglected and all atomic orbitals are treated as spheres when calculating electron repulsion integrals. The directionality of p-orbitals was only included in certain one-electron terms. The intermediate neglect of differential overlap (INDO) retains one-center repulsion integrals between orbitals on the same atom. The neglect of diatomic differential overlap (NDDO) approximation is the least drastic of the three approximations. The directionality of p-orbitals is retained in calculating resonance integrals and three- and four-center integrals in which overlap occurs between orbitals on the same atom are included.

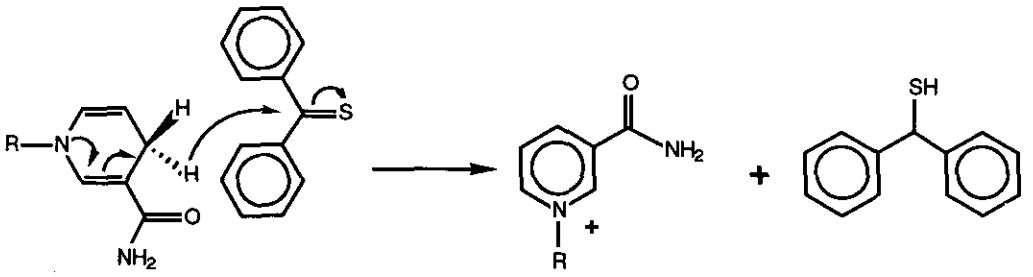
By parameterizing the semiempirical techniques with experimental data, the chemical accuracy of molecular orbital methods was significantly increased.[21] The success of this manipulation can be appreciated by numerous studies showing energetic data within 1.0 kcal/mol of experimental results. Three major approaches have been developed based on this formalism by Dewar. The modified intermediate neglect of differential overlap (MINDO/3) program was a modification of the INDO method in which two-electron one-center repulsion terms were determined by parameters fitted to experimental results.[23] The modified neglect of differential overlap (MNDO) and the Austin Model 1 (AM1) are both semiempirical methods based on the NDDO approximation.[24-26] There are several advantages in using the NDDO system, including the need to parameterize only for atom type rather than for bond type as in the MINDO/3 model. Secondly, systematic errors in treating nonbonded electron associated with INDO-based methodologies are avoided in the NDDO framework. While similar, AM1 is more reliable for certain calculations including hydrogen bonds.

In comparing *ab initio* and semiempirical approaches, the MNDO method compares favorably with *ab initio* calculations at the 4-31G level in the estimation of chemical energy and is superior to minimal basis set calculations.[15] The AM1 method approaches the accuracy of the 6-31G* basis set calculation but in both cases the time required is much shorter.[26] In the case of MNDO and the 4-31G method, the *ab initio* method requires over 1000-fold more computer time than does a similar

calculation using MNDO, meaning that a 3 hour 4-31G project would require 10 sec using the semiempirical technique.

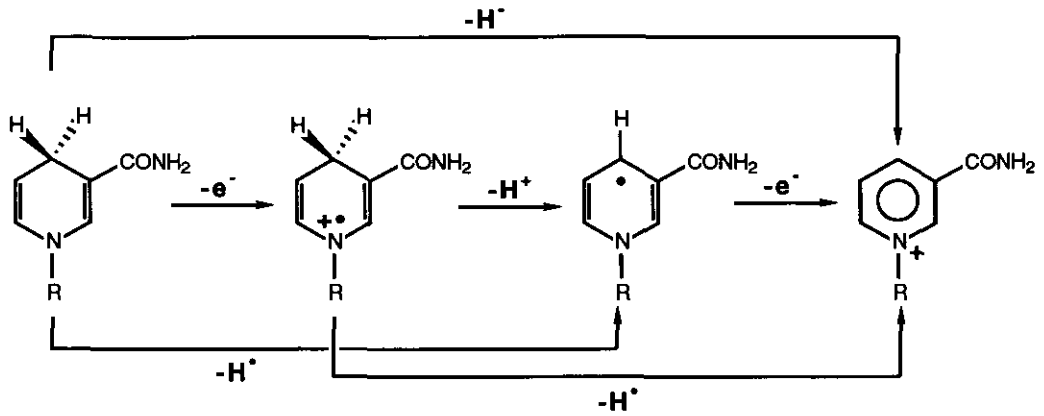
MECHANISM OF HYDRIDE TRANSFER

Experimental Background. The classical work on the reaction of dihydropyridines and hydride acceptors was done by Abeles and Westheimer in the late 1950's.[27] In a study of the reaction between substituted 1,4-dihydronicotinates and thiobenzophenones, the products, i.e., the nicotinamide salt and the mercaptan, were found to form *via* second order kinetics, first order with respect to the dihydronicotinamide and first order with respect to the benzophenone. The rate of oxidation was not affected by oxygen, free radical scavengers or pH suggesting that free radical was not involved in the reaction. The reaction rate was however significantly affected by solvent polarity in such a way so as to suggest the formation of a transition state of higher polarity than the starting materials. Electron withdrawing groups on the thiobenzophenone increase the electrophilicity of the thiocarbonyl function and produced compounds which reacted faster than the unsubstituted parent. Finally, a large kinetic isotope effect was observed (k_H/k_D = 4-5). The collected data were taken to indicate that oxidation was occurring *via* transfer of hydride in a single step.



In 1971, Steffens and Chipman proposed that the reduction of trifluoroacetophenone by substituted 1,4-dihydropyridines was at least a two step process.[28] This suggestion was prompted by the observation that the kinetic isotope effect (k_H/k_D) of the reaction showed a wide range of values inconsistent with a common hydride transfer. In addition, the degree to which deuterium was

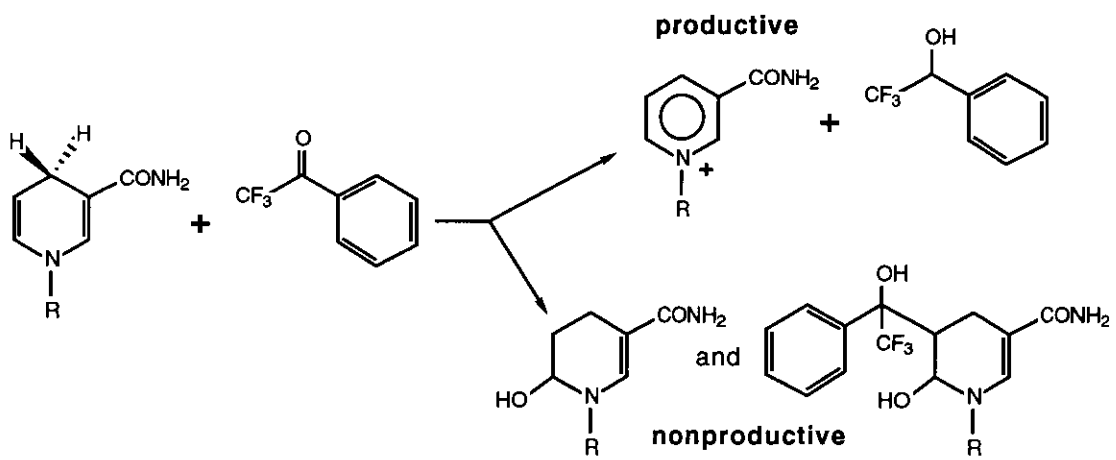
incorporated into the hydroxy products was not consistent with the kinetic isotope effect. These data suggested that a kinetically important intermediate formed on the reaction coordinate prior to product formation. The nature of this intermediate was unclear from these early studies but some speculation that a noncovalent charge-transfer complex was involved was posited. Similar contentions were made by Creighton and Sigman who examined the reaction between substituted 1,4-dihydronicotinamides and *N*-methylacridine.[29] In this system, the isotope effect and isotope partitioning were also found to be inconsistent with a simple bimolecular oxidative mechanism and suggested a kinetically important intermediate which formed from the reactants in a pre-equilibrium step which then collapsed to product. The magnitude of the rate constant for formation of the intermediate was considered, however, to be to low to be indicative of a charge-transfer complex. Later, Hajdu and Sigman studying the same reaction found spectrophotometric evidence of a kinetically competent charge transfer complex upon mixing of the two reactants.[30] It was suggested, however, that formation of a noncovalent intermediate in a pre-equilibrium step followed by product formation was not consistent with kinetic evidence since the formation of the complex would be limited to the overall reaction rate while formation of such species usually approaches the diffusion-controlled limit. Thus, the charge-transfer complex that forms is either anticipatory to a second intermediate on the reaction coordinate or is a nonproductive side reaction. The nature of the possible intermediates on the reaction coordinate was explored by several groups and evidence began to accrue that they were dihydropyridine radical cations. It was shown for example that the NAD(P)H system can undergo oxidation by an initial electron transfer followed by hydrogen atom migration and in fact the nature of the hydrogen migration was further subdivided as a hydrogen radical or as a proton and subsequent electron transfer (see below).[31] It was suggested therefore that the observed charge transfer complex was the vector through which the initial electron transfer was mediated although clearly not all system demonstrated a spectroscopically observable charge-transfer complex.[32] Such hypotheses were bolstered by ESR studies which indicated the formation of radicals in the oxidation process.[33,34] In 1981, Ohno in a series of papers examined the reaction between substituted trifluoroacetophenones and 1-propyl-1,4-dihydronicotinamide and between *N*-methylacridine and substituted 1-phenyl-1,4-dihydronicotinamides.[35,36] The first set of studies found



that the oxidation of dihydropyridines and reduction of the acetophenones was catalyzed by magnesium ions consistent with an initial electron transfer. Furthermore, an analysis of the kinetic and product isotope effects correlated with Hammett substituent constants for both the Mg^{++}-catalyzed and uncatalyzed reaction provided evidence that the reduction consisted of at least two distinguishable steps.[35] The initial process was consistent with an electron transfer to form a dihydropyridine radical cation while the second was associated with a negative Hammett reaction constant and significant isotope effect. This was interpreted to be a proton transfer from the radical cation to form the dihydropyridine free radical necessitating an ultimate electron migration in a non-rate determining step. In the second set of experiments, significant correlations were found between the second order oxidation rate constants for the reaction of *N*-methylacridine and substituted 1-phenyl-1,4-dihydronicotinamides and both the pKa values of the component anilines and the Hammett sigma constants.[36] The linear Brønsted relationship suggested that changes in electron density at the dihydropyridine ring nitrogen were the driving force of the reaction. A plot of isotope effect (k_H/k_D) as a function of substituent constant gave a parabolic relationship with a maximum for the 4-methyl derivative while the isotope partitioning was constant. This pattern was suggestive of a pre-equilibrium step in that the hydrogen atom was only partially involved in the rate determining step. The pre-equilibrium step was insensitive to an isotope effect suggesting an initial electron transfer followed by a rate determining proton or hydrogen atom abstraction. Work on the degenerate

reaction between 1-benzyl-1,4-dihydronicotinamide and 1-benzylnicotinamide salt oxidation also supported the formation of a radical cation intermediate in the redox reaction.[37,38]

The multiple step oxidation of dihydropyridines was not however to remain unchallenged. For certain reaction such as the oxidation of dihydropyridines by Δ^1-pyrrolidine-2-carboxylic acids, Srinivasan demonstrated that kinetic and isotopic data were consistent with a concerted hydride transfer.[39] In addition, Roberts *et al.* found that several degenerate and nondegenerate oxidoreductions using *N*-substituted acridines, nicotinamides and 3-quinolinamides were well described by Marcus theory.[40] Since this construct applies to a one-step process, in this case hydride transfer, multistep reaction mechanisms would show up as Marcus theory-calculated rate constants far in excess of experimental values which were not observed. Most importantly, conclusions drawn about multi-step oxidations were challenged by Chipman, one of the authors who first championed the stepwise mechanism.[41] Specifically, the reaction of trifluoroacetophenones with dihydronicotinamides was associated with a number of nonproductive side reactions including hydration and reversible adduct formation which accounted for many of the kinetic isotope and product composition discrepancies.



This was countered by experiments in which water was excluded from the reaction mixture precluding hydration and by selecting NAD(P)H model compounds that did not undergo the nonproductive side

reactions. Shinkai, for example, examined the oxidation of 1-benzyl-1,4-dihydroquinoline-3-carboxamide by *N*-methylacridine.[42] Since the quinoline derivative represents a circumstance in which the dihydropyridine is annelated with a benzene ring at the 5 and 6 position, it is unreactive at those positions to possible hydration or adduct formation. Results of these studies indicated significant differences in kinetic isotope effects and isotopic product ratios consistent, again, with a multi-step mechanism. Bruice, however, argued that the apparent discrepancy between isotope effects and product composition was the result of three phenomenon: isotopic scrabbling, neglect of the secondary isotope effect and hydride tunneling.[43,44] Scrambling occurs when the isotope is transferred from the dihydropyridine to *N*-methacridine to give the acridan which further reacts degenerately with a second molecule of *N*-methylacridine to introduce the label into the oxidant. This could alter the ratio of deuterium in the product yielding the inconsistent incorporation data. In addition, most studies had largely ignored secondary isotope effects, that is $k_H{}^D$ and $k_H{}^H$ were assumed to be equal. While such assumptions do not introduce error when examining the interaction of dideuterio and dihydro derivatives, significant problems could occur when monodeuterio systems are examined. Thus, an unaccounted for secondary kinetic isotope effect as small as 3-4% was found to alter the k_H/k_D as much as 20%.[44] Finally, studies on the dependence of the primary isotope effect on temperature suggest some degree of tunneling during hydride transfer in dihydropyridines i.e., the difference in activation energies for transfer of deuterium and hydrogen exceeds the zero-point energy. The tunneling coefficients are sufficiently high to significantly contribute of the overall rate. When these three complicating factors were taken into account, it was contended that there was not even a single case where significant discrepancies between kinetic isotope effect and isotopic partitioning occurred.[44] Thus in the majority of oxidation of dihydropyridines, the mechanism of transfer involved concerted hydride transfer.

Similar conclusions were drawn by Verhoeven based on electrochemical grounds.[45,46] If an initial electron transfer were to occur in the oxidation of dihydropyridines, the ratio of experimentally derived rate constants for dihydropyridine oxidation and the maximum rate for the single electron transfer reaction should be less than one. When these ratios were examined, the only systems that passed

this test involved those in which strong one-electron oxidants such as ferricyanide and ferrocenium ions are present.[46] Dihydropyridines can therefore be forced into sequential oxidative process in the presence of a strong kinetic or thermodynamic one-electron oxidant but in the absence of such conditions, concerted hydride transfer appears to be preferred.

Theoretical Evaluations. For the most part, computational explanations for the mechanism of dihydropyridine oxidation have followed the prevailing experimental suggestions. Thus, Inagaki and Hirabayashi suggested in 1977 that the oxidation of dihydropyridines proceeded by a sequential electron-proton-electron shift.[47] Using frontier molecular orbital approaches, it was suggested that the transition state for the oxidation involves the migration of the hydrogen at C4 as a proton from the radical cation. As experimental data shifted in support of a concerted hydride transfer, more publication suggested that this was the preferred mechanism. Computational approaches have, however, been quite useful in clarifying many of the mechanistic uncertainties mentioned in the forgoing discussion. Tapia *et al.* examined the relative preferences of hydride versus multi-step processes using *ab initio* calculations at the 4-31G basis set level.[48] A number of diabatic schemes were calculated for the degenerate oxidoreduction of cyclopropene/cyclopropenium ion, 1,4-dihydropyridine/pyridinium ion and the formaldehyde/methoxide ion in addition to the interaction of cyclopropenium with methoxide ion to give cyclopropene and formaldehyde and the reaction of methoxide ion with pyridinium ion to give dihydropyridine and formaldehyde. The latter reaction was scrutinized carefully as it was considered to be a useful model for enzymatic oxidation of alcohols. In this diabatic scheme, hydride transfer was preferred over sequential oxidative mechanism. The hydride transfer occurred without a significant activation barrier while radical cation formation was highly endothermic.

While concerted hydride migrations make up the vast majority of dihydropyridine oxidation, sequential mechanisms are still of interest. As indicated previously, strong one-electron oxidants induce sequential oxidation and this is thought to be important in the oxidation of NAD(P)H by the enzyme catalase.[49,50] In addition, sequential oxidation of dihydropyridines is induced by ferrocenium

derivatives and ferrocyanide anions, two potent one-electron oxidants. Miller has characterized the oxidation of 1-substituted 1,4-dihydronicotinamide by ferricenium cations to be first order with respect to each species.[51-53] The rates of reaction are unaffected by pH and there is not a measurable kinetic isotope effect. These results are consistent with an initial rate-limiting electron transfer. The reaction with ferricyanide is not as clear. Various studies have shown that the redox reaction is first order with respect to each compound and that there is a small kinetic isotope effect.[54-56] These data suggest that a simple second order reaction adequately described the mechanism. On the other hand, the observation that added base accelerates oxidation and that added ferrocyanide inhibits the reaction suggests a reaction in which the oxidant reacts with dihydropyridines in a rapid pre-equilibrium step yielding the intermediate radical cation.[57] The radical cation then undergoes a rate-determining proton loss to give a pyridinyl radical which rapidly collapses to product through a nonrate-limiting electron transfer to ferricyanide. In such a case, the initial electron transfer is only partially rate-determining as it controls the availability of the radical cation. Such a mechanism was shown to occur in the ferricyanide-mediated oxidation of 1-methylacridan as the reaction demonstrated general-base catalysis.[49,58] An investigation of the relative importance of electron versus proton loss in examining the ferricyanide-mediated oxidation of dihydropyridines has been examined using both experimental and theoretical paradigms.[59-61] The rate of oxidation of 1-(4-substituted phenyl)-1,4-dihydronicotinamides with ferricyanide was highly correlated with Hammett sigma constants over a reactivity range of 30,000-fold suggesting a common mechanism.[60] Since the initial electron loss is either rate determining or partially rate determining, there should be a significant correlation between the energy required to remove the electron and the log of the second order rate constants for oxidation. Such correlations were indeed found when calculated AM1 vertical or adiabatic ionization potentials were compared with ferricyanide-mediated oxidation rates as shown in Figure 1.

The relationships between hydride transfer and sequential oxidations involving a rate determining electron loss have been of interest and several attempts have been made to differentiate between these two mechanisms using substituent effects. Theoretical evaluations demonstrated that the

energy required removal of an electron from the HOMO (vertical or adiabatic ionization potential) and the energy associated with hydride loss to form the corresponding pyridinium species were highly correlated for a wide variety of structures (dihydropyridine, dihydroquinoline, dihydroisoquinoline) and substituents.[62-64] Interestingly, a group of substituents, characterized as having lone electron pairs available for dihydropyridine ring donation, produced derivatives that were less stable (endergonic) to hydride transfer that would have been predicted based on their adiabatic ionization potential.[63]

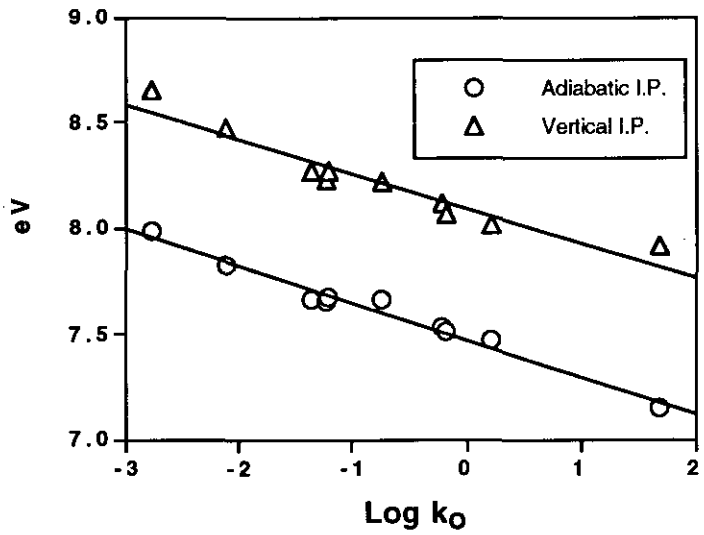


Figure 1. Correlation between AM1-derived vertical and abiabatic ionization potentials for a series of 1-(4-substituted phenyl)-1,4-dihydronicotinamides and the corresponding log of the second-order rate constant for ferricyanide-mediated oxidation.

These effects, as illustrated in Figure 2, were rationalized on ground state effects which control electron loss and effects mediated through an intermediate undergoing a change in symmetry which controls the hydride transfer. In most cases, however, substitution affects electron loss to the same degree that it affects hydride loss suggesting that substituent effect are not useful in differentiating between concerted and sequential mechanism.[57] This is reasonable since both mechanisms involve the development of a positive charge in their respective transition states.

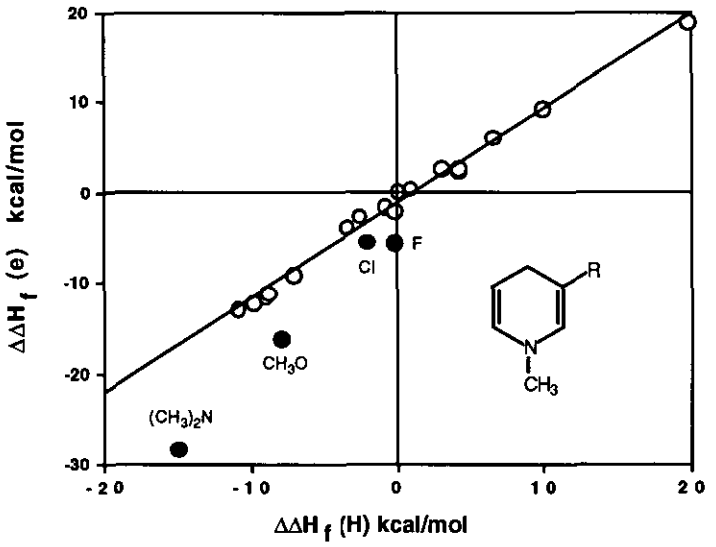
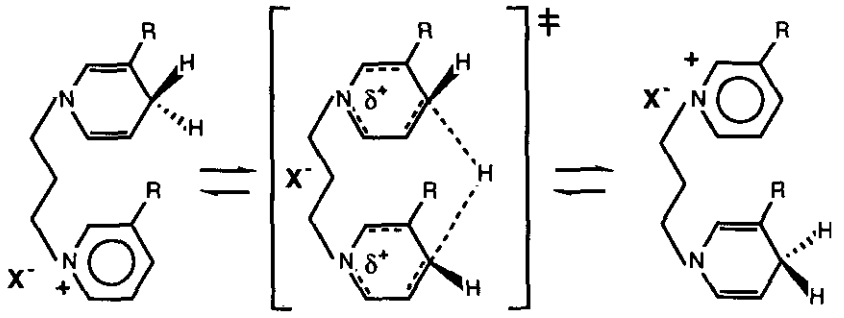


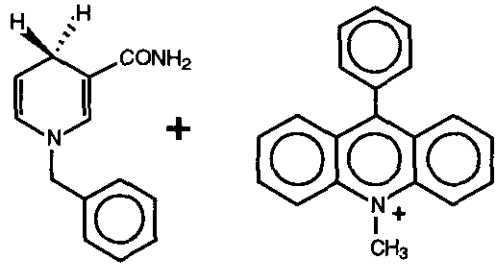
Figure 2. Relationship between the energies associated with electron and hydride removal for a series of 1-methyl-1,4-dihydronicotinamides. The data are given relative to 1-methyl-1,4-dihydronicotinamide (0,0). Four substituents were not well described by the linear relationship which are identified by closed circles.

TRANSITION STATE STRUCTURE OF THE OXIDATIONS OF DIHYDROPYRIDINES

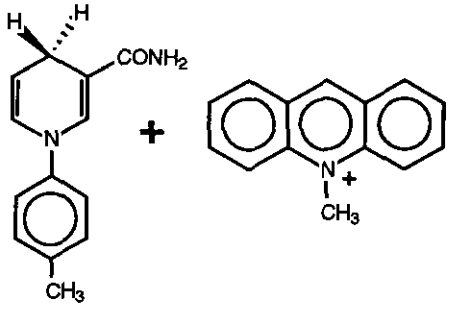
Experimental Background. Transition state structure associated with both enzymatic and nonenzymatic oxidations has been evaluated. In the latter case, the geometry of the hydride transfer i.e., whether the transfer is linear or bent, as well as the juxtaposition of the hydride acceptor and donor have been of interest. In the enzymatic case, X-ray structures have been used to fix hydride donors and acceptors into a particular configuration to view the resulting hydride migration. In the case of nonenzymatic reactions, the observation of π(charge-transfer)–complexes on the reaction coordinate of dihydronicotinates and hydride acceptors such as *N*-methylacridine suggested a model in which the oxidant and reductant were face to face with the hydride transfer occurring in a bent fashion.[30] The issue of hydride transfer geometry in model reaction was stimulated by the studies on the temperature dependence of the primary kinetic isotope effect.[65] This is a controversial semiclassical approach that uses calculated activation energies and the pre-exponential Arrhenius (frequency) A factors to give insight into transition state structure. As reviewed by Verhoeven, three circumstances are described.[46] First, if there is no difference in the activation energy of hydrogen versus deuterium transfer and a temperature independent kinetic isotope effect such that $k_H/k_D = A_H/A_D$ and if the isotope effect is in the range of 1.4 to 6, a bent transition state is assumed. If a linear transition state occurs, a significant difference between the energies of activation for the isotope transfers should occur with the upper limit represented by the difference in the C-H and C-D zero point energies which is approximately 1.15 kcal/mol. The A_H/A_D ratio for a linear transition state is estimated to be between 0.7 and 1.4. If the difference in energies of activation between hydrogen and deuterium is greater than 1.15 kcal/mol, i.e., if it exceeds the zero point energy, it is suggested that hydrogen tunneling is occurring. In ring systems, where face to face transfer of the hydride moiety is sterically constrained, the difference in the energies of activation for hydrogen and deuterium transfer ($\Delta Ea_{(H,D)}$) is within experimental error of zero and the $k_H/k_D = A_H/A_D = 2.7$. These results are fully consistent with the expected bent transition state. On the other hand, reaction between 1-benzyl-1,4-dihydronicotinamide and the *N*-methyl-9-phenylacridine cation give a $\Delta Ea_{(H,D)}$ value of 1.03 kcal/mol, an A_H/A_D ratio of 0.74 and a thermally dependent isotope effect (k_H/k_D) of 4.17 at 298 °K.[46,66] These data are suggestive of a linear transition state. In his study of the oxidation of



1-(4-methylphenyl)-1,4-dihydronicotinamide by *N*-methylacridine, Bruice found a kinetic isotope effect of 4.01, a $\Delta Ea_{(H,D)}$ value of 1.84 kcal/mol and an A_H/A_D ratio of 4.3 which suggests bent transition state with some contribution of tunneling.[44]



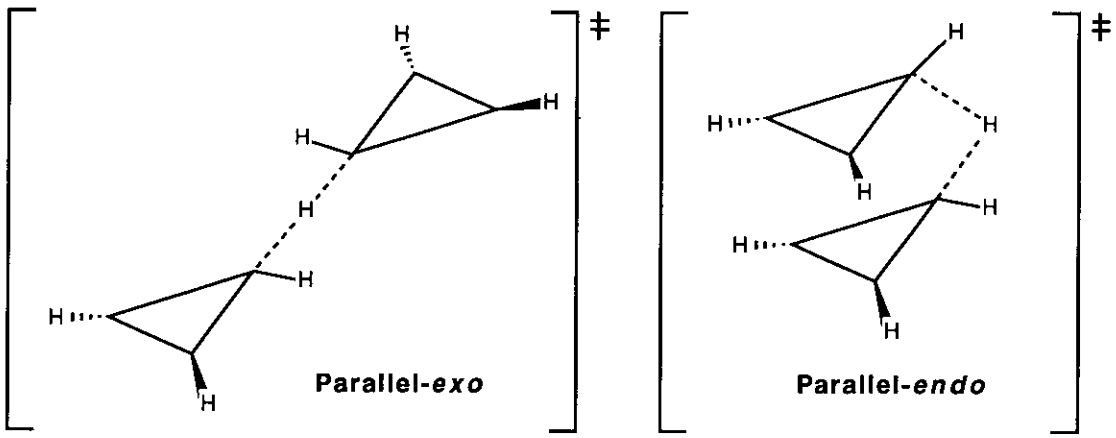
Linear Hydride Transfer (Verhoeven *et al.*)



Bent Hydride Transfer with Tunnelling (Bruice *et al.*)

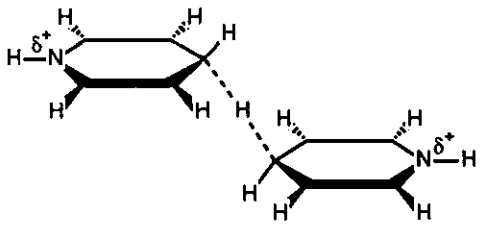
Theoretical Evaluations. In 1981, Donkersloot and Buck examined the reactions between a number of hydride donors such as cyclopropene, dihydropyridine and tropilidene and the cyclopropenium cation which served as a hydride acceptor using the MINDO/3 and the STO-3G approximations.[67,68] In the degenerate cyclopropene–cyclopropenium reaction, two initial geometries were considered, one in which the cyclopropene rings were stacked on top of one another (parallel-*endo*) leading to a bent hydride transfer and a second configuration in which the annular components were arranged

antiparallel (parallel-*exo*) and the hydride transition was linear. The transition state was then calculated by adjusting the distance to or from the incoming or departing hydride species. In the case of the parallel-*endo* configuration, a transition state could not be found since attempts to bring the two reaction components together resulted in molecular reorganization. In contrast, the parallel-*exo* conformer could be well described with a transition state culminating when the migrating hydride was equidistant between the two rings as required by microscopic reversibility and the observed C_{2h} symmetry. The MINDO/3 results suggest that the hydride is 1.30 Å from the departing carbon and away from the carbon accepting the hydride species while the STO-3G calculations place the midpoint at 1.29 Å.[67]

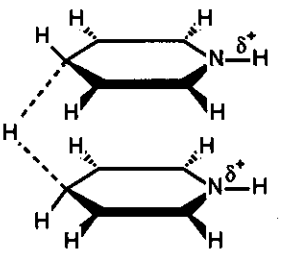


The hydride migration is characterized by a progressive change in atomic charges and positions. The hydrogen adjacent to the incoming hydride gradually bends out of the cyclopropenium ring plane while the hydrogen adjacent to the departing hydride bends into the ring plane consistent with the rehybridization. When the nondegenerate reaction between dihydropyridine and the cyclopropenium cation is examined, the hydride transfer is, of course, no longer symmetrical. Calculations suggest that the hydride transfer is parallel-*exo* with a linear hydride transfer and that the C4 carbon bends toward the incoming cyclopropenium cation. The degenerate reaction between 1,4-dihydropyridine and the pyridinium cation was studies by Verhoeven using the MNDO framework.[46] Several

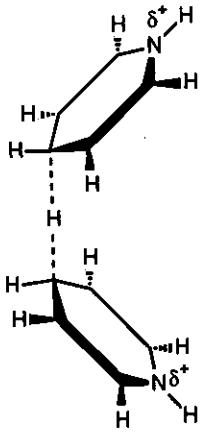
conformations of the reaction were considered. In the first, the pyridine rings were constrained to a parallel configuration and the energy of the hydride transfer calculated in a parallel-*endo* (bent) or parallel-*exo* (bent or linear) supermolecular conformation. The parallel-*endo* transition state displayed C_{2v} symmetry and gave a MNDO heat of formation of 290 kcal/mol with a hydride transferring angle of 158°. The parallel-*exo* transition state in which a linear hydride transfer occurred was found to be the most likely. This transition state was characterized by C_{2h} symmetry and a MNDO heat of formation of 264 kcal/mol, more than 25 kcal/mol more stable than the parallel-*endo* conformation that was restricted so that the rings were parallel. This energy difference clearly suggests a linear transfer.



Parallel-*exo*



Parallel-*endo*



Parallel-*endo* (tilted)

It was also noted however that the rotation of annular components of the low energy parallel-*exo* conformation around the linear axis of the transferring hydride species occurred almost without activation producing a parallel-*endo* rotomer. This latter configuration differs from the constrained example in that the pyridine rings are no longer parallel and the hydride transfer is linear. The MNDO heat of formation of the tilted parallel-*endo* conformer is within a kcal/mol or so from the parallel-*exo* case suggesting that either could be expected to occur. Thus in unconstrained systems, i.e.,

nonenzymatic oxidoreductions, a linear hydride transfer seems to be preferred. Similar conclusions were drawn from an MNDO study of the hydride transfer between 1,4-dihydropyridine and 1,1-dicyanoethylene. Bodor *et al.* expanded these results in an examination of the degenerate reaction between 1-methyl-1,4-dihydronicotinamide and the corresponding 1-methylnicotinamide cation using the MNDO and AM1 methods.[69,70] The addition of the carboxamide function breaks the symmetry observed in the pyridinium/dihydropyridine case suggesting four starting points rather than two. The four transition states that were considered included two *endo* conformations in which no constraints were placed geometry. In one of the conformers, the amide functions were *syn* (*endo-syn*), resulting in the transfer of the pro-*S* dihydronicotinamide hydrogen to the *re* (A) pyridinium face, while in the second, the carboxamide functions were *anti* with respect to one another (*endo-anti*) resulting in the transfer of the pro-*S* hydrogen to the *si* (B) face of the nicotinate salt. The second set of conformations was designed with the pyridine rings antiparallel, again with no constraints on ring parallelism. In the *exo-syn* case, the carboxamides were placed on the same side of the supermolecule and the hydrogen transfer represented a pro-*S*→*re* migration while the *exo-anti* example, in which the amide functions were on opposite sides of the supermolecule, displayed pro-*S*→*si* enforced stereospecificity. These conformations were used as starting points and fully optimized with the only constraint being that the hydride transfer was symmetrical. In all cases, the supermolecule reduced the extent of steric repulsion between the *N*-methyl and carboxamide functions by adjusting the dihedral angles associated with the two pyridine rings. The MNDO heats of formation for the optimized transition states were 177.2, 178.7, 182.7 and 185.2 kcal/mol for the *endo-syn*, *exo-syn*, *exo-anti* and *endo-anti* conformations, respectively.[69] The lowest energy conformer which started with the carboxamide functions *syn* had rotated to maximize separation. The fact that conformers did not converge to the same structure may be related to local minima imposed by the rotation barrier around the axis of the transferring hydride. While in the pyridine/dihydropyridine case rotation of the pyridine rings with respect to one another was facile,[46] significant energy barriers were encountered when the nicotinamide/dihydronicotinamide pair was considered meaning that the initial conformation is important in obtaining the global minima.[69] Using the more favorable transition state, the energy of activation was found to be 30.7 kcal/mol when the

MNDO method was applied and 9.3 kcal/mol using the AM1 approach. The latter value was within a kcal/mol or so from experimentally obtained results. The higher MNDO value may be related to the tendency of this method to exaggerate atomic repulsions, a shortcoming corrected in the NDDO-based AM1 method.[25] The most favorable transitions state could be bent along the axis of the transferring hydrogen with only moderate energetic costs i.e., distortion 180° to 150° increased the energy of the transition state by 3 kcal/mol.

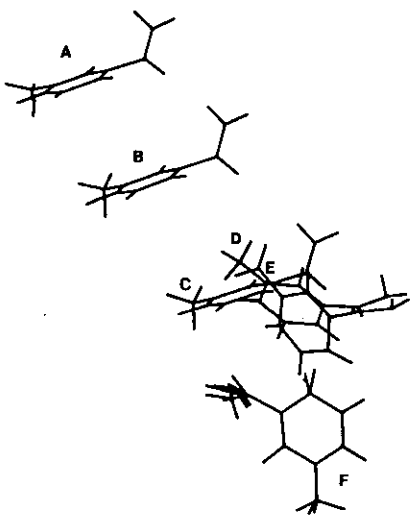


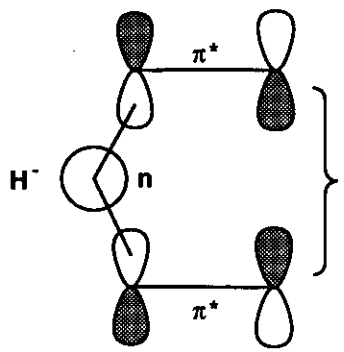
Figure 4. Structural representation of the interaction of 1-methyl-1,4-dihydropyridine (F) and the 1-methylnicotinamide cation (A-E) at various distances between the transferring hydride and the nicotinamide salt C4 carbon. The distances given are 15Å (A to F), 10Å (B to F), 4Å (C-F), 2Å (D to F) and 1.4Å (E to F).

The trajectory leading to the transition state was examined for the degenerate process by bringing the dihydropyridine and pyridinium components together from an initial distance of 100Å.[69] The components were oriented *endo-syn* at this distance and the reaction coordinate was defined as the axis of the transferring hydride. At large distance, the individual components behave as the isolated

molecules. The initial approach vector generates a bent C4(dihydropyridine)–H–C4(pyridinium) angle (250°). As the components approach each other, a energy minima is observed at a separation distance of approximately 4Å (Figure 4). This minima represents a stabilization of 8.3 kcal/mol relative to the infinitely separated species. The structural and electron features of this reaction coordinate minima are consistent with a charge-induced dipole complex. The carbonyl function of the dihydropyridine is oriented toward the incoming positively charged pyridinium species and is polarized with negative charge accumulation on the carbonyl oxygen and positive charge on the carbonyl carbon. The reaction coordinate complex marks the beginning of the final stages of hydride transfer with the C4(dihydropyridine)–H–C4(pyridinium) angle linearizing. At distances less than 2 Å, the dihydropyridine C4 rehybridizes with lower p_z and higher σ contributions to the highest occupied molecular orbital (HOMO). The MNDO predicted transition state is observed at a C4-hydride distance of 1.396Å with a linear hydride transfer.

Simple models for hydride transfer were examined by Wu and Houk using *ab initio* calculations at the 3-21G and 6-31G* basis set levels.[71] These included the degenerate reaction between methylamine and the methyleniminium ion and the nondegenerate reaction between 1,4-dihydropyridine and methyleniminium cation. Both parallel-*endo* and parallel-*exo* transition states were considered. In contrast to semiempirical results which suggested that the linear hydride transfer is most economical energetically, the *ab initio* findings for this reaction indicated that the parallel-*endo* conformation in which a bent hydride transfer (154-149° depending on the basis set) occurs was the most favorable. The bent transition stated is more stable than the linear parallel-*exo* case by 2.7 kcal/mol without and 5.7 kcal/mol with inclusion of electron correlation at the MP2 level. For the dihydropyridine–methyleniminium case, the bent parallel-*endo* transition state was also found to be 1.4 kcal/mol more stable than that produced by the parallel-*exo* conformation. In addition, the transition state for this reaction is associated with a slight bending of the dihydropyridine into a shallow boat in the direction away from the incoming electrophile. In this way, the pseudoaxial hydrogen is transferred.

The preference for the bent hydride transfer was rationalized in terms of orbital overlap. In the transition state, three groups of orbitals are involved, the hydride ion with a filled nonbonding (n) orbital and the interacting double bond systems with a π and π^* orbital. In the transition state, the HOMO of the hydride interacts with similar phases i.e., bonding combinations, of the double bond π^* orbitals in such a way so as to maximize overlap (see below). This is done most efficiently when the π^* orbitals are parallel and the angle of the transferring hydride is bent. In the linear case, the planes of the π orbitals are tilted reducing interaction energy. In the parallel-*exo* arrangement, optimization of orbital overlap is precluded by geometry suggesting no advantage to either a linear or bent transfer.

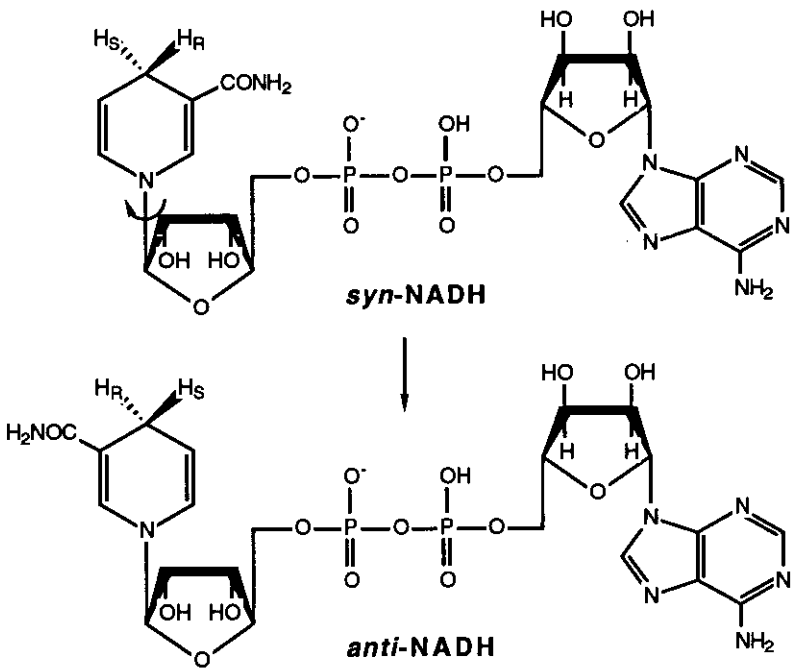


overlap is maximized when π^* are parallel (bent transition state)

STEREOSELECTIVITY OF ENZYMATIC NADH OXIDOREDUCTIONS

Experimental Background. In 1953, Westheimer *et al.*, found that the two C4 hydrogens of NADH were not equivalent enzymatically and that oxidoreductases that utilized NAD(P)H as a coenzyme transferred only one of the two enantiotopic hydrogens.[72] Thus, while the C4 carbon of dihydronicotinamide is not chiral, replacement of either of the hydrogens would lead to an optically active compound.[2] The C4 hydrogens are therefore designated as pro-*R* or pro-*S* indicating the prochirality of NADH and other substituted dihydronicotinamides. The fact that a particular enzyme will transfer only one of the two hydrogens atoms has lead to the classification of pyridine nucleotide-linked enzymes into two stereospecific classes.[73] Those that transfer the pro-*R* hydrogen of the dihydronicotinate are termed A-stereospecific enzymes while those transferring the pro-*S* have B-

stereospecificity.[2,73] When a substrate is oxidized, A-type enzymes transfer hydrogen atoms only to the *re* side and B-type enzymes to the *si* side of NAD(P)$^+$. Another important observation based on X-ray crystallographic studies is the orientation of binding of NADH to the enzyme. In A- type enzymes, NADH is bound in an *anti* configuration, that is to say, the nicotinamide carboxamide is on the opposite side of a plane perpendicular to the dihydropyridine ring while the coenzyme is bound *syn* to B-type enzymes.[74] In the latter case, which represent a 180° rotation of the dihydronicotinamide around the ribosidic bond, the carboximide and ribose substructures are on the same side of the molecule.



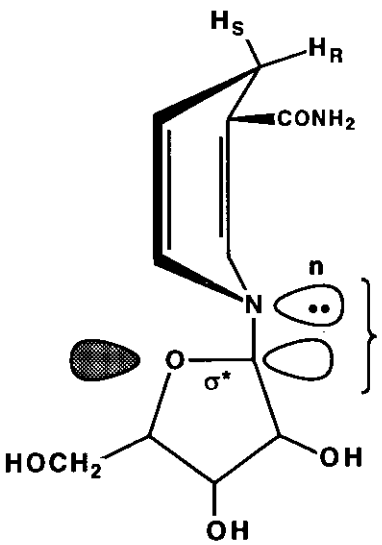
Two hypotheses have been forwarded to explain the stereospecificity of NADH dehydrogenases.[73,74] According to the first, the specificity maybe the result of simple structural differences between two precursor enzymes which prevent hydride transfer from one side of the coenzyme. A-and B-specific enzymes evolved and both types have been conserved over time. In 1986, Schneider-Bernlöhr *et al.*, suggested that ethanol and polyol dehydrogenases that utilize zinc in the catalytic site and which

have a 40,000 dalton subunit have a common A-type enzymatic precursor while those dehydrogenases which do not use zinc as a catalyst and which are associated with a shorter (25,000 dalton) subunit evolved from a B-type enzymatic precursor.[76] One the other hand, Benner and colleagues, have put forth the suggestion that dehydrogenases are enzymes that have evolved to conform to stereoelectronic principles.[74,77-79] This theory was prompted by the observation that A- and B-type enzymes have selective affinity to alcohol-carbonyl substrates of specific stabilities.[74] A-stereospecific enzymes reduce carbonyl compounds that are relatively unstable i.e., those in which the $-K_{eq}$ as defined below is large ($>10^{11}$).

$$K_{eq} = \frac{[\text{carbonyl}][\text{NADH}][H^+]}{[\text{alcohol}][\text{NAD}^+]}$$

Conversely, relatively stable ketones or aldehydes with $-K_{eq}$ values of $<10^{11}$ are reduced by enzymes transferring the pro-S hydrogen (B-type). The hypothesis as developed by Benner is based on four assumptions.[74] First, since X-ray data indicate that NADH in the *anti* conformation is bound to A-type enzymes and *syn* NADH to B-enzymes, the *anti* bound NADH transfers the pro-*R* hydrogen to substrate and *syn* bound NADH transfers the pro-*S* hydrogen. Second, it is assumed that enzyme bound *anti*-NADH is a weaker reducing agent than the corresponding bound *syn*-NADH. Third, according to Knowles, optimal catalytic activity of an enzyme is achieved when the free energy of the bound intermediates is closely matched by the bound substrates.[80] In the case of dehydrogenases, such matched internal thermodynamics is suggested by the fact that NADH is bound more tightly than NAD^+ and that carbonyl-containing compounds are bound more tightly than the corresponding alcohols.[75] Finally, it is assumed that dehydrogenases fall into the category of evolutionary optimized enzymes. The structural contentions of the hypothesis are raised as a result of the anomeric effect.[81,82] The anomeric effect has multifarious application but has been very useful in explaining the conformation of carbohydrates. In pyranoses and other systems, electron-withdrawing substituents at the anomeric carbon are more likely to assume an axial conformation compared to the cyclohexyl analogs where equatorial conformations are preferred.[81] The explanation for this axial

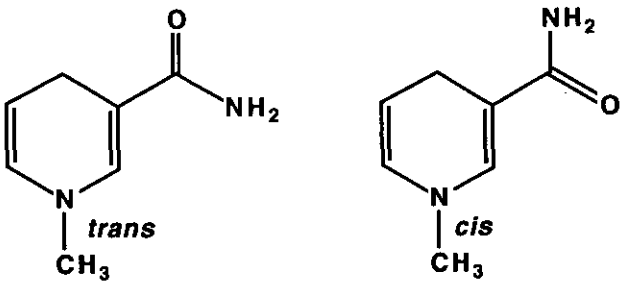
stabilization is that the configuration provides for maximization of oxygen lone pair (n) overlap with the anomeric carbon-substituent antibonding (σ^*) orbital. In this configuration, the oxygen lone pair is antiperiplanar to the axis of the anomeric carbon-substituent bond. In the case of dihydronicotinamide ribosides, the stabilizing effect is structurally reversed since it is the lone pair on the dienamine nitrogen which interacts with the σ^* orbital of the ribosidic oxygen-anomeric carbon bond.[74] This overlap can be optimized by slight deplanarization of the dihydropyridine ring into a shallow boat which increases C-N double bond character.



In the case of the *anti* conformation, the dihydropyridine ring is distorted so as to make the pro-*R* hydrogen pseudoaxial. In the *syn* rotomer, the pro-*S* hydrogen becomes pseudoaxial. The pseudoaxial hydrogens are presumably easier to transfer than the second α-hydrogen. Thus, the transferring hydride is put into close proximity with the substrate in a form that is activated by a slight deplanarization of the dihydropyridine ring.

Theoretical Evaluation. When considering influences that appear to affect the stereospecificity of dehydrogenases that utilized NADH as a cofactor, conformation is an important parameter with

several structural features of primary interest. These include (1) the torsion angle of the carboxamide function of the nicotinamide derivative, (2) the conformation of the dihydropyridine substructure of NADH and model compounds and (3) the conformation of 1-substituents of the dihydronicotinamides including riboses and ribose derivatives as well as models for these carbohydrates. In the case of the carboxamides, most of the early theoretical studies of dihydropyridines were designed to reproduce X-ray configurations with application to stereospecificity coming later. Experimentally, the carboxamide torsion angle was found to be *cis* to the dihydropyridine ring in some crystal structures such as those of *N*-benzyl- and *N*-propyl-1,4-dihydronicotinamide.[83] In contrast, the X-ray crystal structures of dehydrogenase-bound NAD^+ indicate that the carboxamide is *trans* to the pyridinium ring.[84,85]



Studies by Kuthan and associates using semiempirical approaches at the extended Hückel theory (EHT), CNDO, CNDO/2, PCILO, MINDO/2 and NDDO level found model dependence in the structural results.[86-93] The CNDO/2 and MINDO/2 level found that while the carboxamide function is predicted to be *cis* with respect to the dihydropyridine ring, consistent with experimental data, that the conformation of the corresponding pyridinium salt is poorly modeled.[86] Better results were obtained with the NDDO frameworks which predicted a global minima at 150° out of the pyridine ring plane for nicotinamide, consistent with the experimental value of 156°.[87] Interestingly, calculations at this level indicate that while the structure in which the carboxamide is *cis* to the dihydropyridine ring is most stable in the case of 1-methyl-1,4-dihydronicotinamide, the *trans* rotomer is more stabile in the case of nicotinamide.

Interest on the conformation of the carboxamide function was greatly stimulated by the proposal of Donkersloot and Buck that this parameter has a direct bearing on the stereospecificity of hydrogen transfer.[68,94] In semiempirical (MINDO/3) and *ab initio* (STO-3G) studies of the hydride transfer from 1,4-dihydronicotinamide to CH_2OH^+ and the cyclopropenium cation as well as the degenerate hydride transfer between cyclopropenecarboxamide and the cycloprepeniumcarboxamide cation, several configurational events were observed. First, the carbonyl oxygen of the hydride source rotates towards the approaching positively charged hydride acceptor in response to electrostatic interactions. The dipole established by the carbonyl group then provides a mechanism for lowering the energy of the transition state for the hydride transfer. In this context, asymmetry is introduced into the molecule by the orientation of the carboxamide group with the hydride transfer occurring most easily on the side of the dihydronicotinamide to which the carbonyl oxygen is oriented.[94] It was suggested that enzymes may induce stereospecificity by binding NADH in such a way so as to orient the carboxamide function in the direction of the desired hydride transfer i.e., to yield pro-*R* or pro-*S* migration. When a hydride is transferred to NAD^+, the same conditions would apply, that is orientation of the nicotinamide carbonyl oxygen toward to incoming hydrogen is favored over that in which the carbonyl oxygen is oriented to the opposite pyridinium face. Experimental evidence is supportive in that for *anti*-NADH, which is bound to A-stereospecific enzymes, the carbonyl of the pyridine is deplanarized by 30° in the coenzyme-bound crystal structure of horse liver dehydrogenase. A criticism of this proposal is that the carbonyl function in the model system orients toward the approaching cationic species presumable due to Coulombic attraction. Such pressures may not occur in the enzyme case requiring other conformationally directing effect in the enzyme active site.

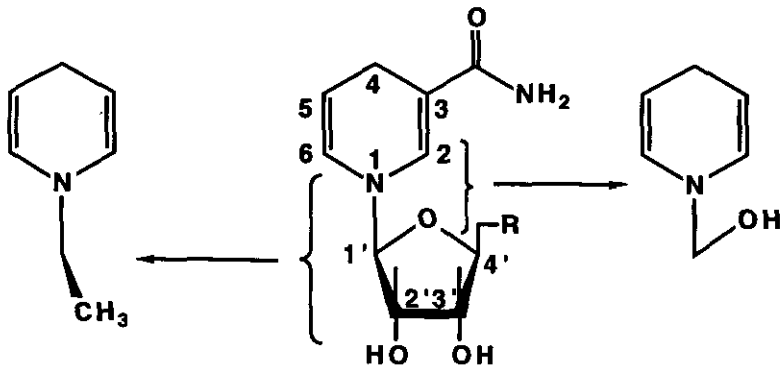
Later, Cummins and Gready, using *ab initio* methods at the STO-3G, 3-21G and 6-31G levels found that the most stable configuration of the carboxamide for both 1-methyl-1,4-dihydronicotinamide and the 1-methylnicotinamide salt was predicted to be *cis* with a secondary minima for the 180° (*trans*) rotomer.[95] At the 3-21G and 6-31G, the difference in energy between the *cis* and *trans* conformation is 3.1 kcal/mol in the case of the pyridinium species and between 4.1 and 4.7 kcal/mol in the case of

the dihydronicotinate. In addition, calculations of the *cis-trans* transition state suggest that rotation of the carboxamide function is more difficult in dihydronicotinamides than in the nicotinamide salts due to electron delocalization into the C3–carbonyl carbon bond. Having noted this, the barrier height itself is small. Wu and Houk completed a similar study at the 3-21G and 6-31G* basis set levels and included electron correlation at the MP2 level in some calculation.[85] While the preference of the *cis* conformer over the *trans* was confirmed, the difference became smaller with the use of larger basis sets with the MP2/6-31* model giving *cis-trans* energy differences of 0.9 and 1.0 kcal/mol for protonated nicotinamide and 1,4-dihydronicotinamide, respectively. The stabilization of the *cis* conformation over the *trans* was attributed to favorable interactions between the carbonyl oxygen (δ^-) and the C2 carbon of the pyridine ring (δ^+). The estimated barriers to rotation were qualitatively similar to those found by Cummins and Gready with activation energies being approximately 3.4 kcal/mol higher in the dihydronicotinamide as compared with the protonated nicotinamide. The higher barrier was attributed to disruption of π-conjugation.[85] In the optimized *trans* conformation, there is significant pyramidalization of the amide nitrogen and the amide function manifests a dihedral angle that significantly varies from 180°. Both of these perturbation are associated with low energetic cost and allow the molecule to decrease steric interaction between the amide NH_2 and the pyridine C2–H. The low barrier heights to carboxamide rotation provide an avenue for external forces to contribute to carboxamide orientation *via* hydrogen-bonding, hydrophobic interaction and other environmental/enzyme pocket effects. This is consistent with the proposal that the carboxamide can reorient to allow for breaking of C_s symmetry and increasing the proclivity for the hydrogen on the same side of the dihydropyridine face as the carbonyl oxygen to be more preferentially transferred.

The conformation of the dihydropyridine component of NAD(P)H has also been of interest. From the forgoing argument, deviations from planarity of the NADH coenzyme may be responsible for enzymatic stereospecificity. On the other hand, most X-ray crystal structures indicated that the dihydropyridine portion of substituted nicotinamides is within experimental error of planarity. Nmr data suggest some nonplanar character in NADH but these studies were complicated by the apparent stacking of the adenine bases which may have induced conformational changes.[96] The configuration

of the dihydropyridine ring has been examined in isolation using several theoretical methods. Bodor and Pearlman found that 1,4-dihydropyridine is within 0.5° of planarity as calculated using the MINDO/3 approximation.[97] While the same conclusions were reached by Raber and Rodriguez using the 3-21G split-valence basis set, it was also noted that the dihydropyridine structure was quite flexible and that deviations from planarity of 20° were associated with an energetic cost of only 1.4 kcal/mol.[98] Studies by Hoffman and Cimiraglia, however, suggested a boat structure for the dihydropyridine ground state with the 6-31G* basis set indicating that the C4 methylene carbon deviated 5.5° and the ring nitrogen 8.4° from the plane defined by the four remaining sp^2-hybridized carbons.[99] The energy required to planarize this conformation was, however, only on the order of 0.5 kcal/moL. The very small transition energy suggests that a number of factors associated with enzyme binding or the environment of the binding site could act to induce a conformational change in the dihydropyridine ring. The same flexibility is not present in the oxidized cofactors as deviation from planarity of the pyridinium ring is associated with significant energetic costs.[98] Wu and Houk examined ring puckering in dihydronicotinamide using 6-31G* basis set with inclusion of electron correlation (MP2). Calculations indicate that the dihydropyridine substructure is slightly deplanarized into a shallow boat with a modicum of pyramidalization of the ring nitrogen.[85] This latter manifestation has been observed in certain recently obtained X-ray crystal structures including that of 1-(methyoxymethyl)-1,4-dihydronicotinamide.[83] There appeared to be little effect of dihydropyridine ring puckering on the conformation of the carboxamide group.

Wu and Houk also examined *N*-ethyl- and *N*-hydroxmethyl-1,4-dihydropyridine and the corresponding pyridinium salts as models of the NADH-NAD^+ couple especially aimed to exploring interactions between the dihydropyridine ring and the riboside.[75] The *N*-hydroxymethyl and *N*-ethyl derivatives were selected to mimic the *N*-anomeric carbon–furanosyl oxygen bond and the *N*-anomeric carbon–2' carbon of the *N*-riboside, respectively and calculations were performed at the 3-21G and 6-31G* basis set level.



In the case of the pyridinium derivatives, the lowest energy conformation for the hydroxymethyl group was one in which the C–O bond is in the plane of the pyridinium ring. The barrier to rotation is, however, very small. This was accounted for by hyperconjugative stabilization of the pyridinium species which occurs at planarity and steric interferences which favor the hydroxymethyl function in a perpendicular arrangement. In the case of the *N*-ethyl derivative, the lowest energy conformation is clearly one in which the substituent is perpendicular to the plane of the pyridinium ring. In extrapolating these findings to NAD^+, it is expected that the lowest energy conformation is one in which the C1' and C2' atoms are perpendicular to the plane of the pyridinium ring while the furanose oxygen and anomeric carbon form a bond that is in the plane.[75] This orientation is in keeping with X-ray structure of coenzyme bound NAD^+. In the case of the reduced models, both the hydroxymethyl and ethyl substituents prefer a perpendicular conformation which is consistent with crystal structures of various 1-substituted 1,4-dihydronicotinamides. The perpendicular orientation is attributed to the anomeric effect which, as mentioned earlier, is a result of maximization of overlap between the dihydropyridine lone pair and the C–O antibonding (σ^*) orbital. In the case of the ethyl substituent, the perpendicular rotomer prevails due to steric interaction. In extrapolating to NADH, it is expected that the C–O bond will assume the perpendicular conformation.

Rotation of the *N*-substituents in these dihydropyridine has an effect on ring puckering and ring nitrogen pyramidalization, the latter two events being highly correlated. Perpendicular orientations of

the hydroxymethyl function and a planar orientation of the ethyl substituents induce the largest degree of ring puckering with one of the C4-hydrogens becoming pseudoaxial. In the case of NADH, perpendicular orientation of the C1'–O bond provides a planar orientation of the C1'–C2' bond and, in the *anti* conformation, will result in the pro-*R* hydrogen becoming pseudoaxial. In the case of the *syn* conformer, interactions will favor a pseudoaxial conformation for the pro-*S* hydrogen. Raman spectroscopy is consistent with these assignments in that upon binding of NADH to various A-dehydrogenases , the pro-*S* hydrogen demonstrated a shift of 20 cm^{-1} to higher wavenumber.[96] Such differences in absorption spectra are consistent with dihydropyridine deplanarization as the equatorial hydrogens in similar ring systems are generally shifted to higher wavenumbers.

The proposed differences in conformation minima for NADH (C–O perpendicular with the dihydropyridine ring) and NAD^+ (C–O planar with the pyridinium ring) may be important in their stereospecificity. As indicated, enzymatic catalysis is optimized when the binding energy of the bound intermediates matches or is brought closer together than the free energy of the components in solution.[80] Since enzymatically mediated dehydrogenations have very small K_{eq} values, that is the *equilibrium is to the left, factors that would increased binding on the right side* (ketone/aldehyde and NADH) of the equation will act to equalize the internal binding energy.[75] In X-ray crystal structures, the ribosyl C–O bond is generally perpendicular to the dihydropyridine ring and this correlates with the observation that NADH is more strongly bound to various dehydrogenases than is the oxidized coenzyme, NAD^+. If NADH is strongly bound and NAD^+ is weakly bound, the internal thermodynamics of the system are more closely matched.[75] A second point made by Wu and Houk is that, based on model compounds, the orientation of the ribosidic C–O bond significantly reduces the energy of the transition state with respect to pro-*R* hydrogen transfer in *anti*-NADH and with respect to the pro-*S* hydrogen in *syn*-NADH.[75]

Studies of *N*-(1,β-ribosyl)-1,4-dihydronicotinamide and the corresponding nicotinate salt were conducted by Bodor *et al.* using the MNDO framework.[69] In comparing the dihydropyridine portion of the riboside and 1-methyl-1,4-dihydropyridine, several important differences were discovered. First, a

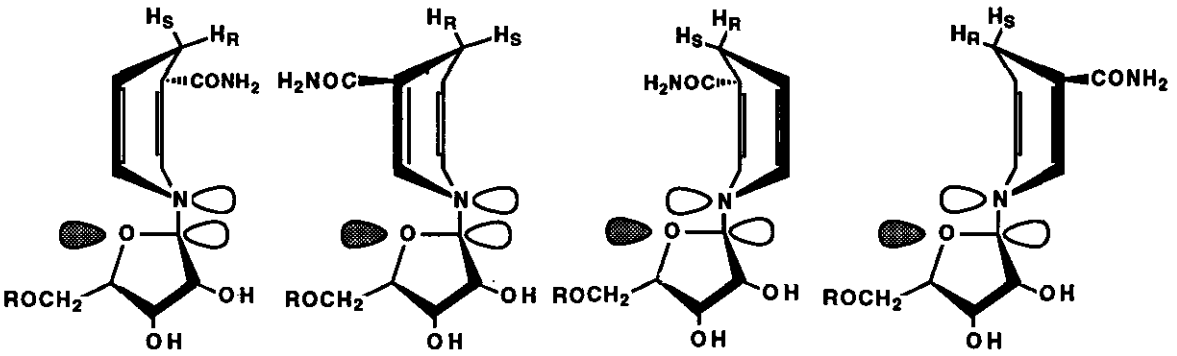
greater differentiation between the enantiotopic hydrogens in the case of the riboside compared to the simpler model was observed. Thus, while the atomic orbital coefficient contributions to the HOMO were -0.194 and +0.196 in the case of the pro-*R* and pro-*S* hydrogens, respectively, of 1-methyl-1,4-dihydronicotinamide, they were +0.201 (pro-*R*) and -0.180 (pro-*S*) in *N*-(1,β-ribosyl)-1,4-dihydronicotinamide. Second, the atomic orbital contribution by the ring nitrogen is decreased form 0.529 in 1-methyl-1,4-dihydronicotinamide to 0.289 in *N*-(1,β-ribosyl)-1,4-dihydronicotinamide. Using the AM1 framework, Bruice found that the dihydropyridine component of *N*-(1,β-ribosyl)-1,4-dihydronicotinamide was planar in the ground state but was fairly flexible.[17,100] When the amide and riboside twist angle are adjusted to 170° and 80°, respectively which are the values observed from X-ray crystal structures for enzyme bound NAD(P)$^+$ or NAD(P)H, deplanarization of the C4 carbon and the ring nitrogen by 10° each produce a structure which is only 1.7 kcal/mol less stable than the planar benchmark. These results are consistent with the reported flexibility of the dihydropyridine ring. In addition, deplanarization of the corresponding pyridinium species was found to be difficult. The C–O bond of the riboside function was predicted to be within 10° of planarity in the case of the pyridinium species and approximately perpendicular (80°) in the 1,4-dihydropyridine case. As stated previously, X-ray structures of enzyme bound NAD(P)$^+$ or NAD(P)H indicate riboside conformations close to the 1,4-dihydropyridine minima (-87° to -140°) for A-type (*anti*-NADH) enzymes. The effect of the carboxamide twist angle with respect to the dihydropyridine ring was similar to that previously described with the global minima at the *cis* and local minima at the *trans* configurations.[100] Furthermore, local minima are identified at 150° and -150° corresponding to an orientation in which either the pro-*R* or pro-*S* hydrogens are coplanar with the carboxamide function.

The interaction of NAD$^+$ and NADH have also been modeled in the active site of the enzyme using appropriate crystal structure data and molecular mechanics in combination with either semiempirical or *ab initio* optimizations. These studies have included evaluations of NAD$^+$ and NAD$^+$ analogs in the active site of horse alcohol dehydrogenase,[101,102] 1-ribosyl-1,4-dihydronicotinamide in the active sites of dogfish muscle lactate dehydrogenase, D-glyceraldehyde-3-phosphate dehydrogenase, *L.*

casei dihydrofolate reductase and porcine heart maleate dehydrogenase and the interaction of NAD(P)H models with catalase.[17,100] Beijer and colleagues examined various NAD^+ analogs including those in which the 3-carboxamide function was replaced with thioamide, an acetyl function, a formyl group and a nitrile.[101] The active site of the horse liver alcohol dehydrogenase enzyme and the substrate structures were translated from X-ray crystal structures to a molecular mechanics program (AMBER) and optimized with the catalytic zinc ion and a cage of 44 amino acids were fixed as to their position. For the substrates, the MNDO method was used to calculate the atomic charges with other parameters (harmonic force constants, rotational barriers, etc.) derived from the literature. The structure of NAD^+ bound to the enzyme active site by the AMBER force field is very close to the X-ray structure with the carboxamide twisted 34° out of the plane of the pyridinium ring. Other derivatives demonstrated different twist angle with the thiocarbonyl 47° out of the pyridinium ring plane, the acetyl group, 6.5° and the formyl substituent 9° out of the pyridine plane. The values of the dihedral angles for the carboxamides and related substituents in the enzyme active sites are roughly correlated with the catalytic activity of the enzyme in the oxidation of isopropanol, a substrate whose oxidation by dehydrogenases is limited by the rate of hydride transfer.[101] It was also noted that the 3-cyano-NAD^+ analog is inactive in this reaction. Since this group is linear, it cannot orient out of the pyridinium plane and therefore cannot, presumably, stabilize an approaching hydride species.

One challenge that has been raised to the proposal by Benner is that only two conformations were considered to be important for NADH in the active site, those being *anti*- and *syn*-antiperiplanar (the nitrogen lone pair with respect to the C–O ribosidic bond). The structures in which the pyramidal nitrogen is inverted i.e., the *anti*- and *syn*-periplanar systems are not considered even though X-ray and nmr data suggest that they are involved with certain enzymatic reactions (see below, from Ref. 17). Almarsson and Bruice examined the interaction of several dehydrogenases modeled using the $CHARM_m$ mechanics package to investigate these and other points.[17,100] In the studies, the AM1 optimized structure for *N*-(1,β-ribosyl)-1,4-dihydronicotinamide was placed in the enzyme active site of several dehydrogenases or models thereof. As pointed previously, while most X-ray crystal

structures of dihydronicotinamides demonstrate a *cis* conformation, the enzyme bound NAD(P)$^+$ or NAD(P)H have the carboxamide group *trans* to the dihydropyridine ring. To examine this



phenomenon, the AM1-optimized dihydropyridine-pyridinium cation were placed in a simple model for the enzyme active site. This involves a superimposition of *N*-(1,β-ribosyl)-1,4-dihydronicotinamide on the NADH structure in coenzyme-bound dogfish muscle lactate dehydrogenase, of formaldehyde on the pseudosubstrate of the enzyme (oxamate),of protonated imidazole on the histidine 193 amino acid (the source for the proton in the dehydrogenation) and of a methylguanine molecule on the arginine 106 residue position. In this supermolecule, the dihydropyridine portion of the riboside was planar and the hydride ion was gradually directed toward the carbonyl carbon of formaldehyde. The lowest energy transition state involved intermediate hydride transfer to the carbonyl carbon of formaldehyde and almost complete proton transfer from the imidazole nucleus. This reaction had an enthalpy of reaction of approximately 4 kcal/mol and an activation energy (AM1) of 31 kcal/mol.

In a study of reduction of protonated formaldehyde, hydrogen bonding occurs between the dihydronicotinamide carbonyl oxygen and the proton of the formaldehyde carbonyl oxygen. This hydrogen bond holds the substrate in close proximity to the hydride donor. The estimated activation enthalpy energy for this process is 27 kcal/mol.[17] If the amide is *cis*, the hydrogen bond is poorly formed and the activation energy is destabilized by 10 kcal/mol. This may account for the observed

enzyme-bound conformation which is not at the (*cis*) global minima. If the formaldehyde substrate is not protonated, the transition state is of much higher energy suggesting that acid catalysis is a *sine qua non* for reduction. These results suggest that the enzyme as modeled by the protonated imidazole / methylguanine / formaldehyde supermolecule provides catalytic reduction at a rate similar to that of the pre-protonated substrate. Both rotation of the sugar residue and inversion of the ring nitrogen so that the antiperiplanar conformation (pro-*R* transfer) is converted to the periplanar conformer (pro-*S* transfer) are not associated with significant changes in transition state energy. Manipulation of ring puckering in the supermolecular enzyme site model did provide for some decrease in the energy of the transition state. If the C4 carbon and ring nitrogen are elevated 10° over the plane defined by the remaining four dihydropyridine ring carbon atoms, the pro-*R* hydrogen becomes pseudoaxial and activation energy for hydride transfer is reduced by 5 kcal/mol over that of the planar dihydropyridine ring. Since it requires 1.7 kcal/mol to effect this deformation, the net benefit in hydride transfer is a little more than 3 kcal/mol. The reason of the increased propensity for hydride ejection may be associated with the increased p-character of the C4 carbon which maximizes overlap with the departing hydride ion. An even more favorable kinetic system is available if the C4 carbon is deformed 15° and the ring nitrogen 5° above the dihydropyridine plane as the activation energy is 6 kcal/mol less than the planar components. After hydride transfer, the deformed pyridinium derivative, which is more highly constrained to planarity, relaxes with energy release of 16 kcal/mol.

Studies using the X-ray crystal structure of various enzymes downloaded into a molecular mechanics package ($CHARM_m$) either bound to NAD^+ or NADH, in the presence or absence of a substrate were then completed. In dogfish muscle lactate dehydrogenase, the oxidized coenzyme, NAD^+, assumed an *anti* configuration in the active site with the C–O ribosidic bond coplanar with the pyridinium ring. The amide group oscillated widely around 150° but a barrier to rotation to more stabile *cis* conformation was enforced by hydrogen bonding to the hydroxy group of serine 161, the carbonyl oxygen of valine 136 as well as to water molecules in the active site.[17] When NADH is examined, the ribose function assumes an conformation in which the C–O bond of the riboside is orthogonal to the dihydropyridine ring (-109° to -155°) and in which nicotinamide is *anti* to the riboside. These is also

evidence for weak interactions between the carbonyl oxygen and the proton on histidine residue 193 as well as other potential hydrogen donors. After proton transfer from histidine to the bound substrate, which comes very late in the reaction, the substrate is reoriented due to the breaking and forming of hydrogen bonds.

When a dynamic simulation is run, there is a statistical predominance for deformation of the dihydropyridine substructure into a shallow boat with the pseudoaxial pro-*R* hydrogen oriented toward the substrate. The bulky isoleucine residue 249 on the opposite face of the active site pocket chides deformation in the direction cited so that the pro-*R* hydrogen becomes pseudoaxial, and prevents the C4 atom from moving behind the dihydropyridine plane which would transform the pro-*S* hydrogen into a pseudoaxial conformation. The distance between the carbonyl carbon of the substrate and the pro-*R* hydrogen is 2.7Å which is the approximate sum of the two van der Waals radii suggesting facile formation of the transition state. In the other enzymes examined including the porcine heart maleate dehydrogenase, *L. casei* dihydrofolate reductase and lobster muscle D-glyceraldehyde 3-phosphate dehydrogenase, similar trends were found. The C4 carbon was observed to deform in the direction of the binding pocket for the substrate and each enzyme contained a bulky amino acids on the B-side of the active site which forced the pro-H_S-C4 carbon system to bend away from the B-side and toward the substrate resulting in the observed anisotropy.[17]

Results of this study suggest that the energy of the *syn* and *anti* conformers of NADH are not significantly different suggesting that Benner's hypothesis in which the *syn*-NADH conformation was bound by B-stereospecific enzymes to allow of reduction of more stabile aldehydes and ketones is unlikely. Another finding from the molecular dynamic evaluations was that the twist angle of the carboxamide group varied widely with the carbonyl oxygen spending significant amounts of time oriented towards both the pro-*R* and pro-*S* sides of the dihydropyridine ring. This being the case, the suggestion made by Donkersloot and Buck that the nicotinamide carbonyl function, which was suggested to be preferentially oriented towards the substrate, acting to assist hydride transfer *via* a dipole-dipole repulsion may not operate in these systems. Finally, the data suggest that

stereospecificity in these systems is in part mandated by enzyme structure with large bulky amino acids present on the B-side of the cavities. These amino acids act to induce the boat transition that may be stabilized by the anomeric and other effects. Hydride transfer from the shallow boat was found to be significantly lower than from the planar dihydropyridine component.

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