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Lecture Notes in Bio-Organic Chemistry

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Mechanistic Models of Asymmetric Reductions

Springer-Verlag
Berlin Heidelberg GmbH

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ISBN 978-3-540-16440-1 ISBN 978-3-642-48868-9 (eBook)
DOI 10.1007/978-3-642-48868-9

2152/3140-543210

FOREWORD

Bio-organic Chemistry has come of age - the sign of this is the start of a new series of Lecture Notes that are the product and substrate of spreading this line of modern knowledge among graduate students and research workers in such fields as mechanistic biochemistry, biomimetic organic chemistry, biotechnological application of enzymology, to name only a few examples of how many frontiers are opened and borders lifted - just at the time when the demand for a "Synthetic Biology" and "Molecular Biotechnology" is increasing - fields that have been neglected for (too) long a time by "classical" chemists in curricula and imagination.

We hope that through this first volume, which points in the several directions mentioned above, the profile of the undertaking will become clear and that it will find resonance among the scientific community interested in the thoughtful application of chemical and physical concepts to biochemical and molecular-biological problems.

The Editors

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Mechanistic Models of Asymmetric Reductions

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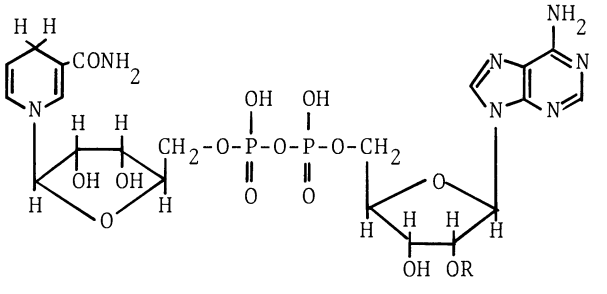
A. Ohno and S. Ushida

1 INTRODUCTION

Oxidation and reduction are the most fundamental processes in both biological metabolism and organic reactions. Organic chemists have developed a variety of reducing agents with the hope of extending their reducing power and of improving the selectivity to be utilized for modern organic synthesis. However, much better reductions had been taking place in living organisms for a long time before people developed organic chemistry. These reactions are fascinating because of their outstanding features with respect to reactivity and selectivity, which can be considered as results of catalysis by an oxidoreductase. Namely, in general, biochemical reactions proceed rapidly and selectively under mild conditions without using a high temperature and strong acid or base as a catalyst. In addition, it should be noted that most enzymatic reactions are stereospecific. As the knowledge of these biological reactions increases by a great many investigations in biochemistry, organic chemists are encouraged to mimic such excellent reactions for their own systems. These attempts have been aimed not only at developing a new type of reducing agent with excellent properties for organic synthesis, but also at obtaining information on the mechanism of enzymatic reactions by using simplified models for the complex biological systems.

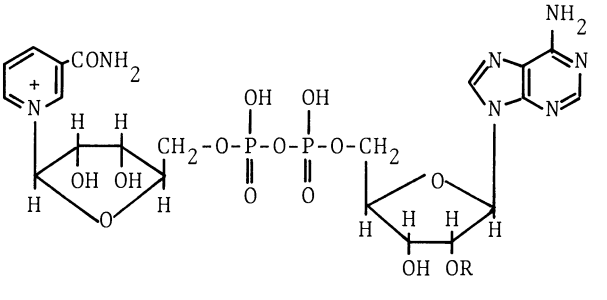
In biological systems, coenzymes such as nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD) are used as redox reagents and react directly with substrates in a reaction field constructed by an apoenzyme. However, the reactivity and selectivity exerted by the coenzyme alone are quite low. The excellent features of enzymatic reductions depend greatly on the function of the apoenzyme which exhibits the catalytic properties. Therefore, it is important to clarify the role of the apoenzyme in detail. Biochemical investigations have revealed the three-dimensional structures of many enzymes as well as the sites of functional groups for catalysis. Within the large molecule of an enzyme, only a few parts seem to be indispensable for the catalytic activity. The function of the remaining parts seems to be to set these catalytic groups, which are located apart from each other in a polypeptide chain (primary structure) of the enzyme, into appropreate positions and to provide a reaction field so that they can exert the catalytic effect. Namely, if a few catalytic groups are arranged appropriately with respect to the substrate by a proper molecular design, such excellent reactions as exerted by an enzyme can be realized artificially. This means that a large molecule such as an enzyme may not necessarily be required to achieve a high degree of catalytic activity. Notwithstanding, it should be noted that an enzyme is a multifunctional molecule, and to exert other functions, such as complexation with a substrate or allosteric effects, the size of the molecule is important.

Among many kinds of enzymatic reactions it is relatively easy to simulate the ones in which coenzymes act as reagents, because the actual reaction site in an enzyme can be readily mimicked in a small molecule (or molecules) by extracting the active moiety in the coenzyme with a small part of the apoenzyme which is important for the catalysis. As a simulation of the reaction with an oxidoreductase, reductions by model compounds of NADH and NADPH coenzymes have been investigated. Hereafter we will denote these coenzymes as NAD(P)H instead of NADH and NADPH. Since a variety of substrates are reduced rapidly and stereoselectively under the catalysis of dehydrogenases, simulated reactions have been intended to accomplish such reactions without enzymes. Achievement of asymmetric reduction is one of the most important subjects in this field. A variety of asymmetric reductions making use of various reagents that have capabilities for asymmetric induction toward the substrate have been reported. In this article we will discuss enzymatic reductions from a stereochemical point of view and describe simulations by model systems. Mechanisms for induction of asymmetry will also be discussed.



NADH : R = H

NADPH : R = PO_3H_2



NAD^+ : R = H

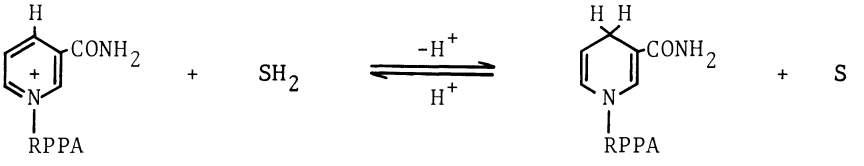
$NADP^+$: R = PO_3H_2

2 NAD(P)H AS A COENZYME

Nicotinamide adenine dinucleotide and its phosphate derivative are widely distributed as coenzymes for biological redox reactions. Structures of their oxidized and reduced forms are depicted above. Within the coenzyme molecule, the nicotinamide moiety which is linked with a ribose moiety through a β-glycosidic bond* acts as a shuttle redox reagent by interconverting between 1,4-dihydropyridine and pyridinium cation structures. The remaining part of the coenzyme mainly acts as the binding site for the apoenzyme.

In the oxidation of a substrate, the pyridinium cation in the oxidized form of the coenzyme accepts two electrons and one proton to be reduced to the corresponding 1,4-dihydropyridine which reduces the substrate in the reverse reaction. The stoichiometry of the redox reaction is depicted in Scheme 1, where SH_2 and S indicate the reduced and oxidized states of the substrate, respectively.

Scheme 1



[RPPA represents the dinucleotide moiety of NAD(P)H]

It has been established that the reduced form of the coenzyme is neither a 1,2- nor 1,6-dihydropyridine derivative but a 1,4-dihydro-type. The 1,2- and 1,6-isomers have no activity toward the reduction. In the reduction by NAD(P)H, one of the hydrogens at the 4 position of the 1,4-dihydronicotinamide ring directly transfers onto the substrate without exchanging with hydrogens of a solvent or of other parts of the enzyme (Westheimer et al. 1951). Direct transfer of hydrogen from the substrate has also been ascertained in the reverse reaction: oxidation of the substrate. The 1,4-dihydropyridine moiety in the reduced form of the coenzyme is stable to air. The stability of NAD(P)H toward molecular oxygen is quite different from that of flavin coenzymes, the reduced form of which is readily oxidized by molecular oxygen. Since the oxidation of the former compound has to break a carbon-hydrogen bond, whereas the oxidation of the latter compound is equivalent to simple dissociation of a hydrogen from a nitrogen, the difference in reactivity of these two compounds is not surprising.

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* The compound α-NAD^+, which contains an α-glycosidic bond between the nicotinamide and the ribose also exists, but this compound has no activity toward the β-NAD^+-dependent dehydrogenases.

3 STEREOCHEMISTRY IN NAD(P)$^+$-DEPENDENT DEHYDROGENASES

3.1 Stereospecific Hydrogen Transfer from NAD(P)H

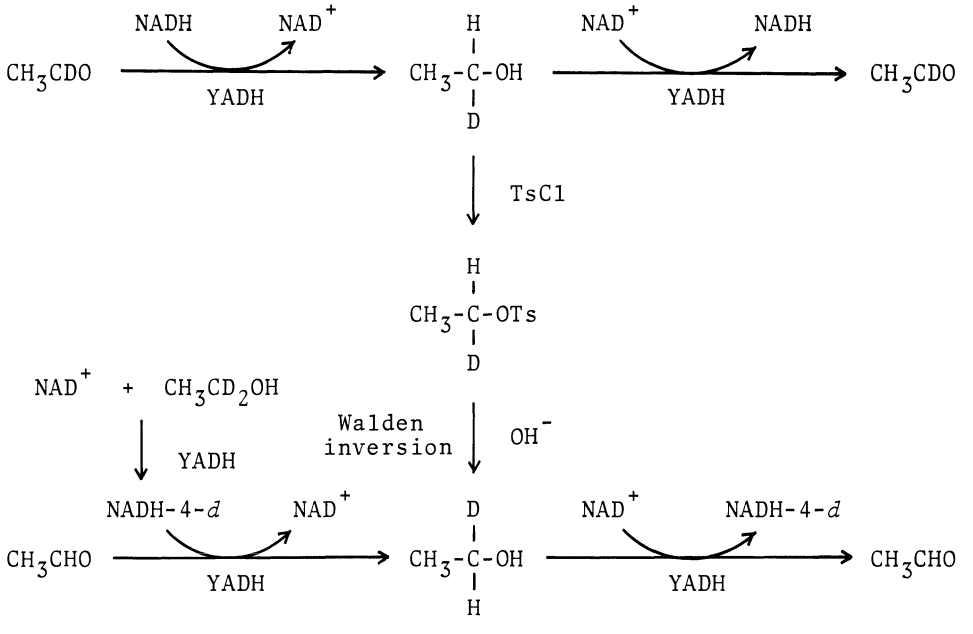
One of the most outstanding features in enzyme-catalyzed reactions is stereochemical completeness. A high percentage of stereospecific reactions, such that only one chiral product is formed in an excellent enantiomer excess (e.e.) and that only one of two enantiomers is available as a substrate, readily take place. In addition, the spectacular feature that an enzyme can also differentiate enantiotopic groups or faces that are nonenzymatically (chemically) equivalent has also been found. For example, enzymes distinguish two hydrogens attached to a prochiral carbon, $>CH_2$. Even the three hydrogens of a methyl group are sometimes nonequivalent within an enzyme. As will be discussed below, such phenomena, which could not be proved without using an isotope labeling technique, tell us that the active site of an enzyme provides a specific environment for the substrate.

3.2 Stereospecificity in an Alcohol Dehydrogenase

In the reaction catalyzed by an alcohol dehydrogenase (ADH), diastereotopic hydrogens at the 4 position of the 1,4-dihydronicotinamide ring in NADH as well as enantiotopic hydrogens in ethanol are clearly discriminated in the course of redox interconversion. This specificity was clarified by Westheimer and his co-workers in a series of their pioneering works with yeast alcohol dehydrogenase (YADH) by means of a deuterium-labeling technique. Loewus et al. (1953b) prepared two kinds of ethanol-1-*d*: one from the YADH reduction of CH_3CDO by NADH and the other from that of CH_3CHO by NADH-4-*d*. This NADH-4-*d* was obtained by the reduction of NAD^+ with CH_3CD_2OH using YADH. These two kinds of ethanol-1-*d*s, which were later proved to be enantiomers of each other, were subjected to reoxidation by NAD^+ on YADH. The acetaldehyde produced from ethanol-1-*d* derived from CH_3CDO contained one deuterium atom per molecule while ethanol-1-*d* derived from CH_3CHO gave nondeuterated acetaldehyde. This means that the hydrogen atom attached to the α-carbon of ethanol transfers directly to become one of the hydrogens at the 4 position of NADH and that the same hydrogen is utilized for the reverse process, reduction.

Next, they subjected ethanol-1-*d*, which was obtained from CH_3CDO by the reduction with YADH, to a tosylation followed by a hydrolysis in aqueous sodium hydroxide. Since this process involves a Walden inversion, the configuration of the reproduced ethanol-1-*d* should be inverted. The oxidation of this ethanol-1-*d* by YADH afforded nondeuterated CH_3CHO. The whole reaction sequence is shown in Scheme 2. Furthermore, reoxidation of monodeuterated NADH (NADH-4-*d*) prepared from CH_3CD_2OH enzymatically afforded NAD^+ without contamination by deuterium. On the other hand, when NADH-4-*d* obtained by the reduction of NAD^+ using sodium dithionite in D_2O was subjected to enzymatic reoxidation by acetaldehyde, the oxidized form of the coenzyme contained about a half equivalent of deuterium at the

Scheme 2



4 position of the pyridinium ring as shown in Scheme 3 (Fisher et al. 1953). These results also support the stereospecificity of the YADH system that only one of the two hydrogens at the α-carbon of ethanol and only one of the two faces of the carbonyl group in acetaldedehyde participate in the reaction.

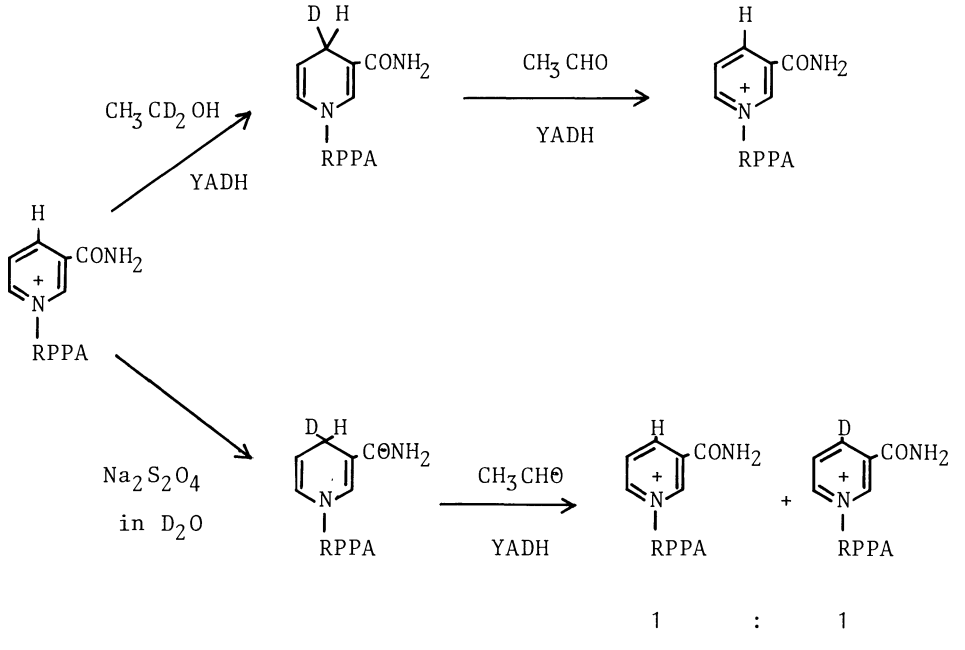
3.3 Stereospecificity in Other Dehydrogenases

The first procedure to study the stereospecificity of ADH was applied to other dehydrogenases. As can be seen in Scheme 4, NADH-4-*d* obtained from the reduction of NAD^+ with CH_3CD_2OH reduced pyruvate with the aid of a lactate dehydrogenase (LDH) to afford lactate which contained one deuterium atom per molecule at the 2 position. On the other hand, the deuterium content in the lactate obtained from the reduction by chemically prepared NADH-4-*d* was 0.58 per molecule (Loewus et al. 1953a). This result indicates that both ADH and LDH utilize the same side of the nicotinamide ring for the redox interconversion.

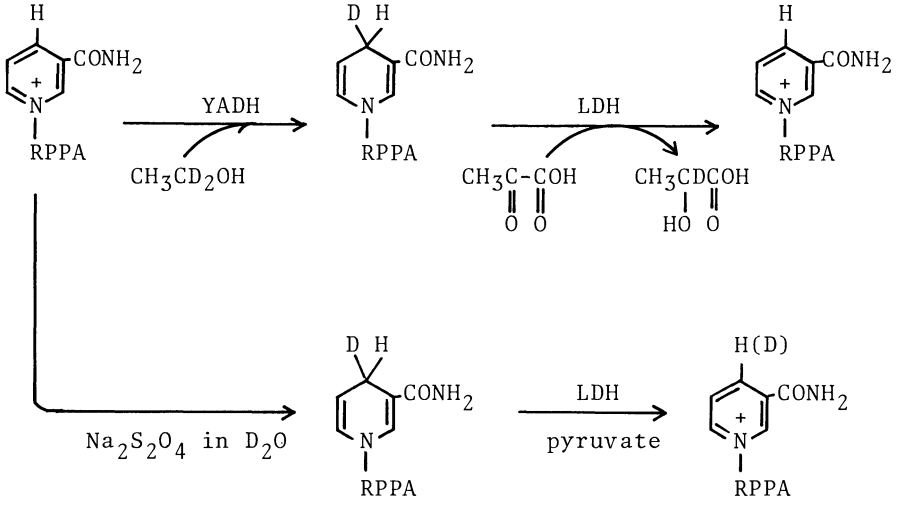
Later, dehydrogenases that utilize the other side of the nicotinamide ring were found. These enzymes are, for example, β-hydroxysteroid dehydrogenase from *Pseudomonas testosteroni* (Talalay et al. 1955, Jarabak and Talalay 1960), the pyridine nucleotide transhydrogenase from *Pseudomonas fluorescens* (San Pietro et al. 1955), and glycerol-3-phosphate dehydrogenase from muscle (Levy and Vennesland 1957).

Thus, NAD^+-dependent dehydrogenases are subdivided into two classes. Dehydrogenases that exhibit the same specificity as ADH

Scheme 3

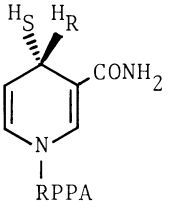


Scheme 4



belong to the A-type and use H_A, whereas those that exhibit the opposite specificity are called B-type and utilize H_B. It was proved such a classification is also applicable to $NADP^+$-dependent

dehydrogenases. According to the modern terminology in stereochemistry, the two prochiral hydrogens should be denoted as *pro-S* or *pro-R* hydrogens as shown in Fig. 1.

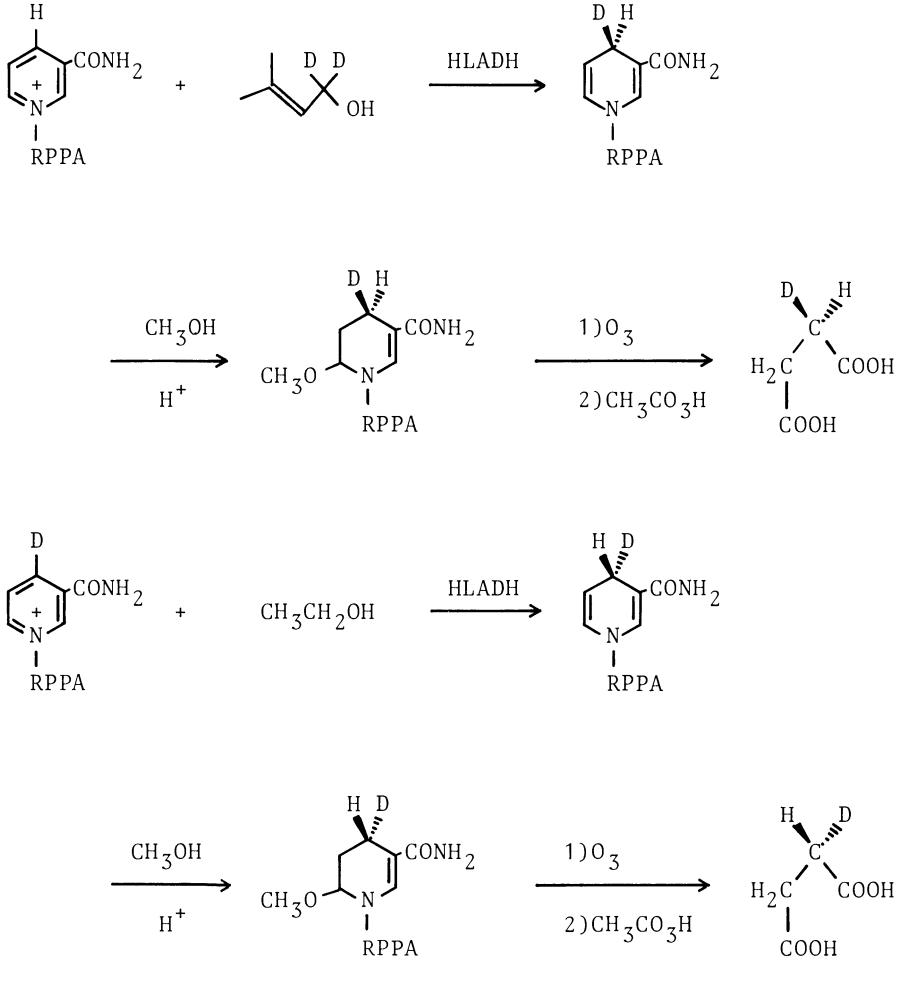


H_S : *pro-S* hydrogen

H_R : *pro-R* hydrogen

Fig. 1. Definition of *pro-S* and *pro-R* hydrogens in NAD(P)H.

Scheme 5

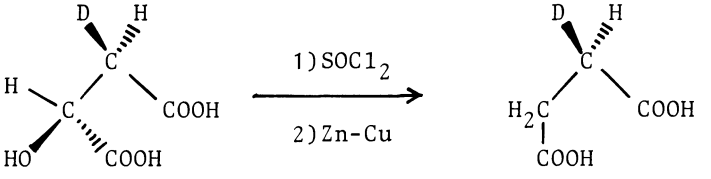


3.4 Stereochemistry of Transferring Hydrogen

Prior to the investigation by Cornforth et al. (1962), it was not clear whether H_R or H_S corresponds to H_A. Using horse liver alcohol dehydrogenase (HLADH) in the reduction of NAD^+ with 3-methylbut-2-en--1-ol-1,1-d_2 they prepared NADH-4-*d* whose H_A position was occupied by a deuterium. The coenzyme with opposite configuration was prepared by the reduction of NAD^+-4-*d* with ethanol. These diastereomeric NADH-4-*d*s were converted into succinic acid-2-*d*s by the addition of methanol in acidic media followed by an ozonolysis and oxidation with peracetic acid as shown in Scheme 5.

The optical rotatory dispersion (ORD) curves of the succinic acids, which were believed to be enantiomers of each other, gave a good contrast. The ORD spectrum of the succinic acid-2-*d* obtained from the reduction with NADH-4-d_A was proved to be identical with that obtained from (2*S*,3*R*)-malic acid-3-*d* according to the procedure shown in Scheme 6.

Scheme 6



(2*S*,3*R*)-malic acid-3-*d* (2*R*)-succinic acid-2-*d*

Therefore, it was concluded that the absolute configuration at the 4 position of NADH-4-*d* whose deuterium was introduced from the substrate on HLADH is "*R*". This means that the H_A can be depicted as H_R.

3.5 Stereochemistry with Respect to the Substrate

As shown above, the prochiral hydrogens in NADH are clearly differentiated by an ADH system. At the same time, the enzyme recognizes the prochiral hydrogens in ethanol to be transferred. The absolute stereochemical course with respect to the substrate was revealed by Lemieux and Howard (1963). Scheme 7 shows that the *pro-R* hydrogen on NADH transfers to the *re*-face of acetaldehyde to become the *pro-R* hydrogen in the 1 position of ethanol and that the same hydrogen returns onto NAD^+ in the reverse reaction.

Thus, it has been proved that an ADH catalyzes the redox reaction between acetaldehyde and ethanol stereospecifically and that both YADH and HLADH exhibit the same specificity toward the coenzyme and the substrate. These two enzymes can also catalyze the reduction of other aldehydes. It has been ascertained that these reactions proceed with the same stereospecificity. Furthermore, ADH can catalyze the reduction of other carbonyl compounds. The scope for the substrate is more limited in the reaction with HLADH compared to

Scheme 7

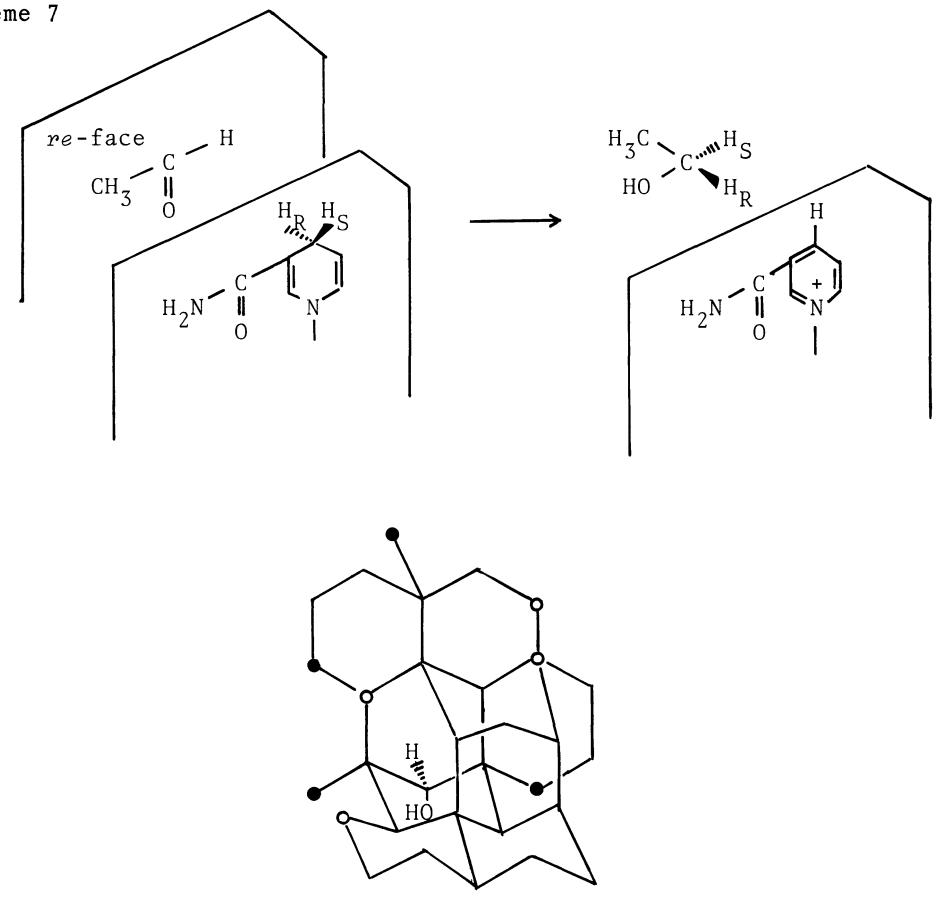


Fig. 2. Diamond-lattice representation for HLADH.

that with YADH. In order to get information on the structure of the active site of dehydrogenase and to achieve stereopecific reductions of carbonyl compounds enzymatically, reductions of a variety of carbonyl compounds with HLADH have been studied. In his pioneering work, Prelog (1964) proposed the diamond-lattice theory for the structure of the active site of the enzyme. He compared the reactivity of a number of monocyclic and bicyclic ketones and alcohols in the redox system with HLADH. The theory predicts the molecular arrangement allowed or forbidden for the substrate to be incorporated into the enzyme. Cyclic ketones are good substrates to investigate the structure of the active site of the dehydrogenase because of their rigid framework. As a result, the active site of the enzyme was drawn as sections of a diamond lattice, as shown in Fig. 2, where the carbonyl group is fixed in a particular position and the undesirable and forbidden positions in the carbon skelton are indicated by o and •, respectively. Other positions are allowed to be occupied.

4 ENZYMATIC REDUCTIONS

4.1 Structure of Dehydrogenase and Substrate Binding

The HLADH is a dimer of molecular weight of about 80000. There are at least two distinctive types of subunits, E (ethanol active) and S (steroid active). These two types differ in the position of six amino acids, which seems to be the origin of the difference in their catalytic properties (Jörnvall 1970a,b). The replacement of only one of the six amino acids by another one results in the appearance of steroid activity (Eklund et al. 1976). It seems that the steroid activity is an intrinsic property of the enzyme rather than the activity toward ethanol because the latter is not a normal bodily constituent (Damgaard 1981).

HLADH contains two zinc ions per subunit. One of the zinc ions is called the "catalytic zinc" and is located in the active site of the enzyme (Sigman 1967). The catalytic zinc can be specifically depleted with concomitant loss of enzymatic activity or substituted by other metal ions with distinctly reconstituted activities. The enzyme is strongly inhibited by metal chelating agents such as pyrazole, which has been well exploited by studies of the reaction mechanism (McFarland and Bernhard 1972, Schmidt et al. 1979). The other zinc ion, the "structural zinc", is used to maintain the three-dimensional structure of the enzyme.

On the other hand, YADH is a tetramer of molecular weight of about 145000 and each subunit has only one zinc ion which is "catalytic". In spite of these differences, the fundamental structures of YADH subunits are considered to be very similar to those of HLADH, based on the comparison of their amino acid sequences.

X-ray crystallographic studies have been made extensively on HLADH and it is known that each subunit is divided into two domains, that is, coenzyme-binding and catalytic domains (Eklund et al. 1974). These domains are separated by a deep cleft. The coenzyme-binding domain is composed of residues 176-318 and has a folding structure essentially the same as found in many other NAD^+-dependent dehydrogenases (Rossman et al. 1975). The catalytic domain is composed of residues 1-175 and 319-374. The catalytic zinc is situated at the bottom of this deep pocket between the two domains and constitutes a hydrophilic center in a highly hydrophobic environment (Brändén et al. 1975). The zinc ion is ligated by two sulfur atoms from Cys-46 and Cys-174 and one nitrogen atom from His-67. The fourth ligand is water or a hydroxide ion, depending on the pH. The catalytic domain in dimeric HLADH undergoes a distinct conformational change upon the formation of a ternary complex. The total change is described as a rotation of the catalytic domains with respect to the central core and it is believed that the front and rear parts move about 6-7 Å. The rotation makes the active site shielded from the solvent.

The role of the catalytic zinc ion was initially considered to be to activate the carbonyl group of the substrate as a Lewis acid (Dunn et al. 1975). Since then, there have been many discussions on the role of the catalytic zinc. Some mechanisms assume an intermediate penta-coordinated zinc and others propose the formation of an outer sphere complex. Recent crystallographic studies on certain ternary complexes strongly support the direct binding of the

substrate to the catalytic zinc in tetra-coordinate fashion. For example, the X-ray diffraction study on crystals from an equilibrium mixture containing predominantly NAD^+ and *para*-bromobenzyl alcohol demonstrated the direct coordination of the true substrate to the catalytic zinc ion during the normal reaction (Bignetti et al. 1979). It was also revealed that *E*-4-(N,N-dimethylamino)cinnamaldehyde (DACA) binds to the catalytic zinc through its carbonyl oxygen in a ternary comlex (Cedergren-Zeppezauer et al. 1982, Jagodzinski and Peticolas 1981, Angelis et al. 1977). It is also proposed that the zinc ion prompts the coordinated water molecule to behave as a general acid-base catalyst (Dworschak and Plapp 1977, Evans and Shore 1980, Bobsein and Myers 1980, 1981).

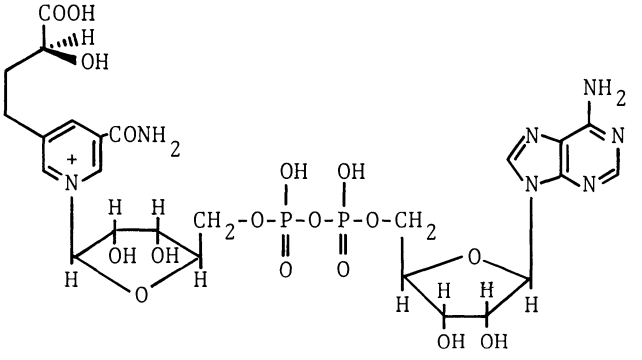
In contrast to the catalytic zinc, the structural zinc ion is inert for binding the substrate, because it is firmly ligated by four sulfur atoms from Cys-97, Cys-100, Cys-103, and Cys-111.

Lactate dehydrogenase (LDH) is usually a tetramer of molecular weight of about 140,000. In contrast to ADH, LDH is not a metalloenzyme (Li et al. 1983). It is suspected that LDH does not require the metal ion catalysis because the substrate for this enzyme is much more reactive toward the reaction than that of ADH. Crystallographic studies on LDH have been done for many apoenzymes and ternary complexes. Usually, each LDH subunit is divided into four domains: N-terminal arm, coenzyme binding domain, loop and helix αD region, and substrate binding (catalytic) domain. The N-terminal arm seems to be engaged in subunit interactions. The coenzyme is placed in the bottom of a cleft with the nicotinamide ring buried deep within the subunit (Adams et al. 1970). Upon the formation of a ternary complex, definite conformational changes occur in the regions around the active site. Consequently, the loop covers the active site pocket. At the same time, the unreactive B side (*si*-face) of nicotinamide is set in a hydrophobic environment. The pyrophosphate moiety of the coenzyme forms an ionic bond with the arginine residue of the enzyme. The conformational change in the enzyme also introduces various charged groups into the vicinity of the substrate binding site. Solvent molecules in the active site seem to be excluded through a hydrophilic gap between the loop and the rigid part of the molecule (Eventoff et al. 1977).

The structure of the productive ternary complex has been deduced from the analysis of various model complexes. An abortive enzyme-NAD^+-pyruvate complex, which was first investigated in detail, was found to be a *binary* complex because it contains a covalent bond between NAD^+ and pyruvate. The complex is reversibly formed by the reaction of enzyme-NAD^+ with the enol form of pyruvate (Griffin and Criddle 1970, Holbrook and Stinson 1973, Arnold and Kaplan 1974, Burgner and Ray 1974, Burgner and William 1978). The nucleophilic attack of pyruvate enol occurs stereospecifically at the 4 position from the A side (*re*-face) of the nicotinamide ring. The significance of this observation upon the mechanism of normal oxidation-reduction with LDH is, however, obscure because the normal reaction requires the keto form instead of the enolate (Tienhaara and Meany 1973). At the same time, both pyruvate and NAD^+ are in oxidized states. Similar formation of an adduct can easily be accomplished by a nonenzymatic base-catalyzed reaction without, of course, any stereospecificity. In the case of the mimetic reaction, further bond formation between the amide nitrogen and the pyruvate carbonyl carbon occurs to give a cyclized product. The cyclization also

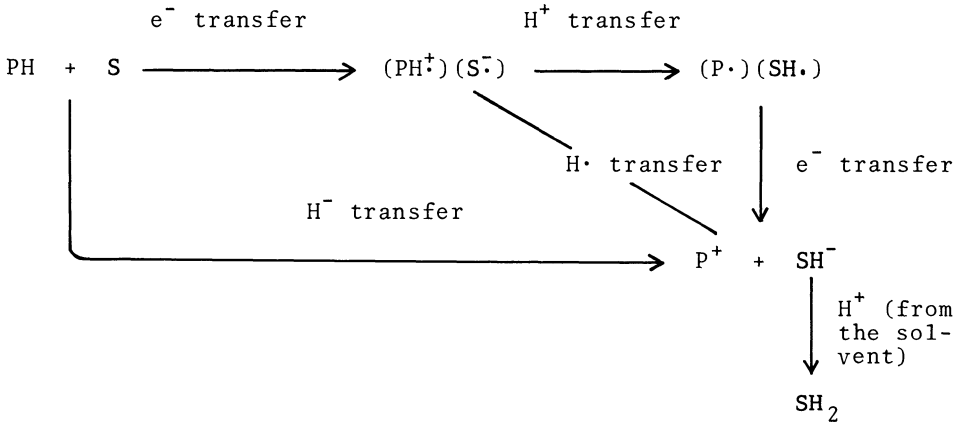
takes place when the adduct formation is performed in the presence of LDH at a raised pH of 10, probably after the dissociation of the first adduct from the enzyme.

The carboxyl group in the substrate forms a salt bridge with the guanidinium group of an arginine residue. The imidazolyl group of a histidine residue is in contact with the alcoholic oxygen of the substrate through a hydrogen bond, thus acting not only as an acid-base catalyst but also as one of the "three points" in the stereospecific oxidation-reduction of the substrate (Südi 1976). The structure of the active site in the reacting ternary complex has been deducedd from the analysis of an active "ternary" complex enzyme-(*S*)-lacNAD$^+$ (Grau et al. 1981). In the structure of (*S*)-lacNAD$^+$, *S*-lactate is covalently bound through a methylene spacer in the 5 position of the nicotinamide ring. This compound undergoes an intramolecular hydride transfer in the presence of pig heart LDH to form 5-(2-oxalylethyl)NADH reversibly (Kapmeyer et al. 1976).



(*S*)-lacNAD$^+$

Scheme 8



4.2 Mechanism of Hydride Transfer

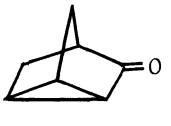
As will be discussed later, two or more mechanisms have been proposed for the "net" hydride transfer reactions in mimetic systems. One is, of course, the one-step "hydride" transfer mechanism and the other is a multistep mechanism involving the initial "electron" transfer process. The latter mechanism is further subdivided into two categories; the two-step electron-hydrogen atom transfer mechanism and the three-step electron-proton-electron transfer mechanism as shown in Scheme 8.

As early as 1957, it was reported that ternary complexes including ethanol-NAD^+-ADH, acetaldehyde-NADH-ADH, and other biological systems exert ESR signals that may indicate the presence of charge transfer complexes as intermediates of enzymatic redox reactions (Commoner et al. 1957). However, the signals were so complicated that they could not be attributed to any particular species. Substituent effects, as well as large kinetic isotope effects, associated with the reductions of substituted and unsubstituted benzyl alcohols with a YADH system, on the other hand, led Klinman to conclude that the reaction does not involve the intermediate (Klinman 1972, 1976). A few years later, however, Klinman and her co-workers studied the α-secondary kinetic isotope effect of the same reduction and concluded that the reaction passes through a radical intermediate (Welsh et al. 1980).

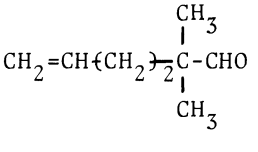
In the reduction of pyruvate to lactate, isoenzymes of lactate dehydrogenase from pig heart and pig muscle exerted no kinetic deuterium isotope effect (Holbrook and Stinson, 1973). The results reveal that the process involving the movement of the hydrogen nucleus does not constitute the rate-determining step. Here, the isomerization of the substrate-NADH-enzyme ternary complex to an "active complex" is suggested to be the rate-determining step. The question of whether the "active complex" corresponds to a "charge transfer complex" or to a "conformationally distorted complex" remains unsolved.

Theoretical considerations on kinetic isotope effects for the reduction with ADH suggest that there is little charge on the carbonyl carbon of the substrate (Cook et al. 1981). The out-of--plane bending of the carbon-hydrogen bond at the 4 position in the dihydropyridine ring and a tunneling effect contribute largely to the large kinetic isotope effect (Cook et al 1981, Huskey and Schowen 1983, Kurz and Frieden 1980).

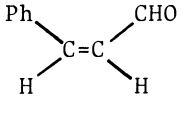
In addition to physical organic techniques mentioned above, product analyses have been done from the viewpoint of reaction mechanism diagnosis. Namely, the reaction with HLADH was investigated by means of several chemically detectable radical probes such as nortricyclanone (NTC), 2,2-dimethyl-5-hexenal (DMHA), and Z-3-



NTC



DMHA



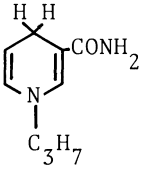
PPA

-phenylpropenal (PPA) (Chung and Park, 1982). These substrates did not afford the rearranged products indicating that there is no radical intermediacy in the reaction. A similar result was obtained in the HLADH oxidation of α-hydroxyalkylcyclopropanes (Maclnnés et al. 1982). It is known that the cyclopropylmethyl free radical isomerizes into the butenyl free radical quantitatively (van Niel and Pandit, 1983), but the whole product from the enzymatic reduction reserved the cyclopropyl moiety.

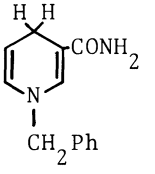
5 ASYMMETRIC REDUCTION BY MODEL COMPOUNDS OF NAD(P)H

5.1 Model Reactions of NAD(P)H Dependent Dehydrogenases

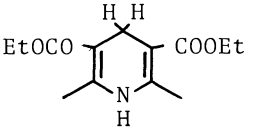
Within a molecule of NAD(P)H, the 1,4-dihydronicotinamide moiety acts as a reducing reagent. Thus, a 1,4-dihydropyridine derivative where the ring nitrogen is substituted by a simple substituent, such as 1-propyl-1,4-dihydronicotinamide (PNAH), 1-benzyl-1,4-dihydronicotinamide (BNAH), or Hantzsch ester (HEH), can be considered as a model compound for NAD(P)H.



PNAH

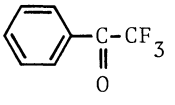


BNAH

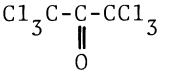


HEH

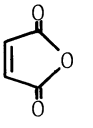
Simulation of the reactions of dehydrogenases using 1,4-dihydropyridine derivatives has been widely investigated (Eisner and Kuthan 1972, Stout and Meyers 1982). These compounds reduce C=O, C=C, C=N, and C=S double bonds as well as flavin and metal ions (Kill and Widdowson 1978, Okamoto et al. 1979). However, they are not so reactive toward the reduction compared with NAD(P)H in a biological system where an apoenzyme participates as a catalyst. The 1,4-dihydropyridine derivatives in a nonenzymatic system can reduce only those substrates like α,α,α-trifluoroacetophenone (TFA), hexachloroacetone (HCA), and maleic anhydride(MA). That is, the substrate should be strongly electron-deficient in order to be reduced.



TFA



HCA



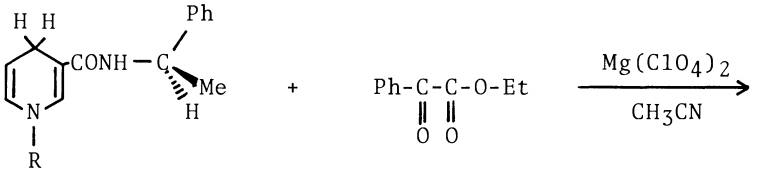
MA

Considering that the reactivity of the 1,4-dihydropyridine moiety extracted from the dehydrogenase system is very low, it is easily noticed that a specific field created by amino acid residues from the apoenzyme is very important for acceleration of the reaction. At the same time, it should be noted that such a reaction field at the active site of the enzyme contributes to the stereospecificity of the reaction. Although NAD(P)H contains chiral centers within the molecule, these chiralities exert no effect on asymmetric reduction of a substrate. Induction of the chirality in an enzymatic system depends on the chiral environment arranged by the apoenzyme. Thus, it is necessary to introduce a chiral environment around the 1,4-dihydropyridine ring of NAD(P)H model compounds to simulate the asymmetric reduction by dehydrogenases.

5.2 The First Asymmetric Reduction

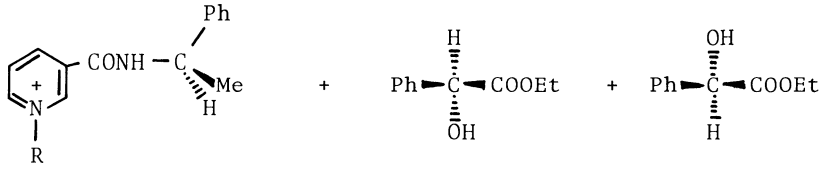
The first example of the asymmetric reduction by an NAD(P)H model compound was reported in 1975 (Ohnishi et al. 1975a). By means of

Scheme 9



R-PNPH : R = *n*-propyl
R-BNPH : R = benzyl
R-Cl_2BNPH : R = 2,6-dichlorobenzyl

EBF



R-PNP^+ : R = *n*-propyl
R-BNP^+ : R = benzyl
R-Cl_2BNP^+ : R = 2,6-dichlorobenzyl

S-EM

R-EM

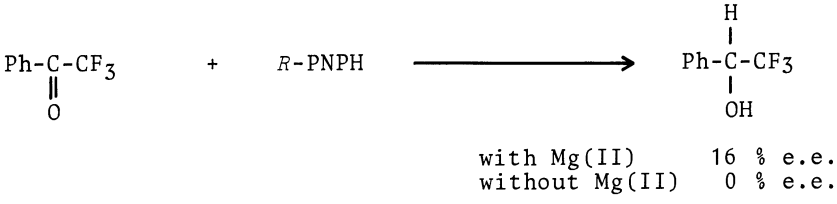
Table 1. Asymmetric reductions of ethyl benzoylformate with chiral model compounds.

Reducing reagent	Configuration of the product	e.e. [%]
R-PNPH	*R*	20
R-BNPH	*R*	18
R-Cl_2BNPH	*R*	11

a 1,4-dihydronicotinamide derivative which contains a chiral α-methylbenzylamine moiety in the carbamoyl side chain, ethyl benzoylformate (EBF) was reduced in the presence of magnesium perchlorate to give ethyl *R*-mandelate (*R*-EM) in 11 - 20 % enantiomer excess (e.e.), as shown in Scheme 9 and Table 1.

This reaction involves several notable features. Although the chiral center is separated from the reaction center (*i.e.*, the 4 position of the dihydropyridine ring) by three atoms, the e.e. value obtained from this system was relatively high. It should also be noted that the bivalent magnesium ion [Mg(II)] is indispensable here, not only for the reduction but also for the induction of chirality into the substrate. It was ascertained that the reduction cannot proceed without Mg(II) (Ohnishi et al. 1975a). The contribution of Mg(II) to the asymmetric reduction is obvious from the following observation: although α,α,α-trifluoroacetophenone was reduced without Mg(II), asymmetric induction was not achieved in its absence, whereas, as shown in Scheme 10, the reduction in the presence of Mg(II) afforded the product in 16 % e.e. (Ohnishi et al. 1975b).

Scheme 10



The metal ion in this system may be regarded as a mimetic apo-enzyme in the sense that it not only accelerates the reduction but also provides an asymmetric field as does the enzyme in biological reactions.

The effect of the bivalent metal ion on the reduction with a 1,4--dihydropyridine derivative as a model of NAD(P)H, particularly on the induction of chirality, was thus found to be quite large. The increase in the stereospecificity is, here, associated with an increase in the reactivity, which is not common in organic reactions. Thus, it will be necessary to discuss the role of the metal ion in the model system before we discuss the mechanism for the induction of asymmetry.

5.3 The Role of Metal Ion

A variety of bivalent metal ions have already been employed in mimetic reactions (Sigman et al. 1978). By the addition of a metal ion, rates of reduction of some substrates such as thioketones and acridinium salts were retarded (Dittmer et al. 1976, Ohno et al. 1978a). Most of the substrates, however, were more easily reduced in the presence of a metal ion (Creighton and Sigman 1971, Ohnishi et al. 1975c, Shirai et al. 1975). As will be discussed below, the role of a bivalent metal ion in model systems seems to be different from the one suggested for the enzymatic system, which is not surprising because the metal ion in a mimetic reaction plays the role of the whole enzyme in a biological reaction. In this sense, Zn(II)

in ADH should be regarded as a part of the enzyme.

The observation of the rate acceleration by a bivalent metal ion such as Mg(II) or Zn(II) in the model system readily leads to the idea that the metal ion may polarize the carbonyl group of the substrate as a Lewis acid to facilitate the reduction (Creighton et al. 1976, Hughes and Prince 1978b, Steevens and Pandit 1983). However, such a function of the metal ion cannot explain the result that the presence of the metal ion exerts an effect for the induction of chirality by PNPH into ethyl benzoylformate. A more intrinsic function, namely, an interaction between the metal ion and the 1,4-dihydronicotinamide moiety is to be considered (Hughes and Prince 1978a, Hughes et al. 1978). In fact, it has been established, based on spectroscopic study, that Mg(II) forms complexes with 1,4-dihydropyridine derivatives. For example, the electronic absorption at around 350 nm due to the π-π^* transition of the dihydropyridine moiety shifts bathochromically and its extinction coefficient increases as the 1,4-dihydropyridine forms a complex with a metal ion (Ohno et al. 1977b). On the other hand, in contrast to the case of acylpyridine derivatives, ethyl benzoylformate has little ability to coordinate onto the metal ion. No spectroscopic change due to the complexation of ethyl benzoylformate with Mg(II) was observed (Ohno et al. 1977b). Therefore, the role of the metal ion in this system might be more than the Lewis acid: the metal ion binds to the 1,4-dihydropyridine to facilitate the electron transfer to the substrate and the substrate is stabilized *at the transition state* by the coordinated metal ion within the ternary complex where, at the same time, the orientation of the reactants is fixed. In this type of complex, Mg(II) would be located between the dihydropyridine and the substrate, as shown in Fig. 3 (Ohno et al. 1977b, 1977c).

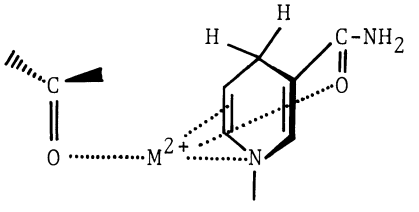


Fig. 3. Schematic representation of the transition state of the reaction in the presence of a bivalent metal ion (M^{2+}).

Such a fashion of the complexation is also supported by infrared spectroscopy, where no appreciable shift of the absorption corresponding to the stretching vibration of the amide carbonyl was observed (Ohno et al. 1977b). Data from ^{13}C-NMR experiments showed a different situation; according to a report by Gase et al. (1976), it seems that Mg(II) coordinates on the amide oxygen of BNAH judging from the down-field shifts of the signal from C2 and the carbonyl carbon and an up-field shift of the signals from C3. Although not all of the spectroscopic data tend to focus into the same result for the position of the coordination of the metal ion, it is certain that the interaction of Mg(II) with the 1,4-dihydronicotinamide plays an important role for the reduction. The association constant is relatively large. For example, that of 1-propyl-1,4-dihydronicotin-

amide (PNAH) and magnesium perchlorate was measured to be about 1.5 x 10^4 M^{-1} in dry acetonitrile at 293 K (Ohno et al. 1984a,b). The corresponding association constant for BNAH is 1.2 x 10^4 M^{-1} (Fukuzumi et al. 1983b).

The difference in the kinetic profiles of the reduction also supports the idea mentioned above. The observed rate constant (k_{obsd}) for the reduction of a substrate, such as ethyl benzoylformate or α,α,α-trifluoroacetophenone, which has low ability for complexation with a metal ion, increases as the concentration of the metal ion increases as depicted in Fig. 4(a) (Ohno et al. 1977c). However, with a substrate which can form a complex with a metal ion, such as 2-acetylpyridine, k_{obsd} changes peculiarly as the concentration of the metal ion increases, as shown in Fig. 4(b) (Gase et al. 1976).

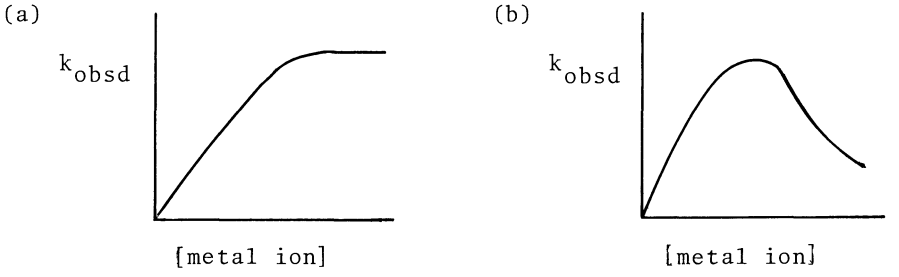


Fig. 4. Rate-metal ion concentration profiles for the reduction of (a) ethyl benzoylformate and (b) a 2-acylpyridine derivative.

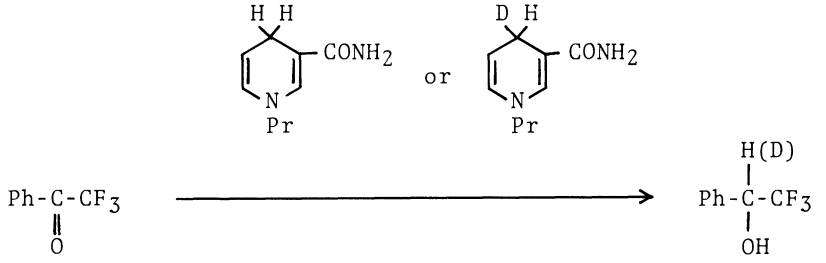
The latter profile was interpreted in terms of the formation of two different types of complexes (Ohno et al. 1980a). In the presence of metal ions in excess, both the substrate and the 1,4-dihydropyridine derivative are complexed by the metal ion. Under such circumstances, the 1,4-dihydropyridine derivative and the substrate cannot come close together because of the repulsive force between two positive charges on the magnesium ions. Consequently, the formation of the ternary complex that is indispensable for the reduction is suppressed. It should be emphasized that the formation of a ternary complex by the chelation with the metal ion is the most important factor for the metal ion-catalyzed model system.

5.4 Mechanism of the Reduction with NAD(P)H Models

Discussion in the previous section raises the question, how the 1,4-dihydropyridine moiety releases a negative species, "hydride", after being coordinated by a metal ion that has an electron-withdrawing property. To answer the question and to consider the molecular arrangement in the transition state of the asymmetric reduction we must deal with the mechanism of the reduction by 1,4-dihydropyridine derivatives.

Based on the fact that the hydrogen at the 4 position of the 1,4--dihydropyridine ring transfers directly to the substrate, a one-step hydride transfer mechanism proposed by Westheimer and his co-workers in early days has been accepted. However it is very curious to consider the polarization where a partial negative charge localizes on a less electronegative hydrogen atom in a C-H bond. In this connection it was suggested that apparent hydride transfer involves an initial one-electron transfer to produce an intermediate which corresponds to an ion radical pair or a charge transfer complex between the substrate and the 1,4-dihydropyridine derivative. The electron transfer mechanism (ET-mechanism) was first suggested by Steffens and Chipman (1971) based on the discrepancy between the kinetic isotope effect associated with the reduction (k_H/k_D) and the deuterium content in the produced alcohol (product isotope effect: Y_H/Y_D) in the reaction of α,α,α-trifluoroacetophenone with PNAH or PNAH-4-*d* (Scheme 11).

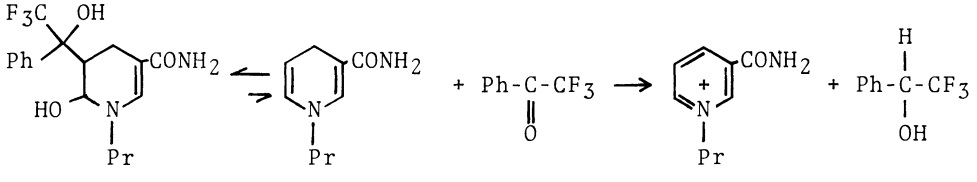
Scheme 11



It was proved later that the discrepancy was caused as a result of a side reaction, shown in Scheme 12, which readily occurred in an aqueous solution (van Eikeren et al. 1979, Chipman et al. 1980).

However, such a discrepancy between two isotope effects was also observed in dry acetonitrile solution, where no side reaction took place (Ohno et al. 1981a). This evidently indicates that a certain step precedes the hydrogen transfer, which can be regarded as an electron transfer step, and that the existence of an intermediate or

Scheme 12



intermediates should be considered. In addition, from the result of the change in ρ-value in the reaction of substituted α,α,α-trifluoroacetophenones with BNAH in the absence of Mg(II), it was proved

that the rate-determining step changes from the electron transfer step to the hydrogen nucleus transfer step as the ability of a substrate for electron acception increases. On the other hand, in the Mg(II)-catalyzed reaction, the migration of the rate-determining step was not observed, which was interpreted in terms of a facile transfer of an electron to the substrate under the catalysis of a metal ion.

A more direct proof of the ET-mechanism is the detection of the intermediate. Ohno and Kito (1972) and Ohnishi et al. (1976b) detected, using ESR, the anion radicals derived from a substrate such as thiobenzophenone or benzil by a one-electron transfer in the course of the reduction. Production of charge transfer complexes between the substrate and 1,4-dihydropyridine derivatives was also reported (Ohno et al. 1981d, Fukuzumi et al. 1982a). It was observed that the anion radical derived from the substrate abstracts a hydrogen from the solvent when the reaction system is designed appropriately. Similar evidence had not been obtained in the usual reductions probably because the electron transfer and the following reactions took place within a cage of the solvent (Yasui et al. 1982, Shinkai et al. 1982, 1983). It was reported that the reduction of diazonium salts and halides proceed through radical chain reactions initiated by a one-electron transfer (Yasui et al. 1983, 1984, Fukuzumi et al. 1982b, 1983a). Substitution of a nitro group by a hydrogen by means of photo-assisted reduction with BNAH (Ono et al. 1980, 1983) and reduction of a disulfide to the corresponding thiol in the presence of BNAH (Oae et al. 1982) are also examples of radical-chain reactions initiated by a one-electron transfer. All of these results support the ET-mechanism. The multistep mechanism is also witnessed by Fukuzumi et al. (1982c, 1983c) and Powell et al. (1984).

It should be noted that the electron transfer process we are now interested in is the process associated with a certain degree of kinetic isotope effect (Okamoto et al. 1977a). The process is also the subject of base catalysis (Okamoto et al. 1977b). In this sense, the process is completely different from a physical process (Martens et al. 1978), in which the movement of an electron is faster than the movement of an atomic nucleus (Franck-Condon principle).

It has also been suggested that the reduction by 1,4-dihydropyridine derivatives proceeds by a one-step hydride transfer mechanism (HT-mechanism), which seems to be contrary to the ET-mechanism described above. This assertion is mostly based on the results from the reduction of cationic substrates such as quinolinium salts, acridinium salts, and protonated Schiff bases (Srinivasan et al. 1982). For example, Ostović et al. (1983) reported that the value of the kinetic isotope effect does not change significantly for the reactions with a series of substrates with which equilibrium constants change widely.

Roberts et al. (1982) concluded that the multistep mechanism involving an electron transfer process can be excluded, considering of the α-value of a Brønsted plot (α=0.5) observed over a wide range of cationic substrates, which agrees with the Marcus theory for atom transfer. The Brønsted α for an atom or group transfer depends on the position of a substituent and the tightness of the transition state (τ) as well as on the resemblence of the transition state to reactants and/or products. The Marcus theory predicts that τ can be related to the rates of symmetrical reactions. Rates and equilibrium constants were measured for the reactions of 10-methylacridane with a series of 1-benzyl-3-cyanopyridinium ions substituted in the

benzyl group and the Brønsted α was found to be 0.37. Using this value, a charge on the in-flight hydrogen was calculated to be about -0.23 (Kreevoy and Lee 1984).

Bunting et al. (1981, 1982a,b, 1984) insisted that the transferring hydrogen carries a partial negative charge, based on the quantitative calculation from the Hammett ρ-values in the reduction of isoquinolinium salts. The authors believe, however, that the Hammett ρ-value is a composite of various physical properties such as solvent polarity, solvation effect, steric effect, and so on, as well as the charge density at the reaction center. Particularly, the tightness of solvation changes depending on the amount of developing charge density (substituent effect) at the transition state (Leffler and Grunwald 1963). Therefore, the Hammett ρ-value is not an appropriate quantity for the calculation of *absolute* charge density at the particular position.

The discrepancy between kinetic and product isotope effects reported by Ohno et al. (1981e) and believed to provide a proof for the three-step electron-proton-electron transfer mechanism was recently claimed by Powell and Bruice (1983). They found that isotopes scramble between the reactant and product in the acridinium /acridane system, and attributed the discrepancy in kinetic isotope effects to this scrambling. It should be noted, however, that the scrambling is not a common phenomenon in the system so far studied.

Since a cationic substrate easily accepts an electron, it is difficult to observe the fast initial electron transfer step even if it is surely present. Namely, the kinetically observable process is only the solely or partially rate-determinig hydrogen transfer step. It should be noted that the evidence provided as the proof of the one-step mechanism is not necessarily proof of the HT-mechanism. Hydrogen may transfer as a proton or a hydrogen atom after donation of one electron to the substrate. Thus, there is no contradiction in concluding that the reaction is composed of three steps, electron--proton-electron transfer, and the importance of each step changes depending on the nature of the substrate. The rate-determining electron transfer and the rate-determining hydrogen transfer are two different extreme situations in a spectrum of the same phenomenon, a "hydride transfer". Most discussions so far concerning whether the reduction involves an initial electron transfer or not should be replaced by discussion on whether the rate-determining step is the electron transfer process or the process for the transfer of the hydrogen nucleus. Namely, the discussion should be focused on whether the activation energy for the electron transfer step is large enough to be observed or small enough to be neglected. Figure 5 schematically shows the energy diagrams for the three-step mechanism: a) reduction where the transfer of a hydrogen nucleus is rate determining and b) reduction where the initial electron transfer is involved in the rate-determining step.

It is the role of a metal ion that should be noted again. Acceleration of the rate by the addition of a metal ion results from the facilitation of the electron transfer step which has a higher barrier than the succeeding hydrogen atom (or proton) transfer process. On the other hand, for the substrate whose reduction is retarded by the addition of a metal ion, the reduction proceeds *via* an energy diagram shown in Fig. 5(a), where the electron transfer step is associated with lower energy of activation than the other step and the metal ion stabilizes the intermediate unnecessarily to result in a larger difference in energy between the intermediate and the rate-determining transition state of the reaction than that in

the reaction without the metal ion. One should notice that the stabilization of a reactant at the ground state results in the *retardation* of reaction and wastes catalytic power. The stabilization of a transition state is the entire and sole way to accelerate the reaction. This is also true in the reaction of proteins. The capacity to bind other molecules is not a special property of an enzyme. Instead, proteins of other classes such as antibodies, ionophores, carrier proteins, and so on, have a highly developed capacity to bind a substrate in the *ground state*. An enzyme is different from other proteins in that it can stabilize the *trnsition state* (Schowen 1978).

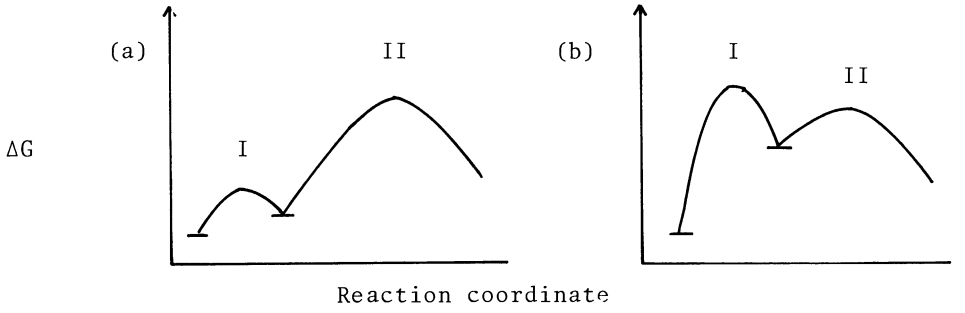


Fig. 5,a,b. Schematic illustration of energy diagrams for reduction; (a) hydrogen nucleus transfer, step (II) is rate determining and (b) initial electron transfer, step (I) is involved in the rate-determining step.

6 STEREOCHEMICAL COURSE OF THE REDUCTION

6.1 Stereochemical Course in the Reduction with PNPH

As mentioned above, it is reasonable to consider that the reduction by a 1,4-dihydropyridine derivative catalyzed by metal ions proceeds *via* a ternary complex as shown in Fig. 3. In the reduction by PNPH, the factor which determines the stereochemistry of the product is the mode of approach of the substrate to PNPH. Namely, the relative orientation between the 1,4-dihydropyridine ring and the substrate in the ternary complex is important. There are many possibilities for the molecular arrangement in the ternary complex as well as for the fashion of the coordination of Mg(II) to PNPH. Here we will discuss what kind of complexation and approach should be considered in order to understand the magnitude and direction of the induced chirality, or the mechanism of chirality induction.

First of all, we assume here a complex where the oxygen of the amide carbonyl in PNPH points toward the 2 position of the dihydropyridine ring and this oxygen and the π-electron system in the 1,4-dihydropyridine moiety coordinate onto Mg(II). The complex of this type was proposed because such complexation seemed to afford the most stable species at the ground state, judging from the spectroscopic data mentioned above. Though it may not be the case at the transition state of the reduction, a large change in the coordination seems to be implausible. The next problem is the mode of the

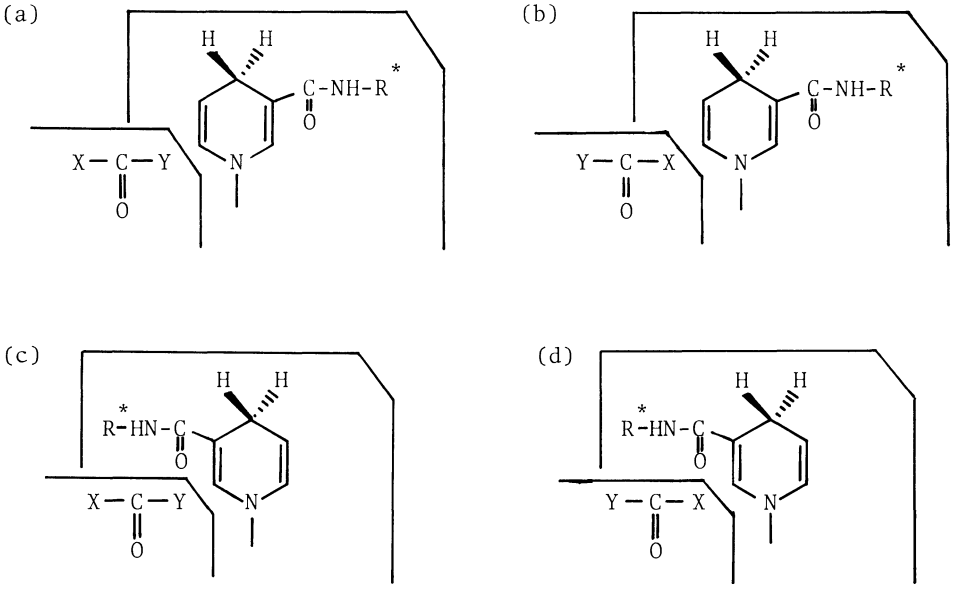
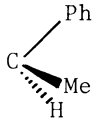


Fig. 6. Four types of approach of the substrate to the 1,4-dihydropyridine moiety.

X = Ph, Y = COOEt, R* = 

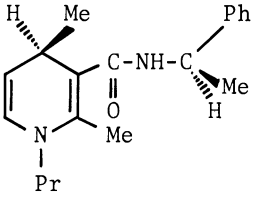
substrate approach to the complex. There are two categories of selection for the approach of the substrate. The first one is the selection of the faces of the 1,4-dihydropyridine ring, which corresponds to the diastereoface differentiation of the prochiral hydrogens at the 4 position. The second involves the selection of the enantioface in the substrate. That is, the "hydride" can choose either the *re*-face or *si*-face of the carbonyl group. As many as four types of approaches must be considered even though the variation of approach is reduced by the assumption that the carbonyl oxygen of the substrate points toward the ring nitrogen due to the binding by Mg(II) (Fig. 6).

Little is known about the factors influencing the molecular arrangement at the transition state of the reduction with PNPH. The most disadvantageous factor stems from the uncertainty on the transferring hydrogen. It is well documented for each dehydrogenase which of the two hydrogens at the 4 position of NAD(P)H transfers, and the method to clarify the stereochemistry of the transferring hydrogen has been well established in biological systems. On the other hand, in the model system, it is difficult to determine experimentally which hydrogen transfers preferentially from the 4 position of a 1,4-dihydropyridine ring, which also makes it difficult to speculate on the arrangement in the transition state of the reduction. At the same time, that both hydrogens are available for the reduction must reduce the stereoselectivity of the model

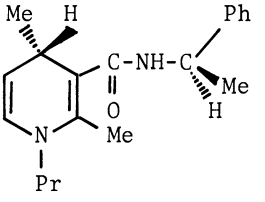
reaction with PNPH, whereas since only one hydrogen is available in an enzymatic system, a high degree of stereoselectivity can be achieved by a biological reduction. Therefore it is expected that if one of the two hydrogens at the 4 position of a 1,4-dihydropyridine derivative were specifically frozen toward the reduction, a high degree of stereoselectivity would be achieved. At the same time, much information about the stereochemical course of the reduction would be obtained.

6.2 Reduction with a Model Which Contains Chirality at the 4 Position

The idea was realized by the substitution of a methyl group for one of the two hydrogens at the 4 position. This means the introduction of chirality at the reaction center. Ohno et al. (1978c, 1979) synthesized N-α-methylbenzyl-1-propyl-2,4-dimethyl-1,4-dihydronicotinamide (Me_2PNPH) which has two chiral centers within a molecule.* By the reduction of methyl benzoylformate with *RR*-Me_2PNPH, one of the four diastereomers of Me_2PNPH, methyl *R*-mandelate was produced in 97.6 % e.e. As shown in Table 2, asymmetric reductions with excellent e.e. were achieved for a variety of substrates.



RR-Me_2PNPH



SR-Me_2PNPH

There are two main characteristic features in the present reduction. First of all, as can be seen from the result that methyl benzoylformate can be reduced without Mg(II), Me_2PNPH exhibits a high reactivity in comparison with other 1,4-dihydropyridine derivatives usually used as model compounds of NAD(P)H such as BNAH, PNAH, HEH, and PNPH. Such activity is due to the electron-donating property of two methyl substituents on the dihydropyridine ring (Bossaerts et al. 1984). Secondly, the chirality which determines the stereochemistry of the product is not the one in the α-methylbenzylamine moiety but that in the 4 position of dihydropyridine ring when the reduction is carried out in the presence of Mg(II). The chirality in the amide side chain exerted an effect only in the reduction of α,α,α-trifluoroacetophenone in the absence of Mg(II).

The reason why such a high degree of enantioface differentiation toward methyl benzoylformate was achieved is that the direction from

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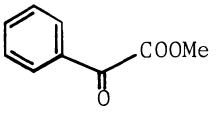
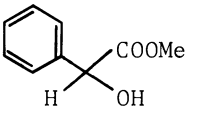
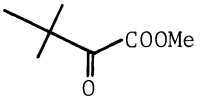
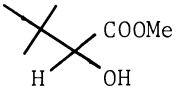
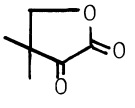
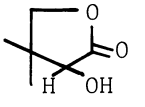
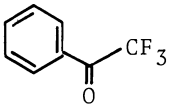
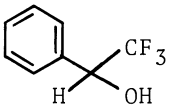
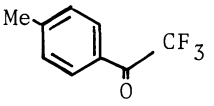
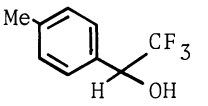
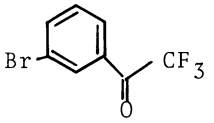
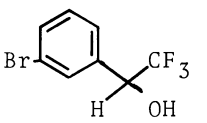
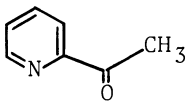
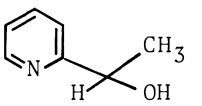
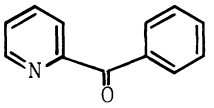
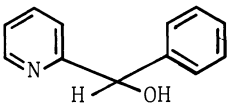
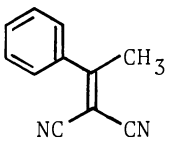
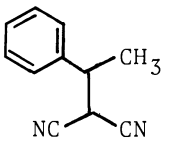
* In the abbreviation of *XY*-Me_2PNPH, *X* represents the configuration at the 4 position and *Y* represents that of benzylic carbon in the carbamoyl side chain.

Table 2. Reduction of unsaturated compounds by Me_2PNPH.[a]

Substrate	Config. of Me_2PNPH	Product	Config. of product	Optical yield [%][b]
	*RR*		*R*	97.6
	SR		*S*	96.5
	SS		*S*	94.7
	RR[c]		*R*	52.5
	*RR*		*R*	>99
	*RR*		*R*	50.6
	*RR*		*R*	70.3(92)
	SR		*S*	70.5
	RR[c]		*R*	63.1
	SR[c]		*S*	41.4
	*RR*		*R*	(95)
	*RR*		*R*	89.2(90)
	*RR*		*R*	62.0
	RR[d]		*R*	47.2
	*SS*		unknown	76.6
	*RR*		*R*	17.0

[a] Reduction was carried out in dry acetonitrile in the presence of magnesium perchlorate.

[b] Values in parentheses are from ^{19}F-NMR spectroscopy.

[c] Reaction without magnesium perchlorate.

[d] Reaction with a half-equivalency of magnesium perchlorate.

which the substrate approaches is perfectly determined as a result of asymmetry at the 4 position. In addition, selectivity in the differentiation of the enantioface of the substrate might be very high. Considering the high e.e. in the reduction of methyl benzoylformate in the presence of Mg(II) (97.6 % e.e.), it is concluded that the orientation of methyl benzoylformate in relation to the 1,4-dihydronicotinamide moiety is almost uniquely defined. If this is also the case for the reduction with PNPH, the low value in e.e. in this system is due to the low selectivity toward the two prochiral hydrogens. Accordingly, it is obvious that specific blocking of one of the faces of the 1,4-dihydropyridine ring is a very important factor for the stereoselective reduction of the substrates not only in the enzymatic system but also in the model systems.

This high selectivity of the orientation of methyl benzoylformate toward the 1,4-dihydronicotinamide moiety largely stems from the coordinating adhesion by Mg(II). Considering the result that the enantiomeric purity of the product in the reduction without Mg(II) falls to 52.5 %, it is obvious that Mg(II) plays an essential role in the asymmetric reduction by Me_2PNPH. How does Mg(II) exert the effect of such a high stereoselectivity? As described before, it is reasonable to conclude that Mg(II) locates between the substrate and 1,4-dihydropyridine ring at the transition state of the reduction and binds both reactants in a most stable arrangement. In such a situation, the polar group in the substrate should overlap with the polar group in Me_2PNPH by the brokerage of Mg(II) as depicted in Fig. 7. By assuming that the carbonyl oxygen (or dicyanomethylidene group in the case of α-methylbenzylidenemalononitrile) of the substrate points toward the nitrogen in the dihydropyridine ring of Me_2PNPH in the transition state, stereochemistry of the products, in every case, can be explained as a result of arrangement that the polar group in the substrate, such as methoxycarbonyl, trifluoromethyl, or pyridinyl, faces the amide moiety of Me_2PNPH.

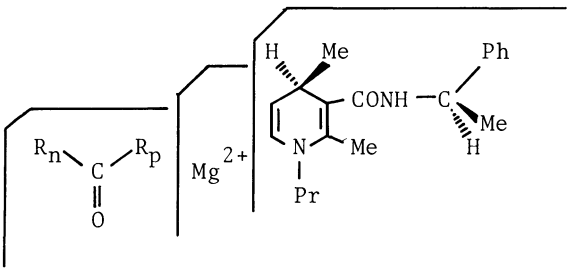
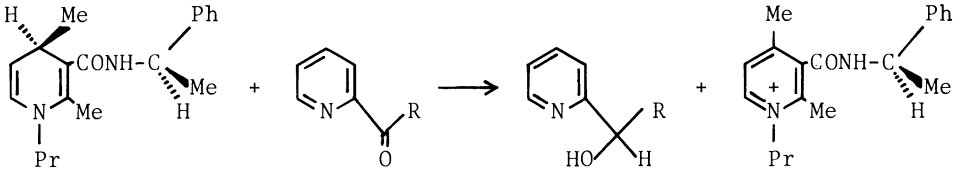


Fig. 7. Preferential orientation between *RR*-Me_2PNPH and a substrate in which R_p and R_n represent the polar and nonpolar substituent, respectively.

The above assumption concerning the orientation of the carbonyl group of the substrate seems to be reasonable because the highly frozen arrangement, which is necessary for high e.e., would be achieved at the transition state: the carbonyl oxygen in the substrate is fixed by the interaction with Mg(II) which, at the same time, has interactions with the amide moiety and the dihydropyridine ring of Me_2PNPH to freeze the conformation. It should be noted that an electronic interaction seems to be more significant than the steric factors in this system. In the case of 2-acetylpyridine, for example, the configuration of the reduced product indicates that the reduction proceeded through a transition state where the polar pyridinyl group faces the bulky carbamoyl side chain of Me_2PNPH despite the fact that the pyridinyl group is expected to be bulkier than the methyl group. Namely, when the substrate approaches the Mg(II) bound to Me_2PNPH, the interaction between the pyridinyl moiety and Mg(II) overcomes the steric repulsion between the two organic molecules.

An increase in the polarity of the methyl group in a series of 2-acetylpyridine derivatives by the introduction of fluorine atoms one by one reduced the stereoselectivity of the reduction as shown in Scheme 13 (Ohno et al. 1981c). The result indicates that, as the number of the fluorine atoms in the substituent R increases, the difference in the polarity between the two substituents of the carbonyl group becomes smaller and the recognition of a difference in polarity becomes difficult.

Scheme 13



RR-Me_2PNPH	R = CH_3	62.8 % e.e.
	R = CH_2F	53.5 % e.e.
	R = CHF_2	30.3 % e.e.
	R = CF_3	16.5 % e.e.

A study with camphoroquinone (CQ) also supported the idea that the dipole-dipole interaction between the substrate and the amide moiety in Me_2PNPH at the transition state controls the stereochemistry in the product (Ohno et al. 1981b). Camphoroquinone is a suitable substrate for studying the effect because the oxygen atom in the reacting carbonyl group is conformationally frozen, and the mode of approach to the reductant is unequivocally defined by a large steric interference of the remaining carbon framework. Camphoroquinone is reduced to form four isomers. The distribution of the reduced

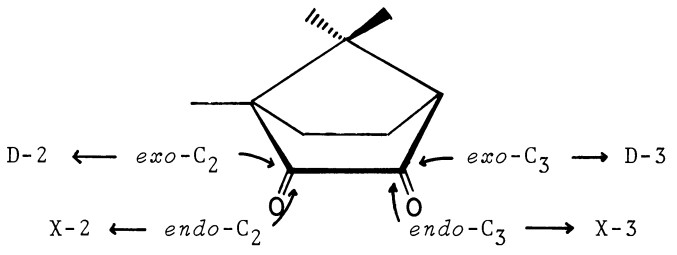


Fig. 8. Four types of "hydride" attack on (-)-camphoroquinone and the corresponding reduced products.

Scheme 14

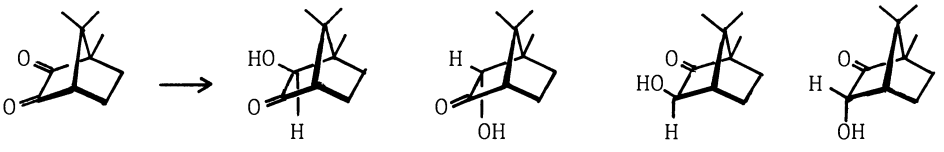


Table 3. Reduction of camphoroquinone with 1,4-dhydronicotinamide derivatives.[a]

Substrate	1,4-dihydro-pyridine derivative	Product ratio[b]			
		X-2	D-2	X-3	D-3[c]
(-)-CQ	BNAH	14	13	16	57
(-)-CQ	PNAH	13	11	24	52
(-)-CQ	*R*-PNPH	15	9	14	62
(-)-CQ	*RR*-Me_2PNPH	8	19	68	5
(-)-CQ	*SS*-Me_2PNPH	20	16	6	58
(+)-CQ	*RR*-Me_2PNPH	21	14	7	58
(+)-CQ	*SS*-Me_2PNPH	7	21	62	10

[a] Reduction was carried out in acetonitrile in the presence of magnesium perchlorate.

[b] Relative intensities of ^{1}H-NMR signals.

[c] X-2, D-2, X-3, and D-3 represent the products obtained from *endo*-C_2 attack, *exo*-C_2 attack, *endo*-C_3 attack, and *exo*-C_3 attack of "hydride", respectively.

products from (-)-camphoroquinone is illustrated in Scheme 14. There exist four directions for the approach of the "hydride" in this reduction, as shown in Fig. 8, where the product corresponding to each approach is also depicted. The ratio of product compositions in the reduction of camphoroquinone with 1,4-dihydronicotinamide derivatives are shown in Table 3. It is recognized in Table 3 that the major product in each reaction with Me_2PNPH is produced through a transition state where the amide moiety in Me_2PNPH faces the unreacted carbonyl group in camphoroquinone (cf. also Ohno et al. 1980b) and this is also true for the second preferable product. In addition, the major product is the one produced by the reduction at the C-3 carbon of camphoroquinone. In spite of the fact that the *exo*-attack is preferred to the *endo*-attack in the reduction with 1,4-dihydropyridine derivatives which have two available hydrogens at the 4 position, *RR*-Me_2PNPH attacks (-)-camphoroquinone from the *endo*-face to reduce the C-3 carbonyl group. This phenomenon is interpreted in terms of an inductive and/or a steric effect of the methyl group at the 1-position of camphoroquinone. It can be concluded, therefore, that the intermolecular arrangement in the transition state such as the one shown in Fig. 9 is responsible for the reduction, where the polar groups face each other with the assistance of coordination by Mg(II).

6.3 Further Comment on the Stereochemistry of PNPH and Its Aanalogues

The stereochemical relationship described above may be able to be extended to the reduction with PNPH or other model compounds that have two C-4 hydrogens. Keeping the assumption that the carbonyl

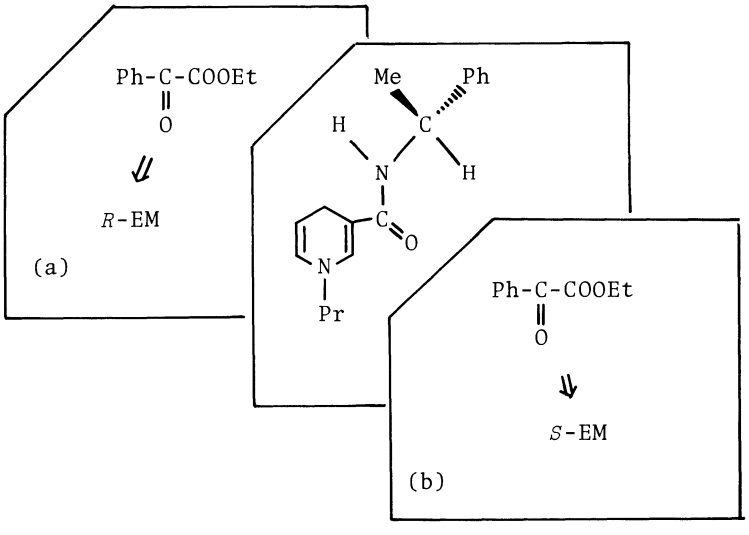
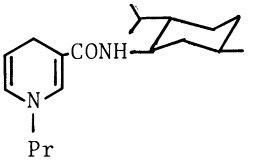
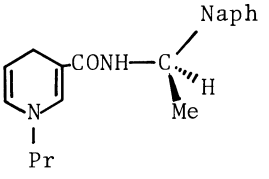
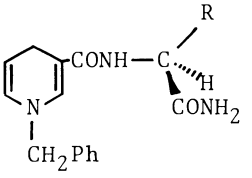
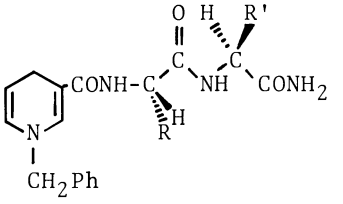
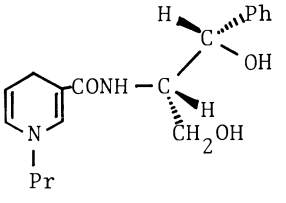
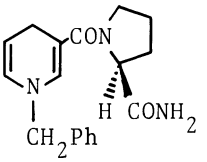


Fig. 9. Plausible molecular arrangement in the reduction with *R*-PNPH where dipole-dipole interaction between the ethoxycarbonyl group in ethyl benzoylformate and the carbamoyl side chain in *R*-PNPH is considered. Ethyl *R*-mandelate is produced through the approach of (a), and ethyl *S*-mandelate is produced through the approach of (b).

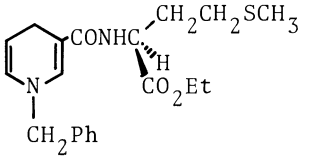
oxygen of the substrate points toward the ring nitrogen of the model due to coordinating participation by Mg(II), one may be able to depict a plausible mode of approach of ethyl benzoylformate to *R*-PNPH as shown in Fig. 9a or b. The other two modes of approach as shown in Figs. 6b and c are excluded by the result that the e.e. value in ethyl mandelate produced is very high in the reduction with Me_2PNPH. Considering that the predominant configuration in the product is *R*,

Table 4. Reduction of ethyl benzoylformate with chiral NAD(P)H model compounds.

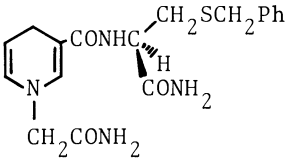
Chiral NAD(P)H model comound		e.e. [%] (config.)	Reference
		26 (*R*)	Ohno et al. 1977a
		28 (*R*)	Ohno et al. 1977a
	R = Me R = ^{i}Bu R = CH_2Ph	47 (*R*) 26 (*R*) 5 (*S*)	Endo et al. 1977
	R = Me R'= Me R = H R'= Me R = Me R'= H	45 (*R*) 35 (*S*) 25 (*R*)	Endo et al. 1979
		29.1 (*R*)	Makino et al. 1980



83.2 (*R*) Baba et al. 1980a



1.4 (*S*)
0.86(*S*)[a] Amano et al. 1982

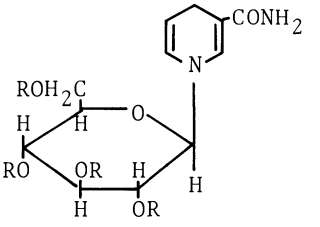


0.5 (*R*)
0.2 (*S*)[a] Amano et al. 1982

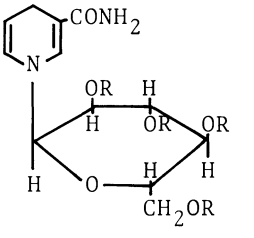
[a] In acetonitrile in the presence of Mg(II).

the approach of type a seems to be more favorable than the other. The above conclusion seems to be in contradiction with the steric effect exerted by the α-methylbenzylamine moiety. That is, the asymmetric induction effected by PNPH was interpreted in terms of the steric block of one enantioface by the chiral α-methylbenzyl group (Ohnishi et al. 1975b, Ohno et al. 1977a). A more complicated situation should be considered, as will be mentioned in the following sections.

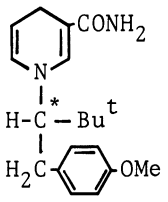
Studies on the asymmetric reduction with 1,4-dihydronicotinamide derivatives that contain chiral centers within their amide moieties have been widely extended after the first report on this subject (Ohnishi et al. 1975a,b). To improve the optical yield and to obtain further insights into the stereochemical course of the reduction, a variety of model compounds have been synthesized and subjected to the reduction. Table 4 lists the optical yields from the reduction of methyl or ethyl benzoylformate which has frequently been used as a substrate for these 1,4-dihydropyridine derivatives.



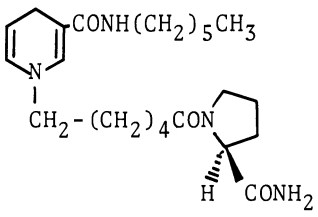
βOH : R = H
βOAc : R = Ac



αOH : R = H
αOAc : R = Ac



1



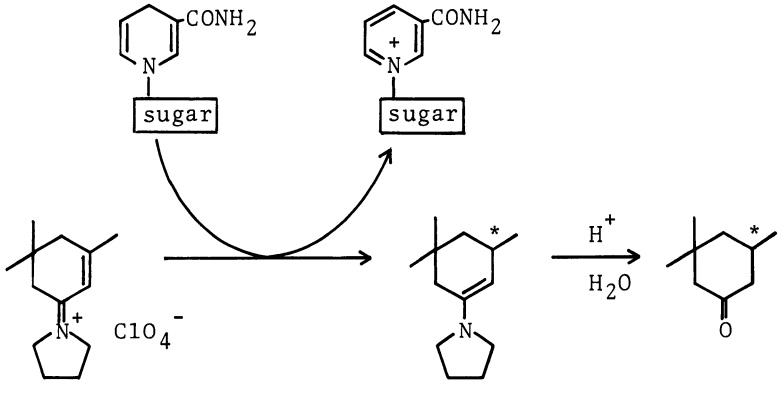
2

Table 5. Reduction of ethyl benzoylformate with 1,4-dihydronicotinamide derivatives containing chiral substituents at the 1 position.

1,4-Dihydropyridine derivative	e.e. [%]	Configuration
βOH	4	*S*[a]
βOAc	3	*S*[a]
αOH	20	*S*[a]
αOAc	19	*S*[a]
R-1	27	*S*[b]
2	90	*S*[c]

[a] From Baba et al. 1980d.
[b] From Van Ramesdonk et al. 1977.
[c] From Hoshide et al. 1983.

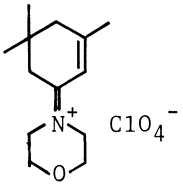
Scheme 15



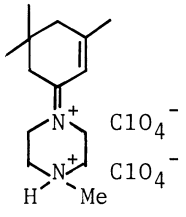
3a

Reduction by a chiral 1,4-dihydropyridine derivative which contains an asymmetric center in the substituent on the nitrogen at the 1 position has also been attempted. Optical yields in the reduction of ethyl benzoylformate are shown in Table 5. It should be noted that 2 which carries a proline moiety exhibited a remarkably high e.e. value.

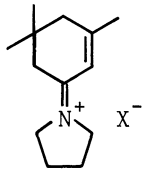
Makino et al. (1977) and Baba et al. (1980e) reported the asymmetric reduction of α,β-unsaturated iminium salts that were produced from isophorone and secondary amines. Since the reduced product can be hydrolyzed to give a saturated ketone as shown in Scheme 15, reduction of this type is equivalent to the reduction of the olefinic double bond in an α,β-unsaturated ketone. Stereochemical results are listed in Table 6. It was found that the iminium salt which afforded the highest e.e. was the one that is derived from pyrrolidine. The e.e. value was independent of the kind of counter anion.

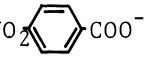


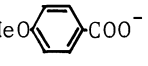
3b



3c



3d: X^- = 

3e: X^- = 

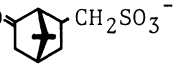
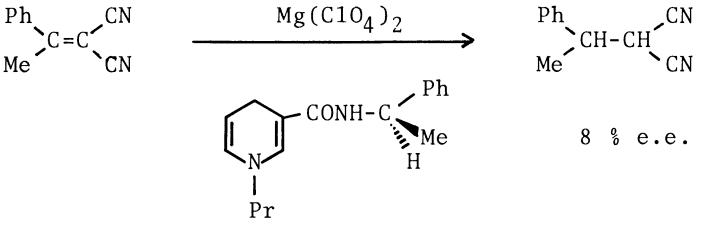
3f: X^- = 

Table 6. Enantiomeric excess in 3,3,5-trimethylcyclohexanone from the reduction of iminium salts with 1,4-dihydronicotinamide sugar pyranosides.[a]

1,4-Dihydronicotinamide	Substrate	e.e. [%]	Configuration
βOAc	**3a**	14	*S*
βOH	**3a**	27	*S*
αOAc	**3a**	15	*R*
αOH	**3a**	14	*S*
βOAc	**3b**	3	*R*
βOH	**3b**	7	*S*
βOAc	**3c**	3	*S*
βOH	**3c**	8	*S*
βOH	**3d**	25	*S*
βOH	**3e**	24	*S*
βOH	**3f**	28	*S*

[a] Reaction was carried out in dry N,N-dimethylformamide at 140°C. From Makino et al. (1977) and Baba et al. (1980e).

Scheme 16



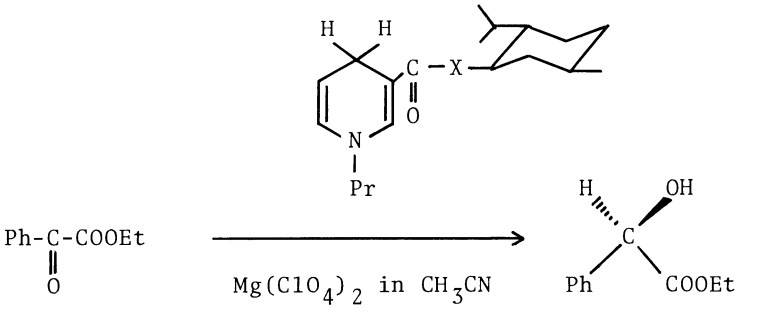
The difference in reactivity between the acylated and free forms of 1,4-dihydronicotinamide sugar pyranosides has also been examined (Baba et al. 1980c).

Asymmetric reductions of other iminium salts (Baba et al. 1976) and olefins are also reported. The first asymmetric reduction of an olefinic double bond by a chiral NAD(P)H model was reported by Ohnishi et al. (1976a). α-Methylbenzylidenemalononitrile was reduced by *R*-PNPH in the presence of magnesium perchlorate in 8 % e.e. (Scheme 16). Again Mg(II) was indispensable for the asymmetric induction in this system.

6.4 Factors That Determine the Stereoselectivity

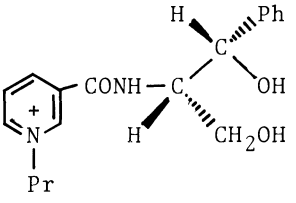
It has been found that the stereochemical course of the reduction of methyl or ethyl benzoylformate by a 1,4-dihydropyridine derivative is controlled by a variety of factors. First of all, the optical yield is influenced by a variation of the atom connecting the carbonyl and α-methylbenzyl groups as shown in Scheme 17 (Ohno et al. 1976). It is concluded that the basicity of the carbonyl oxygen affects the optical yield because the change in the basicity is directly correlated with the ability to coordinate onto Mg(II).

Scheme 17

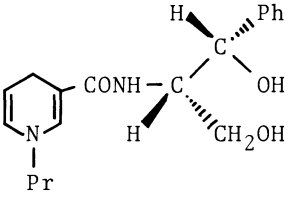


Secondly, the optical yield of the product depends on the molar ratio of the metal ion to the 1,4-dihydropyridine derivative but not on the concentration of the metal ion. Ohnishi et al. (1976c) reported that the optical yield in the reduction of 2-acetylpyridine with PNPH decreases with the increase of the ratio [Mg(II)]/[PNPH]. In contrast, reversed dependency was found for ethyl benzoylformate (Tables 7 and 8). The same dependency was observed in the reduction with PNGH, where the optical yield has a maximum when the molar ratio of Mg(II) to PNGH is 0.5 (Makino et al. 1980).

A much more interesting feature of the present system is the dependence of the optical yield on the conversion of the reduction. The first report on this phenomenon (Ohno et al. 1978b) revealed that the optical yield in the reduction of ethyl benzoylformate with *R*-PNPH changes over the range of 25 % as the reaction proceeds. Moreover, it should be noticed that the configuration of the product isolated at an early stage of the reaction is opposite to that obtained at theend. Because of the well-known fact that 1,4-dihydropyridine derivatives form charge transfer complexes with their oxidized forms, the possibility of the effect of *R*-PNP^+, which is necessarily accumulated in the process of the reduction, was examined. A change in the optical yield was observed by the addition of *R*-PNP^+. However, the change was not so large as to account for the whole shift. An unpublished result from our laboratory suggests that the participation by the produced chiral alcoholate is more significant than that by PNP^+. The three dimensional relationship of the optical yield to the conversion of the reaction and to [Mg(II)]/[PNPH] is shown in Fig. 10.



PNG^+



PNGH

Table 7. Reduction of 2-acetylpyridine by *R*-PNPH.

[Mg(II)] / [*R*-PNPH]	Optical yield [%]
0.5	39
0.6	36
0.7	35
1.0	25
1.3	24
1.7	18

Table 8. Reduction of ethyl benzoylformate by *R*-PNPH.

[Mg(II)] / [*R*-PNPH]	Optical yield [%]
0.3	6.6
0.5	8.6
1.1	19.6
2.0	18.1

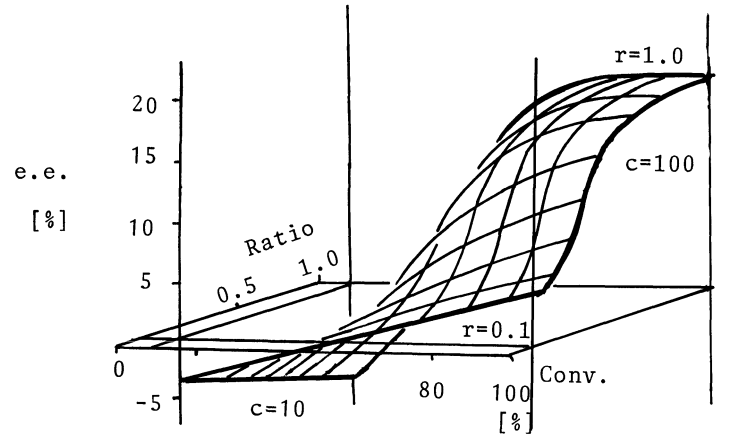


Fig. 10. Dependence of optical yield on the reaction period and molar ratio of the starting materials.

A striking dependence of the optical yield on the conversion has also been observed in other systems. Makino et al. (1980) reported that, in the reduction of ethyl benzoylformate with PNGH, the optical yield of ethyl *S*-mandelate increases as the reaction proceeds (Table 9).

In this system the addition of the oxidized form of PNGH (PNG^+) greatly affects the optical yield in the presence of 0.5 molar equivalent of Mg(II) to PNGH. The e.e. increased to 52 % in contrast to 29% without PNG^+. However, the presence of an equivalent amount of Mg(II) dismissed the effect of PNG^+. The result is summarized in Table 10.

They accounted for the phenomena as follows: as shown in Scheme 18, the reduction proceeds by two different routes. One proceeds though a complex composed of three components, PNGH, ethyl benzoylformate, and Mg(II). The other proceeds through a complex with four

Table 9. Dependence of e.e. on the chemical yield in the reduction of ethyl benzoylformate.

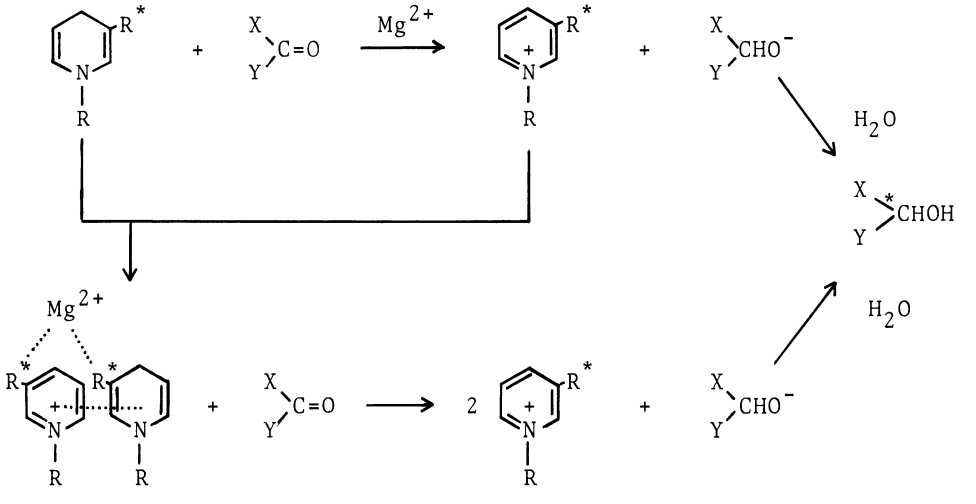
[PNGH]	[Mg(II)]	Reaction time [h]	Chemical yield [%]	e.e. [%]
1.5	0.75	0.5	22	12.8
1.0	0.5	1.0	32	21.0
1.0	0.5	1.5	42	23.8
1.0	0.5	2.0	46	29.1
1.0	0.5	3.0	59	27.6
1.5	0.75	12	69	26.3

Table 10. Effect of additional PNG^+ on e.e. in the reduction of ethyl benzoylformate.

$\frac{[Mg(II)]}{[PNGH]}$	$\frac{[PNG^+]}{[PNGH]}$	Chemical yield [%]	e.e. [%]
0.5	0	59	27.6
0.5	0.5	61	45.8
0.5	1.0	54	51.5
0.5	1.5	54	52.4
1.0	1.0	59	26.5
2.0	1.0	54	19.4

components that involves PNG^+ in addition to PNGH, ethyl benzoylformate, and Mg(II). The former process with low stereoselectivity takes place at the early stage of the reduction. As the reduction proceeds the latter process with high stereoselectivity becomes predominant and the overall optical yield increases. Therefore, when PNG^+ is added to the reaction system, the latter process preferably occurs to result in the enhancement of the optical yield. The high optical yield observed in the latter process is a result of the intermolecular specific blocking of one of the two enantiomeric faces of the 1,4-dihydropyridine ring by charge-transfer type complexation between the oxidized and reduced forms. It is well known that 1,4-dihydropyridine derivatives interact with pyridinium cations as well as with some aromatic compounds. The possibility of such association may be increased by the chelation with Mg(II) at the polar sites of reduced and oxidized nicotinamides. The optical

Scheme 18

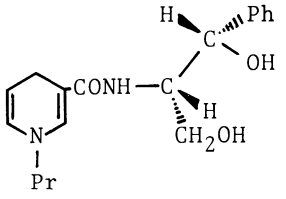
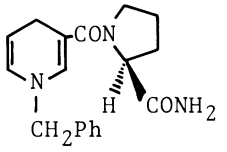


(R^* : chiral carbamoyl side chain)

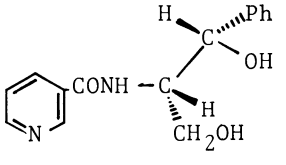
yield in the presence of excess Mg(II) decreases in the reduction of PNGH because the coordination of the hydroxyl group in PNG^+ onto Mg(II) inhibits the formation of the above-mentioned CT-complex (Inouye et al. 1983).

The additive is not necessarily the oxidized form of the reductant. As shown in Table 11, the addition of a chiral compound such as 4 or 5 gave rise to an increase in the optical yield of the produced ethyl mandelate in the reduction with PNGH. These compounds are similar in structure to the reductant, but have two hydroxyl groups in the molecule. The compound with one hydroxyl group such as 6 is inferior to the former compounds (Makino et al. 1979). Complexation between the reductant and additive by the assistance of Mg(II) through the hydroxyl groups was suggested, but detailed information has not been obtained to discuss the effect unambiguously. The addition of some macrocyclic polyethers (7-11) is also effective in enhancing the optical yield, as shown in Table 11 (Baba et al. 1983).

Table 11. Asymmetric reduction of ethyl benzoylformate with chiral NAD(P)H model compounds in the presence of an achiral macrocyclic polyether or a chiral amide compound.[a]

NAD(P)H model compounds	Additive	e.e. [%]	Configuration
	none	26.3	*S*
	4	47.3	*S*
	5	34.2	*S*
	6	19.6	*S*
	none	72.7	*R*
	7	90.9	*R*
	8	61.3	*R*
	9	89.2	*R*
	10	67.1	*R*
	11	80.1	*R*

[a] From Makino et al. (1979) and Baba et al. (1983).



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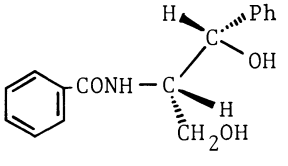

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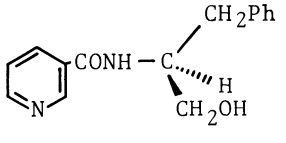

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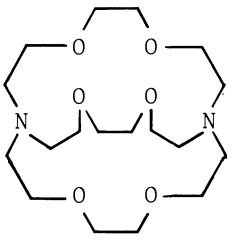

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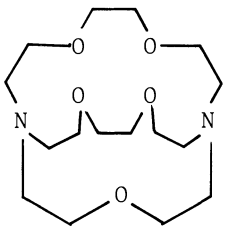

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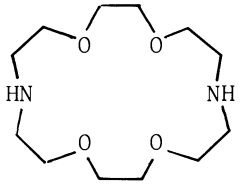

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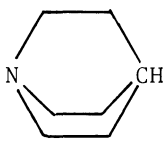

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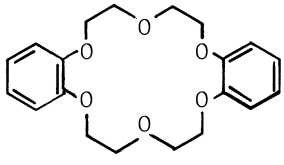

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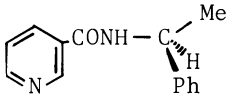

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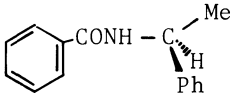

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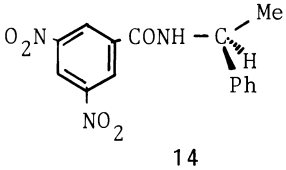
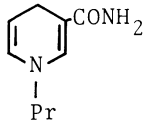
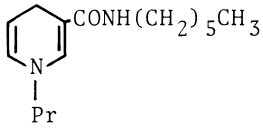
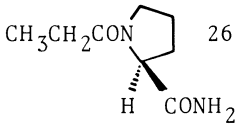


14

Table 12. Asymmetric reduction of ethyl benzoylformate with achiral NAD(P)H model compound in the presence of a chiral additive.[a]

NAD(P)H Mmodel compound	Additive	e.e. [%]	Configuration
	4	12.4	*S*
	5	5.3	*S*
	6	4.8	*S*
	12	0.3	*S*
	13	2.3	*S*
	14	0.9	*S*
	 15	26	*S*

[a] From Makino et al.(1979) and Hoshide et al.(1983).

Asymmetric induction was also accomplished by the addition of chiral additives (4-6, and 12-15) to the reduction system with achiral 1,4-dihydropyridine derivatives as shown in Table 12 (Makino et al. 1979, Hoshide et al. 1983). Here again, the dipole-dipole interaction between the carbamoyl groups in the reductant and the additive seems to play an important role in producing a chiral reducing species.

In addition to the dependence of optical yield on the reaction period and on the molar ratio of Mg(II) to the 1,4-dihydropyridine derivative, it is reported that the optical yield is also affected by the initial concentration of the substrate. The optical yield of 46 % in the presence of 10 % mol of ethyl benzoylformate to BNProH increases to more than 70 % when an equal amount of the substrate is used (Baba et al. 1984). The correlation is plotted in Fig. 11.

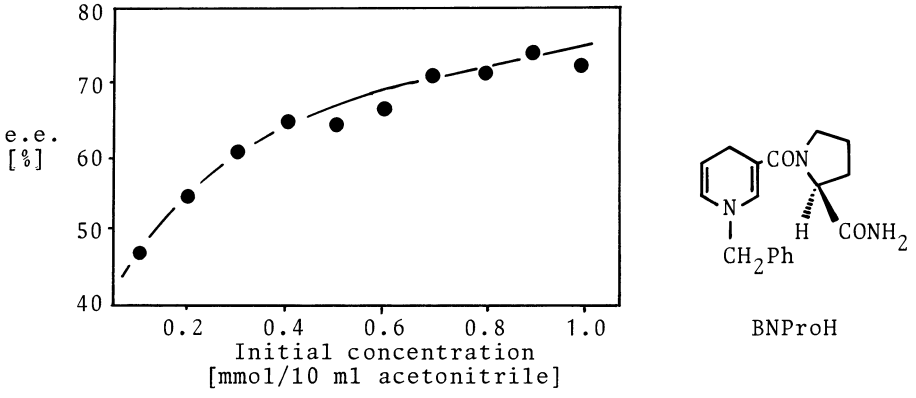


Fig. 11. Dependence of optical yield on the initial concentration of ethyl benzoylformate in the asymmetric reduction with BNPH.

The phenomenon can also be interpreted in terms of the complexation between the reduced and oxidized forms. When the initial concentration of the substrate is low, the reduction without participation of the oxidized form predominates over the reduction through the complex, because only a small amount of the oxidized form is accumulated before all the substrate is reduced. Based on the assumption that the stereospecificity exerted by the complex is higher than that exerted by the reductant alone, it is easy to show that the optical yield increases as the concentration of the substrate decreases, which corresponds to the progression of the reaction. Furthermore, the optical yield is a monotonically increasing function of the initial concentration of the substrate (Baba et al. 1982, 1984).

The idea of complex formation between the reduced and oxidized forms of the reductant will also be applicable to the interpretation of the stereochemistry observed in the reduction with PNPH, where the "hydride" in the sterically hindered side of the 1,4-dihydropyridine ring is transferred (Fig. 9a). That is, the accumulated PNP^+ in the course of the reduction shields the less-hindered side of PNPH

through a charge-transfer-type interaction so that the opposite side of the 1,4-dihydropyridine ring is utilized for the reduction.

It should be emphasized here again that an experiment has revealed that the complex formation cannot account for the whole change in the optical yield and that the chiral alcoholate anion produced by the reduction is a much better ligand toward Mg(II) than the substrate.

Nevertheless, it is a very interesting idea that at least two competing processes are involved in the reduction and that the optical yields from the two processes are different from each other. One should carefully consider the conditions such as the molar ratios of the reactants, the presence or the absence of the additive(s), and the reaction period before discussing a result.

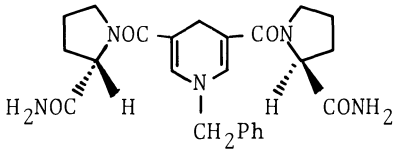
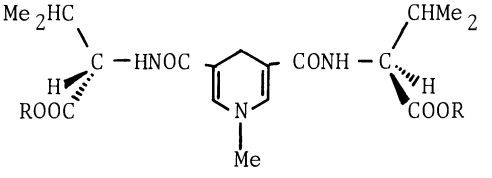
In contrast, such a phenomenon does not appear in NAD^+-dependent dehydrogenases. It is impossible for an NAD(P)H molecule to interact with another molecule of either the oxidized or reduced form of the coenzyme because the reaction site is surrounded by the apoenzyme. However, the origin of the improved stereoselectivity in both model and biological systems stems from the same effect in that one of the enantiofaces of the 1,4-dihydropyridine ring is selectively blocked.

Yet, the authors have to confess that the biological system is far superior to the mimetic one in that the combination of an enzyme and coenzyme tends to *promote* the reaction, whereas the complexation between the reduced and oxidized forms of a model in a mimetic system may result in the retardation. Organic chemists have to make efforts to construct a system in which the reaction is accelerated as a consequence of introduced specificity. In this sense, the Me_2PNPH system is an excellent model of biological reactions.

As has been revealed in the reduction of methyl or ethyl benzoylformate with Me_2PNPH, where the reduction takes place in only one side of the ring, the selective orientation between the 1,4-dihydronicotinamide moiety and alkyl benzoylformate is uniquely arranged to give an excellent e.e. value. In the system of Me_2PNPH, the necessity of the selection of the enantiofaces of the 1,4-dihydropyridine ring is excluded by the introduction of a methyl group at the 4-position. Even for a 1,4-dihydropyridine derivative which has two hydrogens at the 4 position, the same kind of situation can be achieved by the use of a compound which has a C_2 symmetry. A high degree of asymmetric reduction may be accomplished by the use of such NAD(P)H model compounds. Contrary to the expectation, optical yields associated with the reductions were relatively low, as summarized in Table 13, and the values were inferior to those with the corresponding model compound which has one amide side chain.

The result can be rationalized as follows: the most important factor in the choice of the enantioface of carbonyl group in alkyl benzoylformate on which a "hydride" is transferred is the interaction between the alkoxycarbonyl group of the substrate and the amide side chain of the reductant mediated by Mg(II). 1,4-Dihydropyridine derivatives with C_2 symmetry have two polar amide side chains at the 3 and 5 positions that can interact with the polar group in the substrate and the situation for the two side chains is not very different from the viewpoint of polarity. Thus, the selectivity which stems from the steric factor of the two amide moieties is poor.

Table 13. Asymmetric reduction with 1,4-dihydropyridine derivatives with C_2 symmetry.[a]

1,4-Dihydropyridine derivative	e.e. [%]	Configuration
	47.1	*R*
16		
	10	*R*
	18	*R*
17 : R = Me 18 : R = $CH_3OCH_2CH_2$		

[a] From Baba et al. (1980a), Amano et al. (1984), and Jouin et al. (1981).

Recently, an interesting stereochemical feature of the 1,4-dihydropyridine derivative **16** with C_2 symmetry was reported. The configuration of the predominant product and the e.e. values for the reduction of ethyl benzoylformate with model **16** changed with the concentration of Mg(II) as shown in Fig. 12 (Amano et al. 1984). On

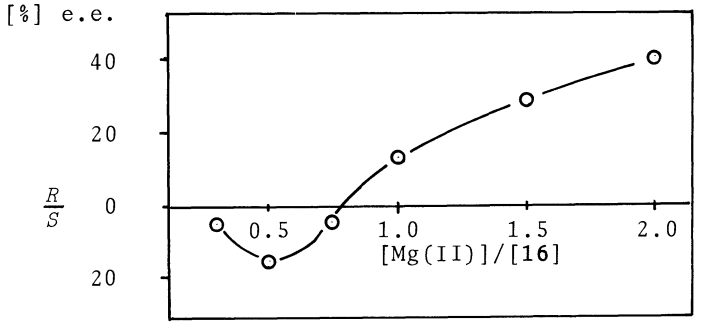


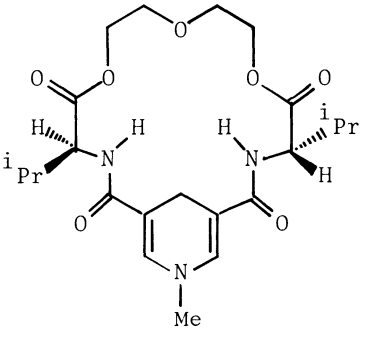
Fig. 12. Dependence of enantiomer excess on the concentration of magnesium ions.

the basis that the different types of complexes are formed between **16** and Mg(II) as the concentration of Mg(II) increases, the phenomenon was interpreted in terms of two reaction routes to the product. From the complex composed of two molecules of **16** and one Mg(II), ethyl *S*-mandelate is formed and from the complex composed of one molecule of **16** and two magnesium ions, ethyl *R*-mandelate is produced.

6.5 NAD(P)H Model Compound Incorporating a Macrocycle

Another type of ingeniously designed NAD(P)H model compound with C_2 symmetry resulted in an improved stereoselectivity. De Vries and Kellogg (1979) synthesized a 1,4-dihydropyridine derivative having a chiral bridge between the 3 and 5 positions (**19**) and subjected the compound to the reduction of several substrates. As listed in Table 14, the optical yields of the products (with the predominancy of *S*-configurations) were good compared to those obtained from C_2-symmetric **16**, **17**, and **18**. A structure of the transition state in which the carbonyl oxygen coordinates onto Mg(II) locating close to the diethyleneglycol bridge was suggested.

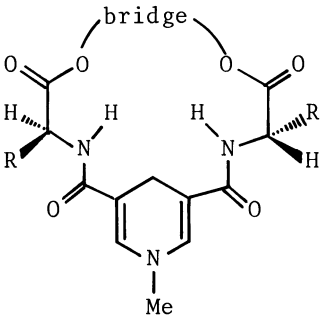
As analogues of **19**, Jouin et al. (1981) synthesized several 1,4-dihydropyridine derivatives (**20** - **22**) with a variety of bridges containing amino acid moieties. The results from the reduction with these compounds are summarized in Table 15.



19

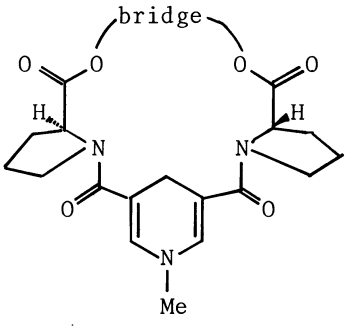
Table 14. Reduction of carbonyl compounds with **19**.

Substrate	Optical yield [%]	Configuration
Ph-CO-CF_3	68	*S*
$PhCOCO_2Et$	86	*S*
$PhCOCONH_2$	64	*S*
PhCOCONHEt	78	*S*



20: R = Me_2CH

21: R = $PhCH_2$

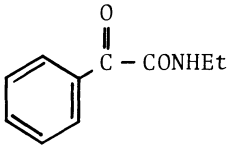


22

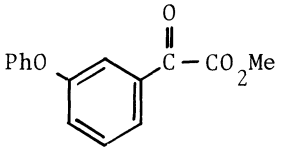
Table 15. Reduction of carbonyl compounds with 20-22.

Amino acid	Bridge	e.e. [%]	(config.)	Substrate[a]
L-valine	$-(CH_2)_2O(CH_2)_2-$	86	(*S*)	EBF
L-valine	$-(CH_2)_2O(CH_2)_2O(CH_2)_2-$	43	(*S*)	EBF
L-valine	$-(CH_2)_2(O(CH_2)_2)_3-$	54	(*S*)	EBF
L-valine	$-(CH_2)_4-$	55-70	(*S*)	EBF
L-valine	$-(CH_2)_5-$	90	(*S*)	EBF
L-valine	$-(CH_2)_6-$	88	(*S*)	EBF
L-valine	$-(CH_2)_8-$	83	(*S*)	EBF
L-valine	$-(CH_2)_{10}-$	53	(*S*)	EBF
L-valine	$-(CH_2)_{12}-$	42	(*S*)	EBF
L-valine	*m*-$CH_2C_6H_4CH_2-$	86	(*S*)	EBF
D-valine	$-(CH_2)_6-$	85	(*S*)	EBF
L-phenylalanine	$-(CH_2)_2O(CH_2)_2-$	87	(*S*)	EBF
L-phenylalanine	$-(CH_2)_2O(CH_2)_2-$	84	(*S*)	23
L-phenylalanine	$-(CH_2)_2O(CH_2)_2-$	60	(*S*)	24
L-phenylalanine	$-(CH_2)_2O(CH_2)_2-$	20	(*S*)	25
L-proline	$-(CH_2)_2O(CH_2)_2-$	none		EBF

[a] EBF denotes for ethyl benzoylformate.



23



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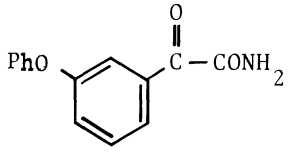


25

By the reduction with these 1,4-dihydropyridines, good to excellent optical yields were obtained except in one case with a proline derivative. The optical yields obtained from the reductions with the corresponding noncyclic derivatives (18) were low (Table 13), which indicates that the cyclic structure is important for a high degree of stereoselectivity (cf. also Paul et al. 1976). The original intention in designing such a macrocyclic NAD(P)H model is to introduce a coordination site for Mg(II) as the mimesis of the active site of the alcohol dehydrogenase. However, by comparison of the result from 1,4-dihydropyridine derivatives with oxygen atoms in the bridge to those without oxygen atoms, it was concluded that the ether bridge is not necessarily required to obtain high optical yields. Instead, ^{13}CNMR spectroscopy revealed that Mg(II) binds to the amide carbonyl oxygen. This result is in accord with that reported by Gase et al. (1976) which was mentioned before. The structure of the transition state postulated is shown in Fig. 13. In the ternary complex, the amide oxygen is forced to point upward in order to coordinate onto Mg(II). The aromatic group in the substrate lies roughly over the 1,4-dihydropyridine moiety and the polar group points toward the bridge. The reason for high enantioface differentiation is explained as a result of steric interference between the amino acid residue in the reductant and the aromatic group in the substrate.

The maximal value was attained in the reduction with 20 with the bridge composed of five methylene groups. With longer bridges, the stereoselectivity of the reduction drops due to an increase in conformational flexibility of the ternary complex. It is interest-

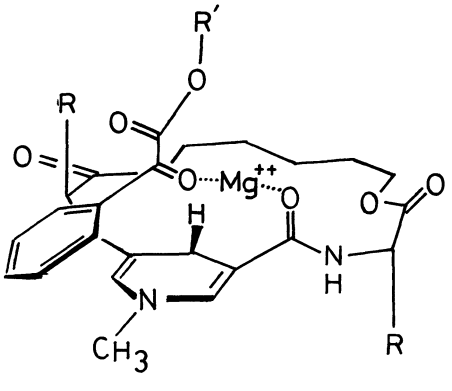
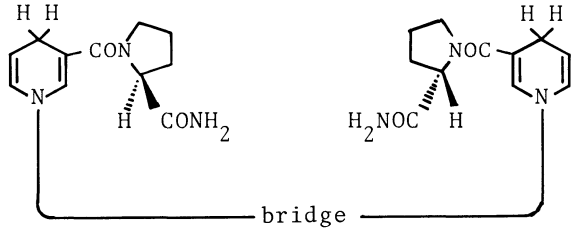


Fig. 13. Structure of the transition state in the reaction with a macrocyclic NAD(P)H model.

ing that noncyclic compounds gave the products in only low optical yields with the reversed configuration (Table 13). The difference in the structure of (and/or factors operating at) the transition state between the closed and the open types must be quite large. Low optical yields in the reduction with a noncyclic model with C_2 symmetry is due to the similarity in polarity of the two amide side chains as mentioned above. On the other hand, in macrocyclic 1,4-dihydropyridines, electronic as well as steric influences exerted by two side chains are quite different in *re*- and *si*-faces of the ring. In such an arrangement, the dipole-dipole interaction between the amide moiety in the NAD(P)H model with a chiral macrocycle and a polar group in the substrate may play an important role, as it did in the reduction with Me_2PNPH.

6.6 Models That Contain Two Chiral 1,4-Dihydronicotinamide Moieties

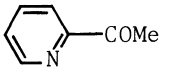
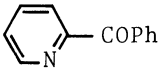
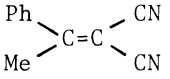
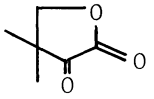
Another successful result in the field of asymmetric reduction with NAD(P)H model compounds is accomplished by bis-nicotinamides of various types. Seki et al. (1981, 1983) and Hoshide et al. (1982) reported a high degree of asymmetric induction using NAD(P)H model compounds that have two 1,4-dihydropyridine moieties within each molecule. Compounds of this type will be abbreviated as bis(NAH), in general. The idea was based on an expectation that a C_2 symmetry in bis(NAH) will create a specific chiral field by an interaction between the two 1,4-dihydronicotinamide moieties with the aid of Mg(II). In their initial report, bis(NAH) in which *ortho*-, *meta*-, or *para*-xylene was employed as a bridge and L-proline attached to the 3 position of the 1,4-dihydropyridine were utilized as the chiral source. As shown in Table 16, *para*-xylene was more effective than *ortho*- or *meta*-xylene as a bridging unit.



bis(NAH)

Table 16. Reduction of several substrates with bis(NAH).

Substrate	Bridge	e.e. [%]	Configuration
Ethyl Benzoylformate	*p*-xylene	98.1	*R*
	m-xylene	34.0	*R*
	o-xylene	36.4	*R*
	$-(CH_2)_4-$	39.9	*R*

	$-(CH_2)_5-$	43.0	*R*
	$-(CH_2)_6-$	95.6	*R*
	$-(CH_2)_7-$	58.7	*R*
	$-(CH_2)_8-$	81.2	*R*
	$-(CH_2)_9-$	63.8	*R*
α,α,α-Trifluoro-acetophenone	*p*-xylene	49.5	*S*
	$-(CH_2)_6-$	13.4	*R*
	*p*-xylene	89.7	*R*
	$-(CH_2)_6-$	66.7	*R*
	$-(CH_2)_6-$	92.7	*R*
	*p*-xylene		*R*
	$-(CH_2)_6-$	24.5	*R*
	*p*-xylene	38.1	*R*
	$-(CH_2)_6-$	33.9	*R*

Similar results were obtained from bis(NAH) bridged by polymethylene chains (Seki et al. 1983). The optical yields from the reduction of ethyl benzoylformate changed with the variation of the length of the carbon chain and the maximal optical yield was obtained in the reduction with the compound bridged by six methylene groups. That the optical yield depends on the structure or the length of the bridge indicates the importance of an interaction between the two 1,4-dihydronicotinamide moieties. It is obvious that the interaction involves a chelation of Mg(II) by the two nuclei because the 1,4-dihydronicotinamide moiety has a large affinity toward Mg(II) as mentioned above. It was ascertained that Mg(II) and bis(NAH) form a 1:1 chelate complex and that the coordination site is primarily the amide oxygen of proline amide. The importance of complexation of this kind in the induction of chirality was also supported by the dependence of the optical yield on the molar ratio of Mg(II) to bis(NAH). The optical yield increased as the relative concentration of Mg(II) increased up to a constant value when the ratio was 1:1. These results were interpreted in terms of the complexation where one of the enantiofaces of the 1,4-dihydronicotinamide ring is specifically blocked as a result of C_2 symmetry to expose the *pro-R* or *pro-S* hydrogen at the 4-position specifically outside the complex (Fig. 14).

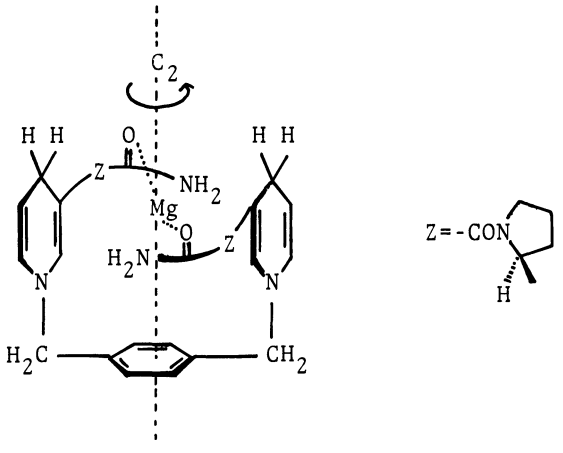


Fig. 14. Transition state for the reaction of bis(NAH).

It was revealed that the optical yield did not depend on the reaction period, in contrast with the case in monomeric 1,4-dihydronicotinamide where the oxdized form produced in the course of the reduction was thought to participate in the complex. This indicates that the intramolecular interaction between 1,4-dihydronicotinamide moieties with the aid of Mg(II) is stronger than the intermolecular interaction between the reduced and oxidized forms.

It is certain that the chiral environment in which one of the enantiofaces of the 1,4-dihydronicotinamide ring is made inactive for the reduction is formed as a result of an interaction between two 1,4-dihydronicotinamide moieties. However, it is difficult to predict a stereochemical course of the reduction from the results accumulated so far. The complex suggested seems to be reasonable as the most stable species at the ground state and can explain the unequivalency of two hydrogens at the 4 position of 1,4-dihydropyridine. However, this complexation seems to be implausible for the transition state because such chelation does not give any idea of the catalytic function of Mg(II). Enantioface differentiation for the reacting substrate cannot be explained by the complexation of this type, either.

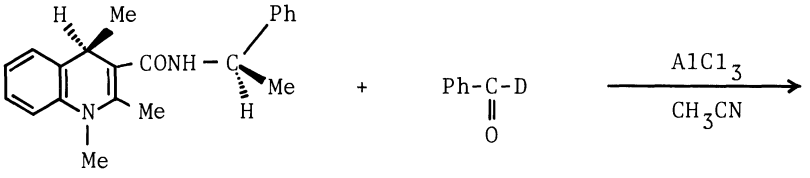
Furthermore, the contribution of the charge-transfer type interaction between the reduced and oxidized forms of a model mentioned before still remains ambiguous, because essentially no reactivity was found for a half oxidized bis(NAH), which still contains one 1,4-dihydropyridine moiety in addition to the pyridinium cation within the molecule.

6.7 Asymmetric Reduction of Nonactivated Substrate

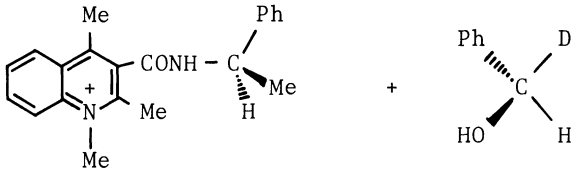
Since the reactivity of 1,4-dihydropyridine derivatives as reducing agents is not so high, only activated unsaturated bonds with electron-withdrawing substituents can be reduced by such reductants. Otherwise, the reaction must be facilitated by the addition of

certain catalysts, such as a bivalent metal ion. Considering the role of Zn(II) in alcohol dehydrogenase, acid catalysis is expected to promote the reduction. However, acidic media cannot be employed for the reduction system because the enaminic double bond in the 1,4-dihydronicotinamide structure is quite susceptible to attack by an electrophile. Shinkai et al. (1978, 1979) utilized 1-benzyl-3--carbamoyl-1,4-dihydroquinoline as a model of NAD(P)H and achieved the reduction of nonactivated substrates such as benzaldehyde in acidic media. Later, Ohno et al. (1982) synthesized a 1,4-dihydroquinoline derivative carrying a chiral α-methylbenzyl group (Me_2MQPH) and subjected it to the reduction of benzaldehyde-1-*d* to afford the corresponding reduced product in 88 % e.e. (Scheme 19).

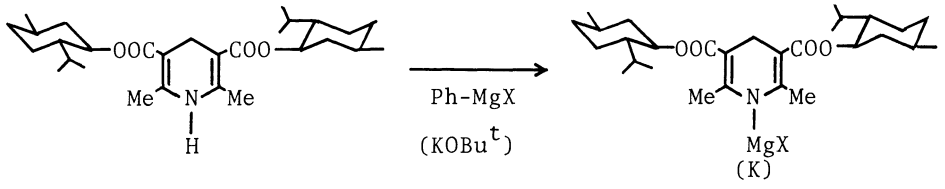
Scheme 19



Me_2MQPH

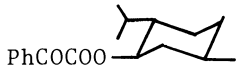
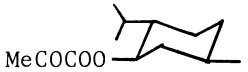
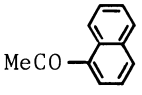


Fushimi et al. (1980) succeeded in reducing nonactivated carbonyl compounds by 1,4-dihydropyridine of Hantzsch ester type (HMH) under the catalysis of a metal ion from potassium alkoxide or phenyl magnesium halide (Inouye et al. 1983). The activation of HMH is accomplished by the replacement of the hydrogen at the 1 position of HMH by a metal ion. The optical yields of some products are listed in Table 17.



HMH

Table 17. Asymmetric reduction of carbonyl compounds by HMH under the catalysis of Grignard reagent or potassium *tert*-butoxide.

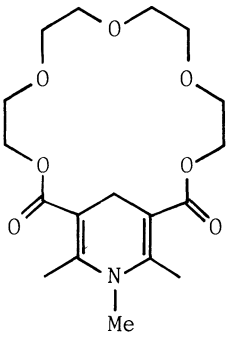
Substrate	Catalyst	e.e. [%]	Configuration
PhCOCOOMe	PhMgI	23	*R*[a]
	PhMgI	48	*R*[a]
	PhMgBr	55	*R*[a]
	PhMgBr	65	*R*[a]
$PhCOCF_3$	PhMgBr	67	*R*[a]
	$PhCH_2MgCl$	40	*R*[a]
$CH_3(CH_2)_5COCH_3$	$KOBu^t$	6	*S*[b]
PhCOEt	$KOBu^t$	34	*S*[b]
	$KOBu^t$	36	*S*[b]
MeCOPh	$KOBu^t$	42	*S*[b]

[a] From Fushimi et al. (1980).

[b] From Inouye et al. (1983).

7 ASYMMETRIC REDUCTION IN A CHIRAL REACTION FIELD

Stereospecificity in the enzymatic reduction with NADH or NADPH as a reducing agent is due largely to the effect from the environment around the 1,4-dihydropyridine moiety. The active site of the enzyme is surrounded by amino acid residues that form a chiral environment so as to achieve the asymmetric reduction of substrates. One of the attempts to mimic the system conveniently is to introduce one chirality into a 1,4-dihydropyridine derivative. This has been successful in constructing a specific chiral field for the reduction with the aid of Mg(II). Based on the fact that an enzyme exhibits its catalytic activity by binding to a substrate with a large association constant, it is expected that a good result will be obtained by introducing a mimic of the binding site within a molecule of a 1,4-dihydropyridine derivative. At the same time, if the binding site incorporates chirality, stereoselective reduction should be achieved. One of the most effective NAD(P)H model compounds of this type is that which has a macrocyclic moiety in the molecule. The compound was designed with the intention of binding a substrate or a metal ion at the macrocyclic moiety. As expected, the acceleration of the rates of the reduction of sulfonium salts (van Bergen and Kellogg 1977, Hedestrand et al. 1978) as well as the binding of other cationic substrates (Piepers and Kellogg 1980) were observed in the reduction by an NAD(P)H model compound with a bridging polyether chain between the 3 and 5 positions of the 1,4-di-



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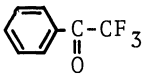
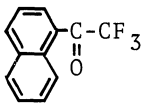
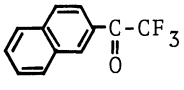
hydropyridine ring (26). The results indicate that the polyether linkage provides an effective reaction field, as enzymes do for the reduction of certain substrates. Asymmetric reductions by NAD(P)H models of this type have already been mentioned. Another type of 1,4-dihydropyridine derivative that has a crown ether structure was reported by Behr and Lehn (1978). The reduction of a carbonyl compound which has a cationic part (ammonium ion) was accelerated by this reductant due to favorable association of the substrate with the polyether moiety.

The approach using cyclodextrin as a binding site has also been developed. Cyclodextrins are widely utilized in biomimetic chemistry as simple models for an enzyme because they have the ability to form inclusion complexes with a variety of molecules and because they have catalytic activity toward some reactions. Kojima et al. (1980, 1981) reported the acceleration in the reduction of ninhydrin and some dyes by a 1,4-dihydronicotinamide attached to β-cyclodextrin. Saturation kinetics similar to enzymatic reactions were observed here, which indicates that the reduction proceeds through a complex. Since the cavity of the cyclodextrin molecule has a chiral environment due to the asymmetry of D-glucose units, these chiralities are expected to be effective for the induction of asymmetry into the substrate. Asymmetric reduction with NAD(P)H models of this type, however, has not been reported. Asymmetric reduction by a 1,4-dihydronicotinamide derivative took place in an aqueous solution of cyclodextrin (Baba et al. 1978), although the optical yield from the reduction was quite low. Trifluoromethyl aryl ketones were reduced by PNAH in 1.1 to 5.8 % e.e. in the presence of β-cyclodextrin. Sodium borohydride works as well (Table 18). In addition to cyclodextrin, Baba et al. also found that the asymmetric reductions can be accomplished in the presence of bovine serum albumin (BSA) which is a carrier protein in plasma.

The idea of using a protein as a chiral source originates from the work by Akabori et al. (1956) who tried an asymmetric reduction of acetoximes from benzoylformate and α-ketoglutarate by means of hydrogenation catalyzed by palladium on silk fibroin. Reduced products were obtained in 30 and 7 % optical yields, respectively.

Another successful result in this field is the reduction of olefins under the catalysis of rhodium ligated by a derivative of biotin (Wilson and Whitesides 1978). The reduction was carried out

Table 18. Asymmetric reduction of aryl trifluoromethyl ketones with PNAH and sodium borohydride.

Substrate	Reductant	Chiral media	e.e. [%]	Configuration
	PNAH	β-CD	3.7	*R*
	$NaBH_4$	β-CD	0	
	PNAH	BSA	46.6	*R*
	$NaBH_4$	BSA	36.2	*S*
	PNAH	β-CD	1.1	*R*
	$NaBH_4$	β-CD	2.6	*S*
	PNAH	BSA	30.0	*R*
	$NaBH_4$	BSA	15.6	*R*
	PNAH	β-CD	5.8	*R*
	$NaBH_4$	β-CD	10.0	*S*
	PNAH	BSA	22.3	*R*
	$NaBH_4$	BSA	38.8	*S*

in the field of avidin, a protein which has a great affinity toward biotin and operates to regulate the concentration of biotin in a living cell.

As shown in Table 18, trifluoromethyl aryl ketones were reduced in 22.3-46.6 % e.e. by PNAH and 15.6-38.8 % e.e. by sodium borohydride in 1.5-1.7 mM solution of BSA. The degree of asymmetric induction was rather high in comparison with those from the reactions with cyclodextrin, which suggests the possibility that such a simple protein as BSA can provide a chiral reaction field, as an enzyme does. As already mentioned, some proteins have a similar (or sometimes greater) affinity toward a molecule in the ground state in comparison with an enzyme. The difference between these two proteins in different classes is the affinity toward a transition state. The enzyme has to bind the transition state more strongly than the ground state.

It should be noted that the difference in the reducing agent results in a change in the configuration of the alcohol produced, except for the reduction of 1-(α,α,α-trifluoro)acetonaphthone. This result indicates that the mechanisms for the induction of chirality with these two reductants are different. Considering the fact that the actual reaction site in a biological reduction by NAD(P)H is constructed by a protein, the BSA system may be regarded as a good model for studying the mechanism of asymmetric reduction operating in dehydrogenase systems. Namely, BSA acts like an apoenzyme in the sense that it provides a chiral reaction field for the achiral reducing agents. It is well known that BSA has the ability to bind a variety of hydrophobic compounds. Therefore, it is a logical conclusion that the binding by BSA is important and that asymmetric reductions occur within a chiral domain of BSA.

Asymmetric reductions of other aromatic ketones by sodium boro-

hydride and sodium cyanoborohydride were also reported by Sugimoto et al. (1978, 1981a). The maximal optical yields in each case are shown in Table 19. More systematic investigations revealed that the system involves several interesting features. First of all, the optical yield of the product increases as the concentration of BSA increases. Maximal optical yield was attained when the molar ratio of BSA toward the substrate was more than 1/3. In BSA there are three domains that can bind hydrophobic compounds (Geisow 1977) and the primary structures of the domains resemble each other. Therefore, the result reveals the importance of binding of the substrate to BSA. Free substrates are reduced, of course, without asymmetric induction. This process may predominate over the reaction from the complexed substrate when the concentration of BSA is lower than 1/3. Hence the molar ratio of 1:3 is the right number corresponding to the three domains. Inhibition of asymmetric induction by the addition of naphthalene also supports the idea that the incorporation of the substrate into the domain of BSA is the origin of asymmetric induction in this system.

Table 19. Asymmetric reduction of carbonyl compounds with sodium borohydride in the presence of BSA.

Substrate	e.e. [%]	Configuration
Acetophenone	45	*R*
α,α,α-Trifluoroacetophenone	36	*S*
Propiophenone	78	*R*
n-Butyrophenone	27	*R*
Isobutyrophenone	66	*R*
Valerophenone	14	*S*
t-Butyl phenyl ketone	21	*R*
1-Acetonaphthone	67	*R*
1-(α,α,α-Trifluoro)acetonaphthone	16	*R*
2-Acetonaphthone	65	*R*
2-Trifluoroacetonaphthone	39	*S*
2-Acetylphenanthrene	21	-
3-Acetylphenanthrene	20	-

The importance of the structure of the BSA molecule on the asymmetric induction is obvious from the phenomenon that the optical yield in the reduction depends largely on the pH of the reaction solution. Dramatic changes in optical yields were observed at pH:3-4 and 7-9, which were attributed to the change in the three dimensional structure of BSA. In addition, the stereoselectivity decreased markedly in a solution of BSA denatured by 0.8 M urea. These results support the idea that the complexation between BSA and the substrate is crucial for asymmetric reduction especially when the concentration of BSA is high. It was suggested that complexation of BSA-BH_4^- type also contributes to asymmetric reduction to afford the product of opposite configuration. However, the contribution of this species to the reaction seems to be small. It can be noted that the binding site for hydrophilic species such as BH_4^- must be different from those for the hydrophobic substrates.

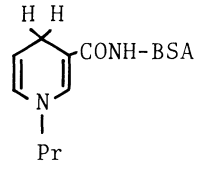
In any case, the asymmetric reduction surely takes place in a chiral environment of the protein. A factor determining the stereochemistry of the product must be an asymmetric binding so as to expose one of the enantiofaces of the carbonyl group selectively to the reductant. It is difficult to clarify the details of the interaction between BSA and the substrate and to elucidate the origin of selectivity. The data listed in Table 17 predicts that bulkiness of the substituent in the substrate is not the only factor for the asymmetric induction.

Chiral field provided by a BSA molecule is utilized in order to achieve other asymmetric reactions. Sugimoto et al. (1979a,b, 1981b) reported the stereoselective oxidation of aromatic sulfides by an achiral oxidizing agent. In the system, the optical yield of the sulfoxide could be enhanced by further oxidation of minor enantiomer of the sulfoxide to the corresponding sulfone within the binding domain of BSA. Ogura et al. (1980) reported the asymmetric oxidation of dithioacetals in a similar manner. Kokubo et al. achieved the stereoselective hydrolysis (1982) and hydroxylation of alkenes (1983) by the use of BSA.

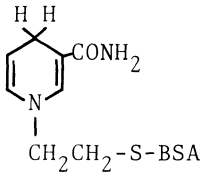
Although BSA is a relatively simple protein in comparison with enzymes, it was found that even BSA can provide a chiral reaction field for reductions. However, the value of e.e. associated with the reduction was not so high. This is probably because not all the reactions take place within the binding domains of BSA and because the enantio-differentiation exerted by BSA through an interaction with the substrate is not strict enough. On the other hand, the reduction catalyzed by a dehydrogenase takes place at a specific reaction site of the enzyme, which, at the same time, provides an excellent reaction field. Since the active site of an enzyme is constructed so as to fit a particular substrate and coenzyme, it is not surprising that the enzyme protein is much superior to a structural protein such as BSA in the recognition of a molecule.

Ohno et al. (1983) synthesized 1,4-dihydropyridine derivatives covalently bound to the BSA molecules, BSA-N-NAH or BSA-S-NAH. This system can be regarded as a model of a holoenzyme where BSA and the 1,4-dihydropyridine moiety correspond to the models of an apoenzyme and NAD(P)H, respectively. If the reaction site is chiral, asymmetric reduction is expected to be achieved. In the reduction of α,α,α-trifluoroacetophenone by BSA-S-NAH, asymmetric induction was observed, as can be seen in Table 20. However, 1,4-dihydropyridine attached to an amino group of BSA (BSA-N-NAH) exerted no ability for asymmetric reduction of α,α,α-trifluoroacetophenone. The basic idea anticipated for this system was to introduce a 1,4-dihydropyridine moiety into a specific chiral field constructed by a polypeptide chain of BSA. Since the BSA molecule has a variety of amino groups, it was impossible to choose a particular amino group for the modification. Attempts to modify a specific amino group of low pKa by changing the pH of the reaction solution were unsuccessful. On the other hand, since BSA contains only one mercapto group, it was easy to set the 1,4-dihydropyridine moiety into a single chiral environment by the modificaion of this mercapto group (BSA-S-NAH) (see also Ohno et al. 1980c).

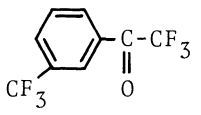
Results listed in Table 20 show that the values of e.e. are low in the presence of excess substrate, which indicates that the reaction of BSA-S-NAH with the free substrate does not exert stereoselectivity. This is in contrast to the fact described above that the reaction between a substrate trapped on BSA and a free reactant ($NaBH_4$ or



BSA-N-NAH



BSA-S-NAH



TFTFA

Table 20. Reduction of α,α,α-trifluoroacetophenone derivatives with BSA-S-NAH.

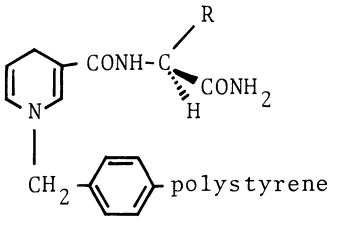
Substrate	[BSA] [mM]	$\frac{[\text{BSA-S-NAH}]}{[\text{BSA}]}$	$\frac{[\text{Substrate}]}{[\text{BSA}]}$	e.e. [%]	Config.
TFA	0.69	0.52	13	5	*R*
TFTFA	0.67	0.48	13	5	*R*
TFA	1.0	0.32	3.1	10	*R*
TFA	1.1	0.29	3.0	11	*R*
TFTFA	1.1	0.32	2.9	17	*R*
TFTFA	1.0	0.32	3.1	15	*R*

PNAH) exerts stereoselectivity. The values of e.e. from the latter system are higher than those from the BSA-S-NAH (Table 18). Thus, it is likely that the factors determining the stereoselectivities in these two systems differ from each other and that, in the modified system, protein-protein interaction between BSA which binds a substrate and BSA which is modified with 1,4-dihydropyridine is important.

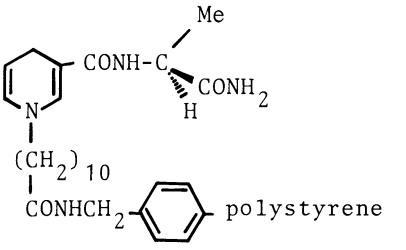
Baba et al. (1980b) attempted an asymmetric reduction by 1,4-dihydropydidine covalently bound to a cyclic peptide, bacitracin, which is an antibiotics and has large affinity to the zinc ion. They intended to enhance the reactivity of 1,4-dihydropyridine by the coordination of a cyclic peptide moiety to a metal ion as well as to achieve the asymmetric reduction by an effect of the chiral field provided by the peptide. However, neither the activity nor the ability for asymmetric induction were so high. The optical yield of ethyl *R*-mandelate in the reduction of ethyl benzoylformate in the presence of magnesium perchlorate was 5.4 % (*R*) in dry methanol only 1.9 % (*R*) in acetonitrile.

The effect of synthetic polymers on the asymmetric reduction by NAD(P)H model compounds was examined. Shinkai et al. (1981) synthesized chiral 1,4-dihydropyridine derivatives bound to polystyrene beads (**27-29**) and subjected them to the reduction of ethyl benzoylformate in acetonitrile in the presence of magnesium perchlorate. As shown in Table 21, the optical yields from the polymeric systems were low compared to those from homogeneous systems. The reductions in homogeneous systems with **30** and **31** gave optical yields of 47 and 5%, respectively.

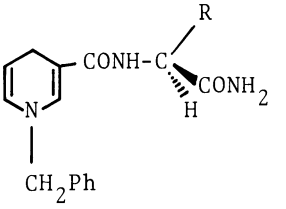
The results were interpreted in terms of the hydrophobic environment constructed by the polymer where the contribution of Mg(II) is smaller than that in acetonitrile solution or of a sterically crowded



27: R = Me
28: R = $PhCH_2$



29



30: R = Me
31: R = $PhCH_2$

Table 21. Asymmetric reduction of ethyl benzoylformate with polymer-bound chiral 1,4-dihydropyridine derivatives.

1,4-Dihydropyridine	Content of 1,4-dihydropyridine [mequiv./g]	e.e. [%]	Config.
27	0.73	1.1	*R*
28	0.60	1.8	*R*
29	0.70	7.3	*R*

reaction center in polymer beads. In fact, the optical yield was enhanced by inserting a spacer between the polymer chain and the 1,4-dihydropyridine moiety as in the case of **29**.

These results indicate that the reaction field constructed by a protein or a peptide which contains a number of chiral centers close to the reaction site does not necessarily induce a high degree of asymmetry. Thus, it is important to set a 1,4-dihydropyridine moiety into a well-designed environment in order to obtain good results.

8 POLAR EFFECT EXERTED BY OTHER ASYMMETRIC REACTIONS

The authors have proposed that the dipole-dipole interaction is

more important than the steric effect in the reduction with the 1,4-dihydropyridine derivative. Especially, this is true when the magnesium ion is present in the reduction system. The idea is different from the traditional one which insists that steric bulk is the most important factor in determining the steric course of a reaction. All of them, the Prelog model (Prelog 1953), Cram model (Cram and Abd Elhafez 1952 a,b), Karabatsos model (Karabatsos and Hsi 1965, 1967, Karabatsos and Krumel 1967, Karabatsos and Fenoglio 1969a,b, Karabatsos et al. 1967), Felkin model (Chérest et al. 1968, Chérest and Felkin 1968), and other similar models are stereo-models for transition states that predict the stereochemistry of the reaction from the viewpoint of a steric effect.

It will now be worthwhile surveying asymmetric inductions where an effect other than steric bulkiness plays an important role in defining the steric course of the reaction.

In the reduction of aromatic ketones with chiral Grignard reagents, it was found that the optical yield of the product was subject to the electronic effect from the *para*-substituents (Capillon and Guétté 1979a,b). Results from combinations of various ketones and Grignard reagents are summarized in Tables 22-25, where the data are arranged in order of increasing difference in the electron--releasing (or -withdrawing) property of two substituents.

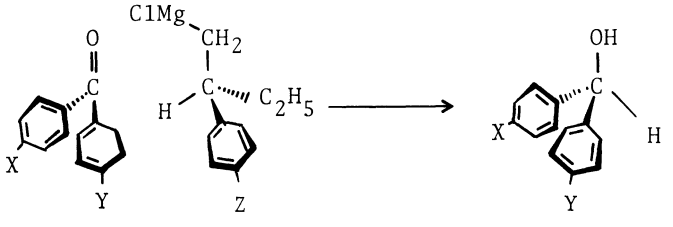
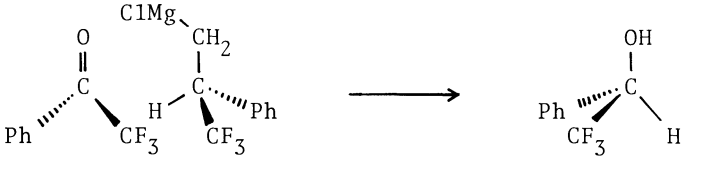


Table 22. Reduction of substituted benzophenones by 2-arylbutylmagnesium chloride.

X	Y	$\sigma_p(x) - \sigma_p(y)$	Optical yield with variation of Z[a]		
			CH_3O	H	CF_3
CH_3O	H	-0.268	- 1.7	+ 1.5	
CH_3	H	-0.170	+ 3.7	+ 5.5	- 4.1
Br	H	0.232	-17	-11	
CF_3	Br	0.31	-15		
CF_3	H	0.54	-32	-27	-15
CF_3	CH_3	0.71	-37		

[a] Optical yield with a positive sign indicates that the configuration of the product is that shown in the above reaction scheme.

The results shown in Table 22 were interpreted in terms of the charge-transfer-type interaction between an aromatic ring in the (substituted) benzophenone and the aromatic ring in the Grignard reagent: since *para*-methoxy substituent is electron-releasing, the electron-rich *para*-methoxyphenyl group prefers to face the electron--deficient side of the ketone at the transition state of the reaction. The unsubstituted phenyl ring in the Grignard reagent seems to like electron-deficient ring of the ketone. There are two exceptions to this generalization. That is, the *para*-methoxyphenyl group faces the electron-rich *para*-methoxyphenyl group, although the optical yield from this combination is quite low, and the *para*-trifluoromethylphenyl group prefers to couple with the electron--deficient *para*-trifluoromethylphenyl group. Although we have no rationalization, there are a few examples of the preferential combination of α,α,α-trifluoromethyl groups. For example, the reduction of α,α,α-trifluoroacetophenone with *S*-4,4,4-trifluoro-3-phenylpropylmagnesium chloride gives *R*-alcohol, which requires that two trifluoromethyl groups to face each other at the transition state of the reduction (Aaron et al. 1967). However, there is no example, to the authors' knowledge, of the pairing of *para*-methoxyphenyl groups.



The results listed in Table 23 indicate that the aryl-alkyl combination is preferred over the aryl-aryl combination. This is probably due to the larger steric bulk of an aryl group compared to that of an ethyl group. The anomalous behavior of the trifluoromethyl group mentioned above operates here again to reduce remarkably the optical yields from the reactions with the Grignard reagents that have this particular substituent. Here, however, no straightforward electronic relationship can be seen.

When both the ketone and the Grignard reagent have an alkyl group in their molecules, steric bulk exerted by the alkyl group becomes important, which is shown in Tables 24 and 25. A detailed discussion is given in a review by Morrison and Mosher (1971).

The authors have considered the reinterpretaion of the electronic effect proposed by Capillon and Guétté and the data listed in Table 22 have been rearranged to those in Table 26, where the data are arranged in order of difference in the substituent σ_m^+ value. Since σ_m^+ and σ_m are values that are free from the resonance contribution of the substituent, the *para*-methoxy group is now regarded as an electron-withdrawing group.

Now, the dependence of the optical yield on the electronic effect of the substituent is apparent. Instead of the *para*-substituent constant, which is a direct measure of the electron density at the reaction center, the *meta*-substituent constant is a suitable measure to the electron density in the aromatic ring. Since it is this aromatic ring which is involved in the intermolecular charge transfer interaction at the transition state of the reaction, we have to deal

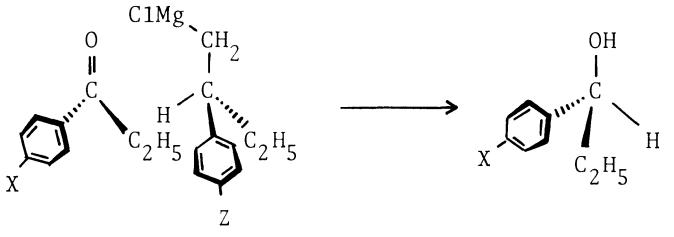


Table 23. Reduction of substituted propiophenones by 2-arylbutylmagnesium chloride.

X	σ_p	Optical yield with variation of Z[a]		
		CH_3O	H	CF_3
CH_3O	-0.268	+51	+51	
CH_3	-0.170	+54	+52	+10
H	0.000	+57	+50	+22
Cl	0.062	+36	+43	
CF_3	0.54	+22	+22	+10

[a] Optical yield with a positive sign indicates that the configuration of the product is that shown in the above reaction scheme.

with the electron density in the aromatic ring instead of that at the reaction center.

It might be interesting to note that UV spectra of a series of substituted benzophenones (and thiobenzophenones) suggest that the phenyl ring substituted by an electron-releasing group is itself located in the plane defined by the carbonyl group, whereas the phenyl ring substituted by an electron-withdrawing substituent is twisted out from this plane (Korver et al. 1965). This fact means that the more electron-deficient aryl group should have a larger steric bulk than the other. Nevertheless, the results listed in Table 22 or Table 26 indicate no relationship between the optical yield and the particular steric bulk, which tells us that the conformation at the ground state retains no meaning in the discussion of the conformation *at the transition state*.

A better correlation of e.e. with the *meta*-substituent constant than with the *para*-substituent constant can also be seen in the reaction of (*S*)-1-(1-naphthyl)-2,2,2-trifluoroethanol with a series of *para*-substitued phenylsulfinyl chlorides (Pirkle et al. 1976). Although, as shown in Fig. 15, Pirkle et al. correlated the e.e. with σ_p of the *para*-substituent, better linearity is seen when the e.e. is correlated with the σ_m value of the corresponding substituent. The

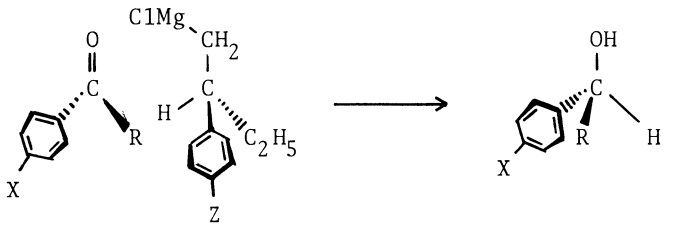


Table 24. Reduction of alkyl phenyl ketones by 2-phenylbutylmagnesium chloride.

R	Optical yield with variation of Z[a]		
	CH_3O	H	CF_3
C_2H_5	+57	+50	+22
$(CH_3)_2CH$	+84	+81	+58
$(CH_3)_3C$	+16	+16	-27

[a] Optical yield with a positive sign indicates that the configuration of the product is that shown in the above reaction scheme.

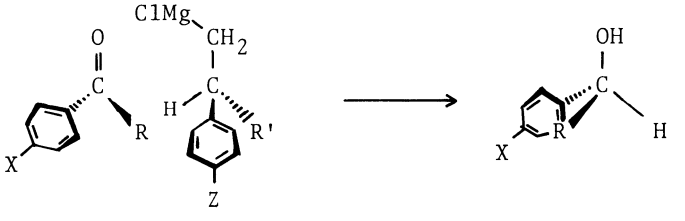


Table 25. Reduction of alkyl phenyl ketones by 2-arylalkylmagnesium chloride.

R	Optical yield with variation of R'[a]		
	CH_3	$(CH_3)_2CH$	$(CH_3)_3C$
CH_3	+38	+47	
CH_3CH_2	+38	+50	+66
$(CH_3)_2CH$	+59	+82	+80
$(CH_3)_3C$	-22	+16	+91

[a] Optical yield with a positive sign indicates that the configuration of the product is that shown in the above reaction scheme.

Table 26. Reduction of substituted benzophenones by 2-arylbutylmagnesium chloride.

X	Y	$\sigma_m^+(x)-\sigma_m^+(y)$	Optical yield with variation of Z		
			H	CH_3O	CF_3
CH_3	H	-0.066	+ 5.5	+ 3.7	- 4.1
CH_3O	H	0.047	+ 1.5	- 1.7	
CF_3	Br	0.115		-15	
Br	H	0.405	-11	-17	
CF_3	H	0.52	-27	-32	-15
CF_3	CH_3	0.586		-37	

choice of σ_m or σ_m^+ may depend on the electron-demanding property of the charge-transfer-type interaction.

The results listed in Table 23 show a somewhat different tendency from those in Table 22. Since the alkyl-aryl interaction (or the alkyl-alkyl repulsion) is much more important than the aryl-aryl interaction here, primarily because of steric reason, the electron density in the phenyl ring is no more the sole factor in this reaction.

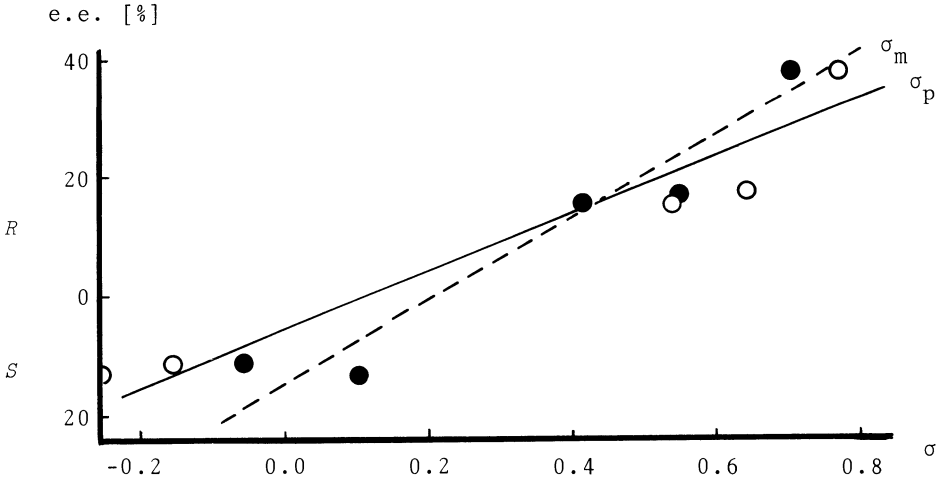


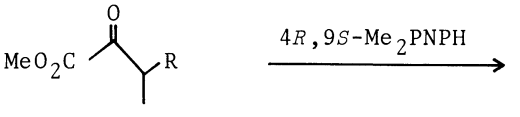
Fig. 15. The enantiomer excess plotted aginst the σ_p or σ_m value of substituent in the reaction of (*S*)-1-(1-naphthyl)-2,2,2-trifluoroethanol with substituted phenylsulfinyl chlorides.

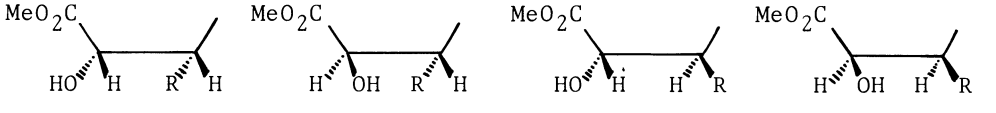
An alkyl group is, in general, a more electron-releasing substituent than an aryl group. Therefore, it is not surprising that an alkyl group prefers to face an aryl group by a charge-transfer type interaction. The interaction is expected to become more important as the electron-deficiency of the phenyl ring becomes larger. However, the charge-transfer-type aryl-aryl interaction also becomes larger with the increase in the electron-deficiency of the aryl group. As a consequence, the optical yield does not appear as a monotonic function of the substituent electronic effect but passes through a maximum value. Here again, the anomaly of the trifluoromethyl group is seen.

8.1 Reduction to Afford Diastereoisomers

Induction of two or more chiral centers by one asymmetric reduction is desirable in organic synthesis. In this connection, the reduction with Me_2PNPH was applied for racemic substrates that have one chiral center in a molecule (Ohno et al. 1984b, Ohno and Yasuma 1985). As has already been described for the reduction of camphoroquinone, substrates of this sort afford all of four possible diastereomers. The diastereomeric composition in the products from the reduction of a series of 3-substituted methyl 2-oxobutanoate is shown in Scheme 20, where the most abundant diastereomer is set at 100.

Scheme 20





R	(2*S*,3*R*)	(2*R*,3*R*)	(2*S*,3*S*)	(2*R*,3*S*)
CH_3CH_2	3.7	100	11.1	83.3
$(CH_3)_2CH$	3.8	100	10.1	78.3
Ph	4.7	100	7.7	27.6
CO_2Me[a]	13.1	100	12.5	23.6

[a] *R-S* notation at the 3 position of this compound is reversed.

The preferential formation of *R*-alcohol holds here again as the strongest tendency of the reduction, which is seen in the 2*R*/2*S* ratio listed in Table 27, and suggests that the requirement for molecular arrangement at the transition state (Fig. 7) is quite strict. The *syn*/*anti*-configuration is not an important factor. Looking at the detailed structure of the selectivity, however, it becomes apparent that the 3*R*-configuration exerts better selectivity than the 3*S*-con-

Table 27. Diastereomeric ratio in the reduction products of 3-substituted methyl 2-oxobutanoate.

R	*syn/anti*[a]	3*R*/3*S*	2*R*/2*S*	d.e. in 3*R*--isomer [%]	d.e. in 3*S*--isomer [%]
CH_3CH_2	44/56	52/48	93/ 7	93	77
$(CH_3)_2CH$	43/57	54/46	93/ 7	93	77
Ph	23/77	75/25	91/ 9	91	56
CO_2Me[b]	25/75	76/24	83/17	77	31

[a] *Syn/anti*-configurations are defined by the relative orientation of R and OH.

[b] *R-S* notation at the 3 position of this compound is reversed.

figuration. The discrepancy of diastereomeric excesses (d.e.) in 3*R*- and 3*S*-isomers of the phenyl-substituted compound (75/25) is due to smaller reactivity of the 3*S*-isomer than the other rather than larger reactivity of the 3*R*-isomer, because the 2*R*/2*S* ratio and d.e. in the 3*R*-isomer for this compound are essentially the same as those for alkyl-substituted compounds. This is a surprising conclusion: in order to form the diastereomers of 3*R*-configuration, the R-group has to face Me_2PNPH at the transition state. Nevertheless, the sterically favorable 3*S*-diastereomer becomes less reactive when the substrate is a bulky phenyl-substituted compound. Some interaction other than a steric effect should be taken into account in order to understand the phenomenon.

Relative reactivities of diastereomers of the substrate with R = CO_2Me seem similar to those of the substrate with R = Ph, considering the *syn/anti* and 3*R*/3*S* ratios for these compounds. However, the 2*R*/2*S* ratio for the former substrate deviates greatly from the ratio for the latter. The magic to make the ratio different from the others lies in the larger proportion of the (2*S*,3*R*)-isomer of this compound (Scheme 20). That is, the *R*/*S*-selectivity of the reduction becomes less strict when the substrate is substituted by a polar group.

There is no appropriate explanation for the diastereomeric differentiation exerted by this reduction. However, it is interesting to note that the selectivity observed in this reduction is quite similar to those observed in the reduction of similar substrates using microorganisms (Oishi and Akita 1983, Nakata et al. 1982). Thus, the recognition of a steric effect in the substrate in an average biochemical process does not seem to differ very much from that in organic ones.

9 DIASTEREO-DIFFERENTIATION AT THE 4 POSITION OF 1,4-DIHYDROPYRIDINE

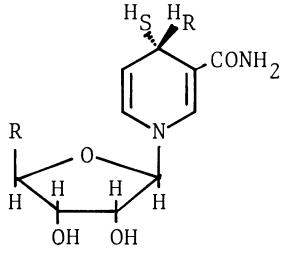
9.1 Explanation of A- or B-Specificity in Dehydrogenases

It is concluded, as described in the previous section, that the most important factor for the stereoselectivity of reduction is the selection of the transferring hydrogen from the prochiral position.

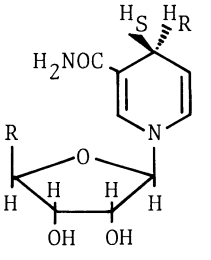
The selection stems from the difference in the properties of the two faces of 1,4-dihydropyridine ring caused by the chiral substituent on carbamoyl moiety. A similar situation is observed in the enzymatic system. In the reduction with NAD(P)H on a dehydrogenase, only one of the prochiral hydrogens at the 4 position is available. The choice depends on the nature of the dehydrogenase. This leads to the classification of dehydrogenases into A- and B-types as mentioned before. It is apparent that such specific restriction for the transferring hydrogen results in the formation of a chiral product in high optical yield. Also in the model system, the specific blockage of one of the enantiofaces of the 1,4-dihydropyridine ring is quite important for a high degree of asymmetric induction. It is of much interest, and of value for further development of asymmetric reduction in the model system, to provide a rational explanation of the origin of such selectivity with respect to the faces of the 1,4-dihydropyridine ring in a coenzyme on a dehydrogenase.

As a consequence of the accumulation of knowledge on dehydrogenases of A- or B-specificity, a rule to predict the stereochemical course of the reduction was proposed by Krakow et al. (1963), which was further extended by Alizade and Brendel (1975).

Recently, Nambiar et al. (1983) proposed an interesting explanation of the difference in stereoselectivity of dehydrogenases. This proposal involves a correlation between the stereoselectivity of dehydrogenases and the thermodynamic stability of their reduced and oxidized substrates. According to the proposal, the value of the equiliburium constant (K_{eq}), which is defined as [ketone]·[NADH]·[H^+] /[alcohol]·[NAD^+], determines the A- or B-specificity of the dehydrogenases. The proposal says that the *pro-R* hydrogen is utilized in the reaction where -log(K_{eq}) is greater than 11.3 and the *pro-S* hydrogen transfers in the reactions where -log(K_{eq}) is less than 11.1. In other words, reactive carbonyl compounds accept the *pro-R* hydrogen and less reactive ones accept the *pro-S* hydrogen of NADH. This proposal is based on the hypothesis that the *pro-R*



anti-NADH



syn-NADH

hydrogen transfers from a 1,4-dihydronicotinamide moiety bound to the apoenzyme in an *anti*-conformation and *pro-S* hydrogen transfers from a nicotinamide moiety bound in a *syn*-conformation and that the reducing power of the *anti*-NADH is weaker than that of *syn*-NADH. They interpreted the selection with respect to the transferring hydrogen in terms of a stereoelectronic effect as follows: As shown in Fig. 16, the 1,4-dihydropyridine ring is distorted into a boat conformation when it is bound to the apoenzyme and the axial hydrogen at the 4 position transfers more easily than the equatorial hydrogen because of the orbital interaction with a lone pair on the ring nitrogen. The axial hydrogen in *anti*-NADH corresponds to *pro-R* and that in *syn*-NADH corresponds to the *pro-S* hydrogen.

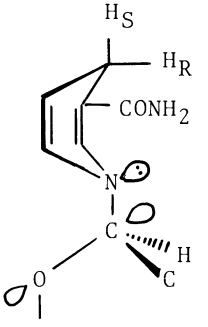


Fig. 16. 1,4-Dihydropyridine moiety in boat conformation where the axial hydrogen at the 4 position transfers more easily than the equatorial one.

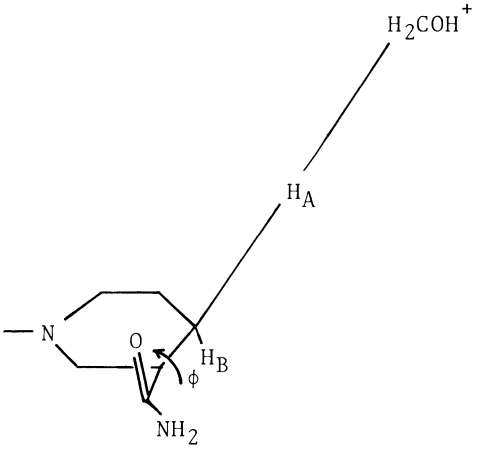


Fig. 17. Geometry of the transition state for the reaction of CDHP with CH_2OH^+.

The hypothesis proposed by Nambiar et al. was immediately opposed. Oppenheimer (1984) rejected the proposal, presenting three cases that

do not fit the correlation and pointing out the ambiguity in the proposal. The proposal does not say unambiguously why the *anti*-conformation is preferred by reactive substrates and *vice versa*. Recent comment by Benner et al. (1985), however, seems acceptable and interesting from the viewpoint of chemical evolution of enzymes.

Another interesting explanation for the stereochemical preferences of dehydrogenases was proposed by Buck and his co-workers. Donkersloot and Buck (1981) calculated the activation enthalpies for the hydride transfer reactions from 3-carbamoyl-1,4-dihydropyridine (CDHP) and its analogous compound 2-carbamoylcyclopropene (CCP) to H_2COH^+ and to cyclopropenium cation ($C_3H_3^+$). Quantum-chemical calculations were carried out by changing the rotational angle (ϕ) associated with the rotation of the carbonyl group in the 3-carbamoyl group (Fig. 17). The activation enthalpy as a function of ϕ showed a minimal value at $\phi=90^o$ for the tarnsferring H_A and at $\phi=270^o$ for the transfer of H_B. The enthalpy-rotational angle correlation is depicted in Fig. 18.

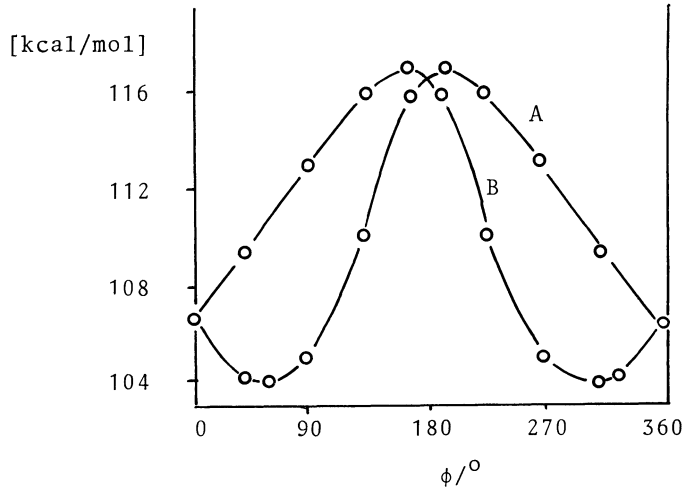


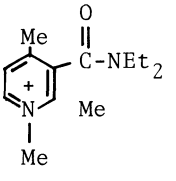
Fig. 18. Activation enthalpy for the rotational state of the system $CP^+...H...CH_2OH$ as a function of the rotational angle ϕ.

The difference between the activation enthalpies for the tranfer of H_A and that of H_B at around $\phi=90^o$ is significant. Here, H_A transfers more easily than H_B. When ϕ equals 270^o, the rate of the transfer of H_B is faster than that of H_A. This difference in the activation enthalpy amounts to 8 kcal/mol for the CDHP-CH_2OH^+ system and 6 kcal/mol for the CDHP-$C_3H_3^+$ system. A similar result was obtained for 1-carbamoylcycloheptatriene as a model compound of a hydride donor (Brounts and Buck 1983).

The result based on the quantum-chemical calculation leads to the conclusion that the hydrogen atom which is located in the same side as where the carbonyl oxygen is included transfers with a low enthalpy of activation in comparison with the hydrogen in the opposite side.

The difference in interaction energy between the negatively charged oxygen atom in the carbamoyl group and the positively charged hydride acceptor is similar to the difference in enthalpy of activation. On the basis of the discussion mentioned above, they explained the stereospecificity in an enzymatic system as follows: The absence of stereospecificity in the nonenzymatic reaction of NAD(P)H which contains chiralities in itself is due to the result of freedom toward the rotation of the carbamoyl group at the transition state. On the other hand, in the active site of an enzyme, the carbamoyl group loses the freedom of rotation by complex interactions with the apoenzyme so that the distorted conformation where the carbonyl oxygen points toward the substrate is frozen and results in the difference in rate of transfer between the two hydrogens at the 4 position. Factors that govern the stereospecificity in an enzymatic system would be much more complicated than in the model subjected to the calculation. The coenzyme in an active site of an enzyme is strongly influenced by environmental potentials and the orientation of the carbonyl dipole may have just a minor importance, if any at all. Indeed, a carbamoyl group at the 3 position of NAD(P)H is not indispensable for the stereospecificity. It was reported that the redox systems with 3-acetylpyridine adenine dinucleotide, thionicotinamide adenine dinucleotide, and 3-cyanopyridine adenine dinucleotide on alcohol dehydrogenase (A-specific) or glutamate dehydrogenase (B-specific) exhibited identical stereospecificity to NAD^+ (Biellmann et al. 1974).

Nevertheless, the proposal is quite attractive in the sense that it includes an interesting suggestion for the mechanism of asymmetric induction in organic chemistry. In order to obtain experimental support for the proposal, Buck and his co-workers investigated the system using model compounds. In molecules of 3-carbamoyl-1,4-dihydropyridine derivatives such as NAD(P)H and its familiar model compounds, the out-of-plane orientation of the carbonyl group cannot be fixed. On the other hand, in a molecule of Me_2PNPH which has two methyl substituents at the 2 and 4 positions of the 1,4-dihydropyridine ring, the carbonyl oxygen must stick out of the plane of the ring due to the steric effect from the methyl groups. As expected, NMR spectroscopy using a shift reagent revealed that the carbonyl group is forced to point out of the plane and that the hydrogen at the 4 position is *syn*-oriented toward the carbonyl oxygen in both *SR*-Me_2PNPH and *RR*-Me_2PNPH. X-ray analysis also supported this conformation (van Lier et al. 1982). The distorted angle of the carbonyl dipole was found to be 65° with respect to the 1,4-dihydro-



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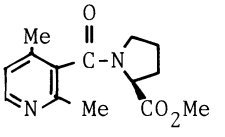

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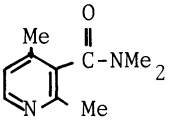


34

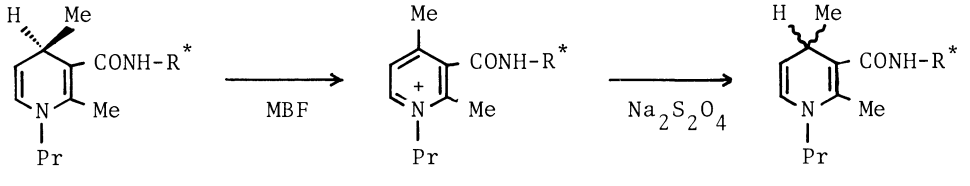
pyridine ring. These results indicate the presence of the axial chirality within the molecules. A derivative of 3-carbamoylpyridinium cation with two methyl substituents at the 2 and 4 positions (32) was separated into two enantiomers as diastereomeric salts of (+)-α-bromocamphor-π-sulfonate (van Hooff et al. 1982).

Another 2,4-dimethyl-3-carbamoylpyridine derivative which has a L--proline moiety at the carbamoyl side chain (33) was found to be a mixture of diastereomers which can be separated chromatographically (Ohno et al. 1985). Van Lier et al. (1983) also reported the separation of enantiomeric N,N-dimethyl-2,4-dimethyl-3-carbamoylpyridines (34), a precursor for 32, by converting it into diastereomeric pyridinium salts of α-bromocamphor-π-sulfonate and subjecting the salts to resolution followed by regeneration of the original pyridine derivative. A chiral detergent was synthesized *via* quaternarization of optically active 34 with *n*-dodecyl bromide (de Weerd et al. 1984).

The axial chirality has not been observed in the pyridine or the pyridinium salt as well as in the dihydropyridine derivatives with a carbamoyl side chain composed of a primary amine. This is also true for Me_2PNP^+ and Me_2PNPH; at least, when uncomplexed with a shift reagent, Me_2PNP^+ exhibits two broad singlets attributable to the methyl groups on the 2 and 4 positions, which means that the free rotation of the carbonyl dipole in this salt is restricted at room temperature but not inhibited completely. The corresponding signals from Me_2PNPH appeared to be sharp singlets indicating that the compound has no diastereomeric faces with respect to the orientation of the carbonyl dipole. Indeed, oxidation of *RR*-Me_2PNPH by methyl benzoylformate (MBF) followed by the reduction of the corresponding salt, 9*R*-Me_2PNP^+, by sodium dithionite resulted in the formation of *racemic* Me_2PNPH (Scheme 21) (Ohno et al. 1985). If Me_2PNPH and Me_2PNP^+ have axial chiralities and one of the diastereofaces of the molecule defined by the orientation of the carbonyl dipole is utilized in the redox interconversion, one stereoisomer of Me_2PNPH will afford only one stereoisomer of Me_2PNP^+ on oxidation, which, in turn, will afford only one stereoisomer of Me_2PNPH on reduction.

Thus, the inhibition by the adjacent two methyl groups for the rotation of the carbonyl group which is the pith of axial chirality in 32-34 is not due to the interference on the carbonyl group but the

Scheme 21

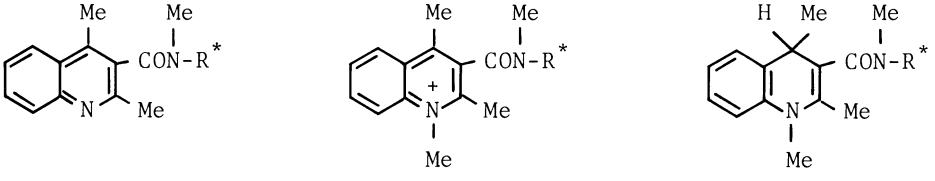


(R^* : chiral α-methylbenzyl group)

interference on the bulky substituent(s) on the amino moiety. The bulkiness of only one alkyl substituent on the primary amine is not large enough to restrict the rotation of the carbonyl group.

9.2 Self-Immolative Transfer of Chirality Between NAD(P)$^+$ and NAD(P)H Models: a Chirality Sink

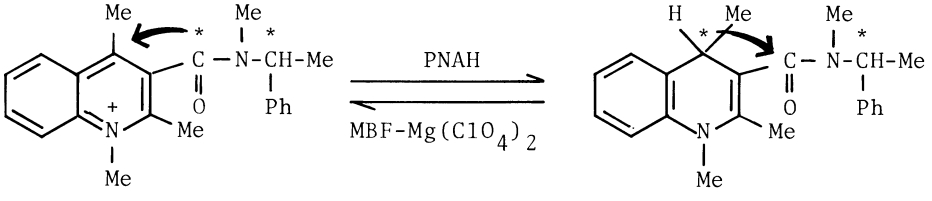
Ohno et al. (1985) attempted to examine the hypothesis by means of the redox interconversion between a 1,4-dihydroquinoline derivative and the corresponding quinolinium cation. It was found that the 2,4-dimethyl-3-carbamoylquinoline derivative with a secondary amine moiety (Me_3QP) and its quaternarized salt (Me_3MQP^+) have stable axial chiralities. Two diastereomers of Me_3QP whose configuration at the amide moiety is fixed to *R* or *S* were separated by column chromatography and quaternarized. The diastereomeric excess (d.e.) in the corresponding 1,4-dihydroquinoline derivative (Me_3MQPH) was about 34% when sodium dithionite was employed for the reductant, whereas it was better than 94% in the reduction with PNAH (Scheme 22).



Me_3QP Me_3MQP^+ Me_3MQPH

(R^* : chiral α-methylbenzyl group)

Scheme 22



Data from NMR spectroscopy have revealed that Me_3MQPH has no axial chirality. That is, the asymmetric centers in this compound are only those at the 4 position and at the benzylic carbon in the α-methylbenzyl group. Interestingly, one of the diastereomers of Me_3MQPH (*RR*- or *SS*-isomer) was stereospecifically converted into one diastereomer of Me_3MQP^+, which has the axial chirality at the carbonyl group as well as the central chirality at the benzylic

carbon, on oxidation with methyl benzoylformate in the presence of Mg(II). The isomer produced by this stereospecific oxidation of Me_3MQPH was the same one as that afforded the starting isomer of Me_3MQPH (Scheme 22). The d.e. was better than 95%. The methyl mandelate produced by the reaction had 99% e.e. On the other hand, oxidation of Me_3MQPH by the ferricyanide ion gave *racemic* Me_3MQP^+.

Although the absolute configuration on the carbonyl dipole has not been clarified yet, it is evident now that the reacting hydrogen in the reaction of the dihydroquinoline derivative has a conformational relationship with the carbonyl dipole provided the substrate is chosen appropriately. The result seems to agree with the hypothesis proposed by Buck and his co-workers. However, unfortunately, since we have no evidence on the absolute configuration of the Me_3MQP^+, we cannot discuss whether the reacting hydrogen has the *syn*-configuration in relation to the carbonyl dipole or whether it is in the *anti*-configuration.

Another factor which should be taken into account is that the oxidation of Me_3MQPH is mediated by Mg(II). Since this bivalent ion can coordinate both the model and oxidant, the orientation of the carbonyl dipole may possibly be restricted during the reaction in the presence of this metal ion. However, at least the reduction process is free from the influence of Mg(II).

In addition, the Me_3MQP^+/Me_3MQPH system has a bulky α-methylbenzyl group on the carbamoyl group. Therefore, it is possible that the conformation is affected by the steric bulk of this α-methylbenzyl group so as to set the carbonyl group *anti* to this moiety and, at the same time, make the *anti*-hydrogen ready to interact with the substrate. As a consequence, the hydrogen which moves during the reaction is that in *syn*-configuration with respect to the carbonyl oxygen, regardless of the amount of enthalpy of activation. The fact that small reagents such as dithionite and ferricyanide ions afforded unsatisfactory results seems to be the support for steric block instead of the enthalpic preference. If this is the case, however, the mechanism of exerting the stereospecificity with this mimetic system very closely resembles that operating in an enzymic reaction. That is, one face of the (dihydro)nicotinamide ring is intrinsically blocked by a bulky residue and the reaction takes place only in the face pointed to by the carbonyl dipole.

In spite of these uncertainties, this mimetic system has provided positive evidence for the relationship between the conformation of the carbonyl dipole and the stereochemistry of the reacting hydrogen, whatever the origin is.

From the viewpoint of organic chemistry, the present system is quite interesting: the system involves self-immolative transfer of the *axial chirality* at the carbonyl group of the oxidized form into the *central chirality* at the 4 position of the corresponding reduced form and *vice versa*. In this sense, this reaction system may be claimed as asymmetric inductions of a brand-new type (chirality sink). There are a few reports of one-way transfer of a chirality into another chirality of different type (Meyers and Wettlaufer 1984).

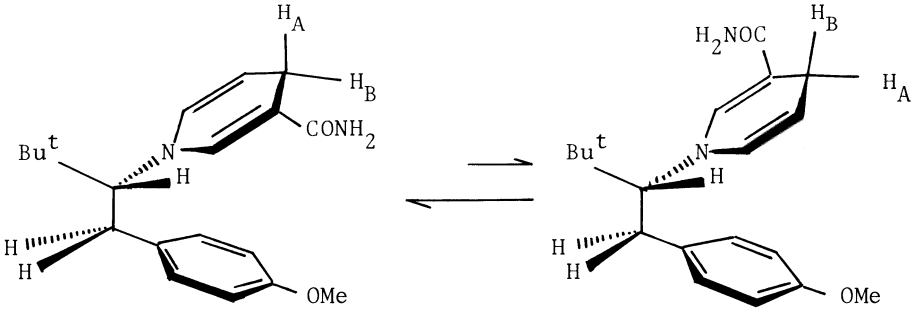
When the diastereomers of Me_3MQP^+ is once resolved, the conforma-

tions and/or configurations of this system might be kept unchanged throughout the shuttle of redox reactions due to the effect of the chirality sink. In other words, a compound of this type may be used as a catalyst for asymmetric reactions without further resolution. The idea was tested with methyl benzoylformate/Mg(II) and PNAH as oxidizing and reducing agents, respectively, and the methyl mandelate obtained was in 99% e.e. in the first cycle and in 90% e.e. in the second cycle.

9.3 Diastereo-Differentiation for Prochiral Hydrogens at the 4 Position

An important factor for asymmetric induction from NAD(P)H model compounds is a difference in reactivity of two prochiral hydrogens at the 4 position of the 1,4-dihydropyridine ring. However, no spectroscopically detectable difference in these two hydrogens had been observed in chiral NAD(P)H model compounds so far mentioned until the study by van Ramesdonk et al. (1977). They detected magnetic unequivalence of the two hydrogens in a 1,4-dihydropyridine derivative (**35**) by means of a NMR spectroscopy. Two protons at the 4 position were equivalent at a high temperature, but their signals were clearly split into an XY pattern at lower temperatures. A detailed analysis of the NMR spectrum has revealed that **35** is composed of two conformers and they are in equilibrium. In both of the conformers, *tert*-butyl and *para*-methoxyphenyl groups are set in *anti*-position. It was concluded from the analysis of chemical shifts of protons on the dihydropyridine ring that one conformer is differentiated from the other by 180° rotation of the 1,4-dihydropyridine ring as shown in Scheme 23. Thus, the anisochronism of two hydrogens at the 4 position stems from the preferential shield of one face of the 1,4-dihydropyridine ring by the *para*-methoxyphenyl substituent. The rotamer **35a** is preferred to the other in a ratio of **35a/35b** of 1.65 ± 0.05.

Scheme 23



35a **35b**

Asymmetric reduction by **35** which contains a chiral center adjacent to the ring nitrogen is also reported (van Ramesdonk et al. 1978).

In the reduction of methyl benzoylformate in the presence of magnesium perchlorate and in the reduction of α,α,α-trifluoroacetophenone, the products were obtained in 27 ± 4 % e.e. and 5 ± 2 % e.e., respectively. It should be noted that the former value of 27 %, which can be translated into a ratio of the enantiomeric composition in the product of *S*/*R* = 1.74 ± 0.15, nearly equals the ratio of the conformers (1.65) mentioned above. In this connection they proposed kinetically controlled induction of asymmetry for this system. The result was discussed on the basis of the equations (1) and (2).

$$k_A/k_B = (p_a k_{exo}+p_b k_{endo})/(p_a k_{endo}+p_b k_{exo}), \quad (1)$$

$$S/R = C(k_A/k_B). \quad (2)$$

In equation (1), the rate constant for the transfer of H_A (k_A) is expressed as a sum of the rate constants from the *exo*-position in the conformer **35a** (k_{exo}) and the *endo*-position in the conformer **35b** (k_{endo}). The molar fractions of **35a** and **35b** are represented by p_a and p_b (= 1 - p_a), respectively. The rate constant k_B is expressed similarly. The left-hand side of equation (1) implies the enantiomeric ratio in the product on the basis of an assumption that the effectiveness of enantioface differentiation with respect to the substrate is the same for each type of encounter of the substrate and the reductant. The selectivity with respect to the enantioface of the approaching substrate relative to the 1,4-dihydropyridine ring is represented in equation (2) by a parameter C.

To explain the experimental coincidence between *S*/*R* and p_a/p_b, the assumptions that C equals 1 and that either k_{exo} or k_{endo} is zero are required. If C is less than 1 or k_{exo} and k_{endo} are of comparable values, the calculated *S*/*R* ratio from the equation becomes smaller than the observed ratio of concentrations of the two conformers. Based on the reason that the above assumption seems to be improbable, the authors concluded that not only the interaction of the substrate with the carbamoyl moiety but also the interaction with other groups in the reductant should be taken into account. However, it does not seem so improbable to assume that C equals 1 because it was revealed from the study with Me_2PNPH that the relative orientation between alkyl benzoylformate and the 1,4-dihydronicotinamide moiety is uniquely set and that the most important factor for the asymmetric induction in the reduction by NAD(P)H model compounds is the differentiation of diastereoface of the 1,4-dihydropyridine ring by the approaching substrate. In addition, the assumption that k_{endo} is much smaller than k_{exo} does not seem so improbable, because the *endo* side of **35** is specifically blocked by a large *para*-methoxyphenyl group to exert the diastereotopic differentiation.

Stereochemistry of the product from this reduction system can be explained in terms of the discussion previously mentioned that methyl benzoylformate is reduced through a transition state where the polar group in the substrate (methoxycarbonyl moiety) interacts with the polar group in the reductant (carbamoyl moiety). This orientation of methyl benzoylformate with the predominant conformer of *R*-**35** which is shown in Fig. 19 should give methyl *S*-mandelate, which is in accord with the experimental result. The low e.e. value in the reduction of α,α,α-trifluoroacetophenone with *R*-**35** is due to the low

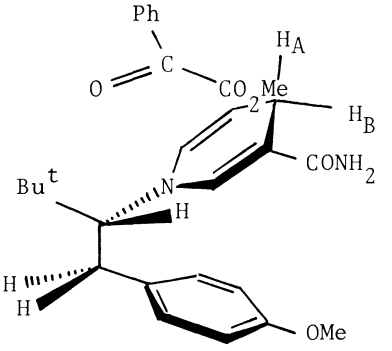
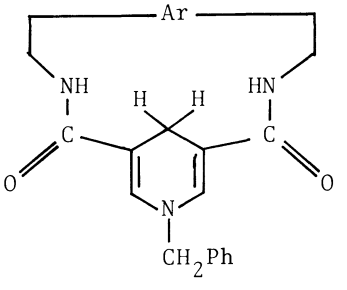
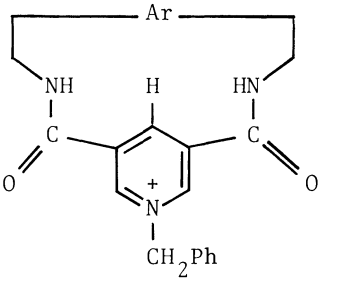


Fig. 19. Plausible transition state for the reaction of 35.



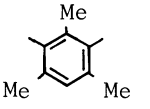
36-40



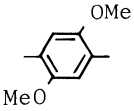
41-45

36, 41 : Ar =

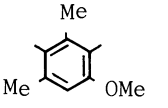
39, 44 : Ar =



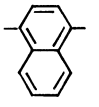
37, 42 : Ar =



40, 45 : Ar =



38, 43 : Ar =

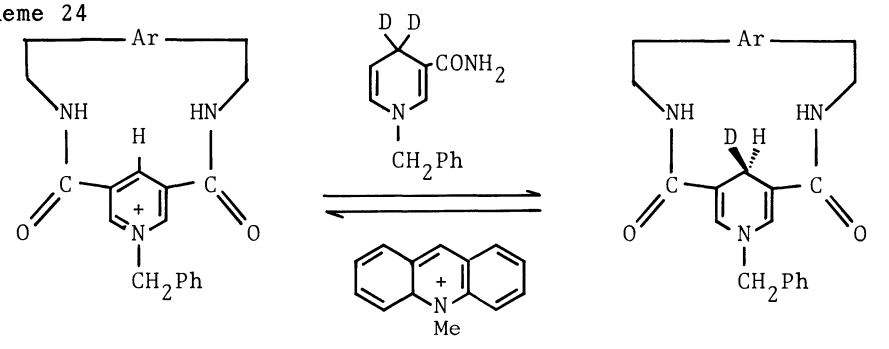


enantio-differentiating ability of this system, which has already been predicted from the result with Me_2PNPH. It was found that the enantio-selectivity with respect to the approaching α,α,α-trifluoro-acetophenone toward Me_2PNPH is relatively poor in contrast with the selectivity for an alkyl benzoylformate. That is, C in the above equation is far less than 1 for α,α,α-trifluoroacetophenone.

Rob et al. (1980, 1984) reported another attractive observation for the differentiation and stereoselective transfer of prochiral hydrogens at the 4-position of 1,4-dihydropyridine in NAD(P)H model compounds. They synthesized five 1-benzyl-3,5-dicarbamoyl-

-1,4-dihydropyridine derivatives bridged by a cyclophane moiety between the two carbamoyl substituents (**36-40**). A distinct anisochronism of the hydrogens at the 4 position was observed for three of them, **38**, **39**, and **40**, as a difference in chemical shifts of the NMR signals from the two protons. The mechanism opperating here to differentiate two hydrogens is similar to that operating in an enzyme. That is, one diastereotopic face of 1,4-dihydropyridine is specifically blocked by a bulky wall. The origin of the magnetic difference between two hydrogens in this model system stems from the difference in the position, axial or equatorial, in the boat-shape distorted 1,4-dihydropyridine moiety. Interest was focused on the relation between the anisochronism observed in the NMR spectroscopy and the diastereo-differentiation in the hydride transfer reaction, and they examined the stereospecificity associated with the reduction of the corresponding pyridinium cations (**41-45**). The reduction of **43**, **44**, and **45** with 1-benzyl-1,4-dihydronicotinamide-4,4-d_2 (BNAH--4,4-d_2) resulted in stereoselective incorporations of the deuterium (Scheme 24). The efficiencies of diastereo-differentiation amounted to 67 % for **43** and to more than 90 % for **44** and **45**.

Scheme 24

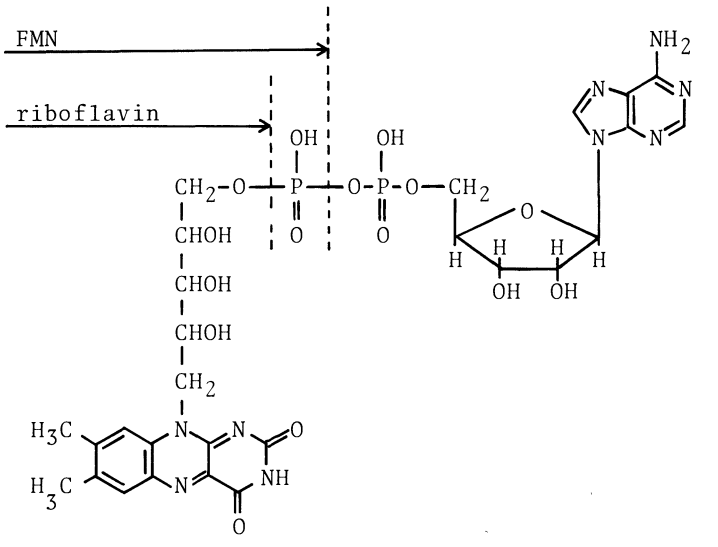


The selectivity toward the hydride transfer from the 4 position of these compounds was also studied for the reduction of 10-methylacridinium cation with **38**-4-*d*, **39**-4-*d*, and **40**-4-*d* that were prepared by the reduction of **43**, **44**, and **45** with BNAH-4,4-d_2, respectively. The deuterium content in the produced 10-methylacridan and the primary isotope effect which was observed in the reduction with these reductants revealed the ratio of the reactivities of the diastereotopic hydrogens as 8.8. The result indicates that the hydrogen which was introduced into the 4 position preferentially transferred in the reverse oxidation. Alternatively, one of the two faces of the dihydropyridine ring was specifically utilized for the redox interconversion in this system.

10 STEREOCHEMISTRY OF FLAVIN-DEPENDENT REACTIONS

10.1 Flavin as a Coenzyme

A variety of reactions that depend on flavin coenzymes are known. Two kinds of flavin coenzymes are utilized as redox reagents in

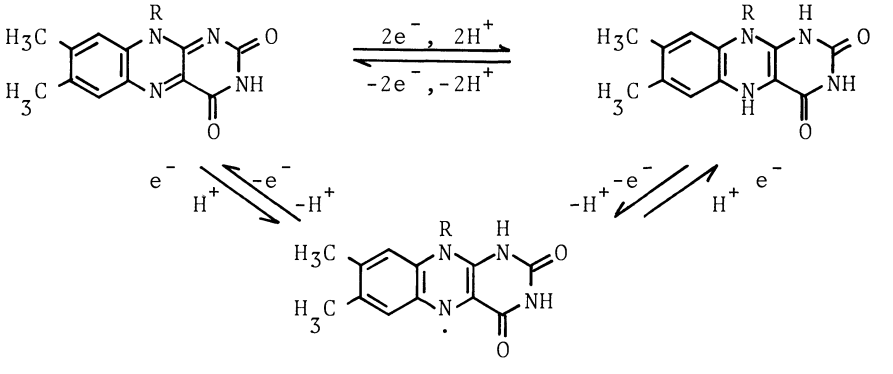


flavin adenine dinucleotide (FAD)

biological reactions. One is flavin mononucleotide (FMN), and the other is flavin adenine dinucleotide (FAD). Oxidized form of FAD is shown here. The structures of FMN and riboflavin (vitamin B_2), which are constituents of FAD, are also depicted. Both FAD and FMN involve the 7,8-dimethylisoalloxazine ring which acts as an actual redox reagent in flavin-dependent enzymatic reactions. Thus, an isoalloxazine derivative whose 10 position is substituted by a simple substituent such as a methyl group is used as a model compound of the coenzymes.

Oxidized forms of the flavin coenzymes, FMN and FAD, accept two electrons and two protons to afford the reduced forms, $FMNH_2$ and $FADH_2$, respectively (Scheme 25).

Scheme 25



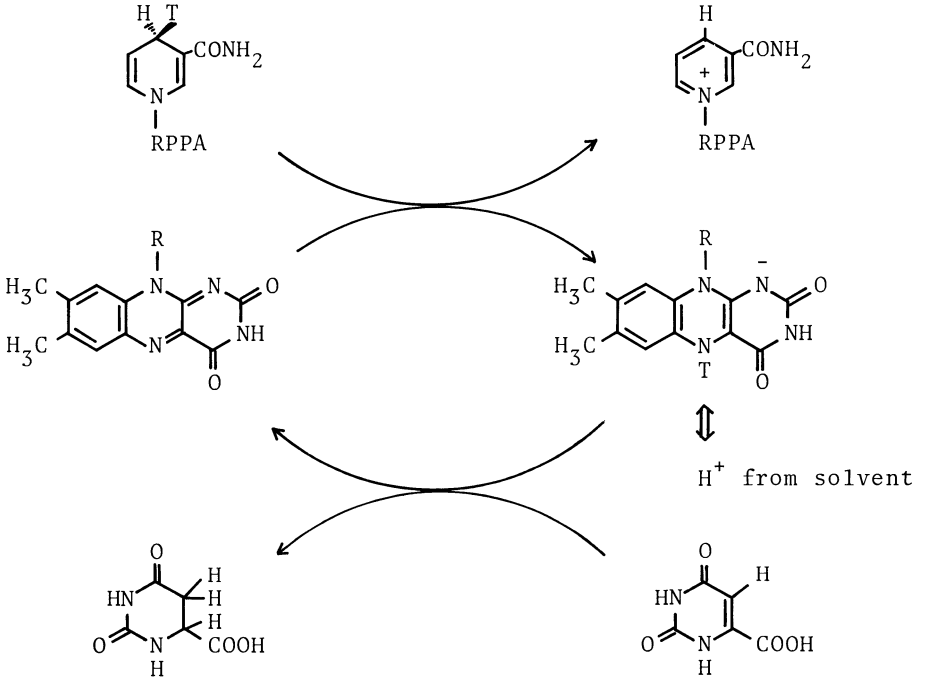
A one-electron-reduction state of flavin, which is regarded as a semiquinone, is also a stable species. In fact, flavin coenzymes can act as either one-electron or two-electron mediator, which is not observed in the system of $NAD(P)^+$-dependent enzymes. In addition to the properties mentioned above, there are several characteristics in flavin coenzymes which are different from $NAD(P)^+$. Firstly, flavin acts as a coenzyme for a wide variety of enzymes such as dehydrogenases, oxidases, and reductases. Secondly, oxidized flavin is a stronger oxidizing agent than $NAD(P)^+$. Finally, the reduced form of the coenzyme can be oxidized directly by molecular oxygen.

10.2 Stereochemistry of Flavin-Dependent Enzymatic Reactions

In the reduced structure of flavin, the hydrogen attached to the nitrogen at the 5 position and being transferred to a substrate is easily exchanged with protons in a solvent. This feature of the flavin coenzyme make it difficult to study the mechanism of the redox system and the stereochemical properties of flavin-dependent enzymes.

In the study of NAD(P)H-dependent reductions, mechanistic and stereochemical features were investigated by using deuterium or tritium. However, such a technique cannot be applied to the study of flavin because the isotope on the 5(N)-position is washed out into the solvent. For example, in the case of dihydroorotate dehydrogenase, which catalyzes the reduction of orotate by NADH through a mediation of FAD, the route of the hydride from NADH to the dihydroorotate produced has been followed, not without difficulty. As

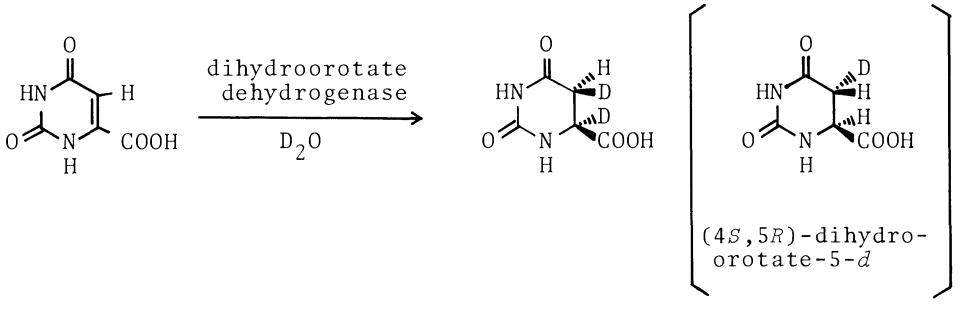
Scheme 26



shown in Scheme 26, in the first step of this reaction, the 5(N) position of the oxidized flavin is believed to accept a "hydride" from NADH.

It has been clarified by the use of tritium-labeled NADH that the *pro-R* hydrogen at the 4 position of NADH specifically transfers onto a "hydride" acceptor. However, the transfer of tritium onto the 5(N)-position of FAD has by no means been proved. In addition, it is unable to be proved whether the hydrogen originating from NADH transfers onto orotate in the succeeding step. Therefore, the only conclusive remark is that the hydrogens at the 4 and 5 positions of the dihydroorotate produced are those originating from the solvent. The stereochemistry of the product was clarified by another method. Dihydroorotate which was produced by an enzymatic reduction of orotate catalyzed by dihydroorotate dehydrogenase in deuterium oxide was compared with authentic (4*S*,5*R*)-dihydroorotate-5-*d* which was derived from (2*S*,3*R*)-aspartate-3-*d*, and it was found that *pro-S* hydrogen at the 5 position of dihydroorotate was introduced from FAD (Scheme 27) (Blattmann and Rétey 1972). This implies that the reduction of orotate proceeds with *trans*-hydrogenation. As for the reverse reaction, dehydrogenation of dihydroorotate, an *anti*-elimination mechanism has also been proved. These stereochemical characteristics in dihydroorotate dehydrogenase are the same as those observed in other dehydrogenases such as acyl-CoA dehydrogenase and succinate dehydrogenase. The evidence for stereoselective reduction of olefinic substrates by flavin indicates that the molecular arrangement of the substrate against flavin is restricted by the environment at the active site.

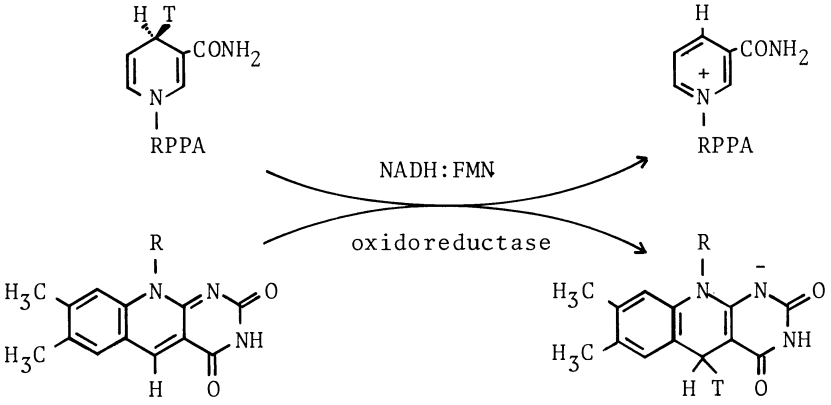
Scheme 27



From the viewpoint of stereochemistry, it is valuable to know whether one of the enantiofaces of the isoalloxazine ring is specifically used for the "hydride" acception and donation or whether both faces are related to the redox mediation. In the case of NAD(P)H in enzymatic systems, two faces of the 1,4-dihydropyridine ring are perfectly discriminated and one of them specifically interacts with the substrate. An attempt to make clear such a stereochemical problem was made using a 5-deazaflavin derivative which is an ananalogue of flavin and has a carbon atom at the 5 position instead of a nitrogen atom. The compound was reduced by NADH to afford the corresponding dihydro form whose hydrogens at the 5 position did not exchange with protons from the solvent (Brustlein and Bruice 1972). This property, which does not appear in the native component of isoalloxazine, has an advantage in the investigation of the mechanism and stereochemical features of flavin-dependent enzymes.

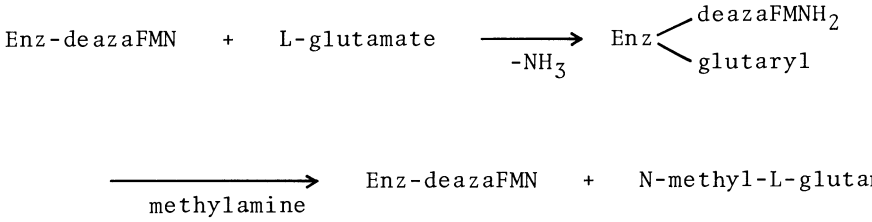
Reduction of the 5-deaza-analogue of FMN under a catalysis of NADH /FMN oxidoreductase was investigated (Fisher and Walsh 1974) and direct transfer of the *pro-R* hydrogen of NADH onto the 5 position of 5-deazaflavin was observed (Scheme 28).

Scheme 28



In the case of methylglutamate synthetase, it has been found by using glutamate-2-*t* that the α-hydrogen of the substrate is incorporated into 5-deazaflavin in the first step of the reaction and that the same hydrogen transfers onto the intermediate bound to the enzyme to afford N-methylglutamate (Scheme 29) (Jorns and Hersh 1974).

Scheme 29

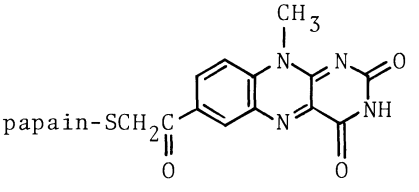


This stereospecific re-oxidation of the deazaflavin moiety indicates that the same side of the isoalloxazine ring is used in the catalytic cycle of the enzyme. A similar result was reported for D-amino acid oxidase (Hersh and Jorns 1975).

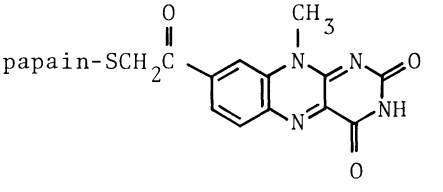
10.3 Model Reaction of Asymmetric Inter-Coenzyme Hydrogen Transfer

Asymmetric hydrogen transfer from NADH or chiral 1,4-dihydropyridine derivatives to flavin in mimetic systems has been attempted. Levine and Kaiser (1980) found that flavin attached to the Cys-25 in papain (flavopapain) discriminates between the prochiral hydrogens on NADH. Flavopapain, in which the active site of the hydrolytic

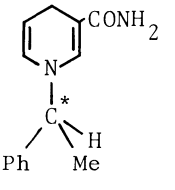
enzyme, papain, is utilized as a mimic of the reaction site of flavin-dependent dehydrogenase, is regarded as a semisynthetic enzyme and exhibits dehydrogenase-like behavior such as saturation kinetics and significant rate acceleration (Levine and Kaiser 1978, Fried and Kaiser 1981, Slama et al. 1981). Experiments with 4*R*-NADH-4-*d*, 4*S*-NADH-4-*d*, and NADH-4,4-d_2 revealed that the *pro-R* hydrogen at the 4 position of NADH transfers onto the isoalloxazine ring in preference to the *pro-S* hydrogen in the oxidation of NADH with flavopapain, **46**. The ratio of the rates for the transfer was calculated to be 7. It was also found that flavopapain, **47**, can discern some kind of chirality in a substrate (Slama et al. 1984). Kinetic parameters (k_{cat}/K_m values) for the oxidations of enantiomeric 1,4-dihydronicotinamide derivatives that contain chiral α-methylbenzyl groups on their carbamoyl side chains (BNPH or PNPH) were different from each other by a factor of approximately 2. On the other hand, discrimination of chirality by **47** was not detected for the oxidation of a chiral 1,4-dihydronicotinamide whose asymmetric carbon is in the α-methylbenzyl group on the nitrogen at the 1 position (MBNAH).



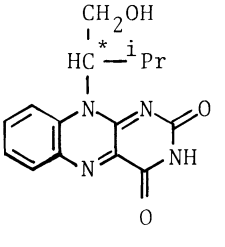
46



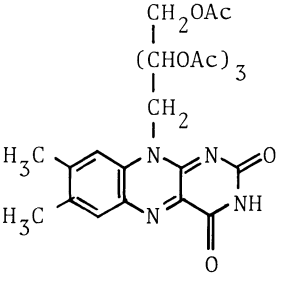
47



MBNAH



48



49

Shinkai et al. (1984) measured the rates for hydrogen transfers from PNPH and MBNAH to isoalloxazine derivatives such as **48** and tetra(O-acetyl)riboflavin (**49**). A difference in the rate constant due to the chirality on PNPH and **48** was observed in the presence of a large excess of magnesium perchlorate in acetonitrile solution, as shown in Table 28. Such a difference was not observed when the reaction was carried out in aqueous solution, with low concentration of Mg(II) in acetonitrile, nor in the absence of Mg(II). Thus, in this system, Mg(II) is important as a bridge between the two reactants at the transition state of asymmetric hydrogen transfer.

Table 28. Reduction of **48** or **49** with NADH model compounds.[a]

Flavin	Additive (mM)	k_R/k_S for PNPH[b]	k_R/k_S for MBNAH[c]
48	$Mg(ClO_4)_2$ (0.2)	1.03	0.92
48	$Mg(ClO_4)_2$ (100)	1.09	0.79
49	$Mg(ClO_4)_2$ (0.2)	1.03	0.98
49	$Mg(ClO_4)_2$ (100)	1.91	1.33
49	Bu_4NBr (100)	0.96	

[a] Reaction was carried out in acetonitrile at 30°C.

[b] Ratio of the second-order rate constant for the reduction with *R*--PNPH to that with *S*-PNPH.

[c] Ratio of the second-order rate constant for the reduction with *R*--MBNAH to that with *S*-MBNAH.

10.4 Asymmetric Reduction by a Model of Flavin Coenzyme

Isoalloxazine derivatives are used as model compounds of flavin. The reduced form of flavin has the ability to reduce certain substrates such as quinones, dyes, aromatic nitro compounds, and activated carbonyl compounds. Model studies are mainly focused on the mechanism of reduction and no asymmetric reduction with an isoalloxazine derivative has been reported so far. One attempt was made at the reduction by a 5-deazaflavin derivative. Successful results were obtained in oxidation of alcohols and amines by 5-deazaflavin as an excellent oxidizing agent (Yoneda and Sakuma 1977, Yoneda et al. 1979). It was noted, in this system, that the reduced form of 5-deazaflavin was slowly reoxidized by molecular oxygen (Yoneda et al. 1981a). Therefore, the reagent can be used catalytically. Since this property is similar to that observed in the isoalloxazine system, 5-deazaflavin derivatives can be regarded as satisfactory model compounds for flavin. At the same time, the structure at the middle ring of 5-deazaflavin also resembles the 1,4-dihydronicotinamide moiety in NAD(P)H. Thus, they can also be considered as model compounds for NAD(P)H. Indeed, reductions of ketones and aldehydes by the dihydro form of the 5-deazaflavin have been reported (Yoneda et al. 1978, 1981b).

An attempt at asymmetric reduction was made using 1,5-dihydro-5-

-deazaflavin. Tanaka et al. (1984) synthesized a 5-deazaflavin derivative which has a chiral α-methylbenzyl group on the nitrogen at the 3 position (**50**), and the compound was converted into the reduced form (**51**) by sodium borohydride. Table 29 lists the result from the asymmetric reduction of ethyl benzoylformate (EBF) into ethyl mandelate (EM) by this compound.

It is interesting to remember that ethyl *R*-mandelate was produced in 20 % e.e. in the reduction of ethyl benzoylformate with *R*-PNPH, whereas *R*-**37** afforded ethyl *S*-mandelate predominantly. The structures of the two reductants are similar but the stereochemical properties are quite different from each other, which indicates that the situation at the transition state of the 1,5-dihydro-5-deazaflavin system is different from that of PNPH. This is perhaps because of the cyclic structure of **51** and of the presence of several heteroatoms in the molecule. A magnesium ion may be able to interact with these heteroatoms.

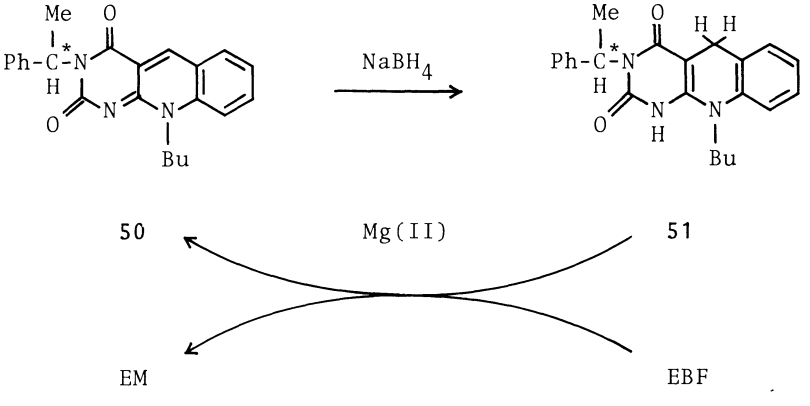


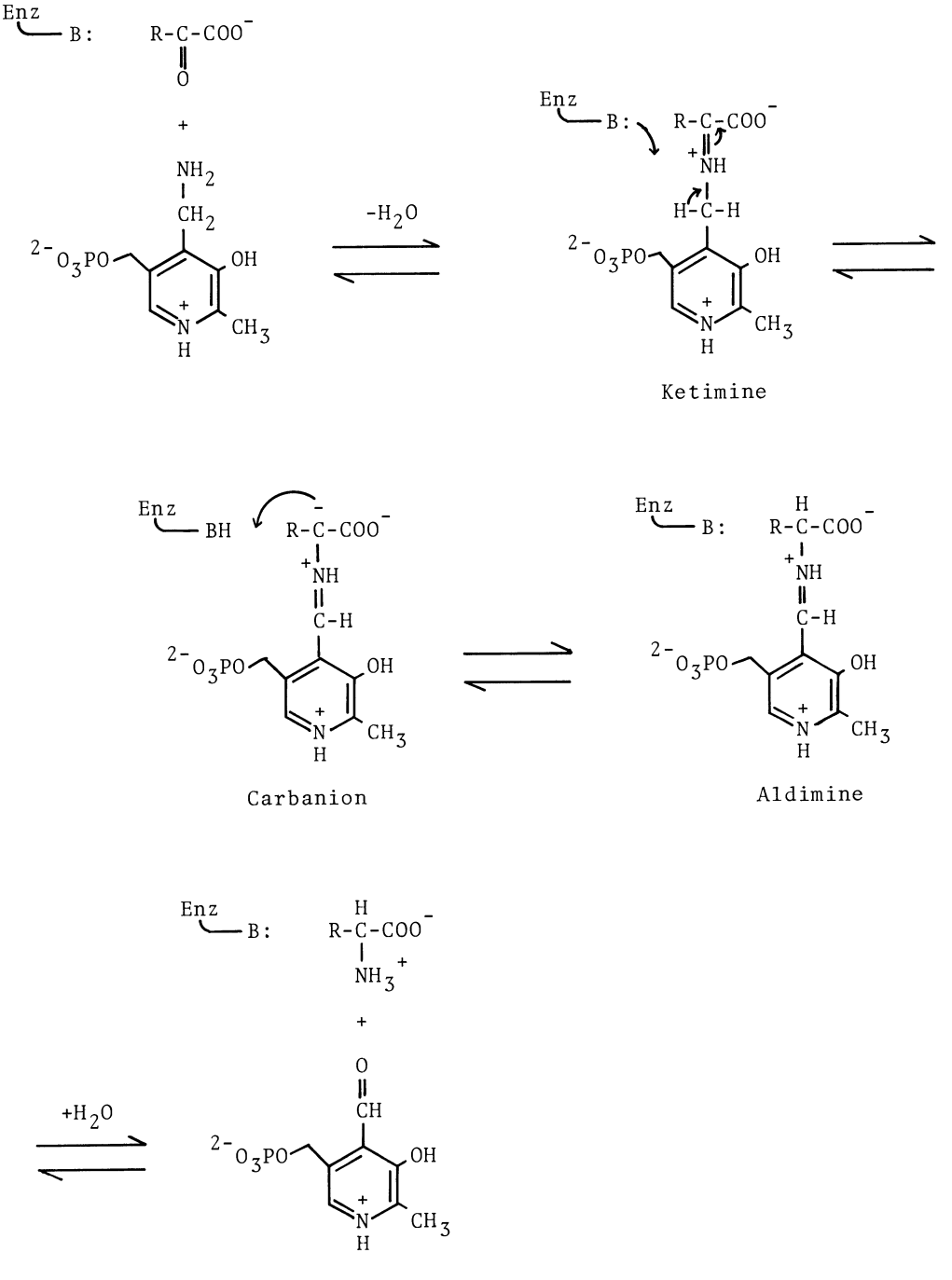
Table 29. Asymmetric reduction of ethyl benzoylformate with **51**.

1,5-Dihydro-5-deazaflavin	Optical yield [%]	Configuration
R-**51**	14	*S*
S-**51**	21	*R*

11 ASYMMETRIC SYNTHESIS OF α-AMINO ACIDS

It has been emphasized that the most important factor determining the efficiency of asymmetric induction by a chiral NAD(P)H-model compound is the effectiveness in discriminating between the *re*- and *si*-faces of the dihydropyridine ring. A similar effect also operates in the stereochemistry of transamination mediated by pyridoxamine, vitamin B_6.

Scheme 30


Enz
B:
R-C-COO⁻
O
NH2
CH2
2- O3PO
OH
N
H
CH3
-H2O
NH
H-C-H
Ketimine
BH
C-H
Carbanion
Aldimine
H
NH3 +
+H2O
CH

In vitamin B_6-dependent transamination, the process in which a Schiff base (ketimine) formed from a keto acid and pyridoxamine rearranges into the corresponding aldimine is the key step controlling the stereochemistry. The configuration of the product is determined by the enantioface chosen by the proton which is going to react with the intermediate carbanion (Scheme 30).

In an enzymatic system, one of the amino acid residues in the active site of the protein acts as a base to abstract a proton from the ketimine, which then behaves as an acid to protonate onto the carbanion center. The net migration of the proton takes place within the same face of the coenzyme. The single-base mechanism operating here is important to keep the stereospecificity of the reaction. Of course, specific steric blocking of one of the two faces of the coenzyme by a wall of active site cleft is the most important factor of all.

A mimetic reaction has been developed extensively and is known to proceed with the aid of a metal ion such as Zn(II). As has been achieved in the stereoselective reductions by NAD(P)H-models in which one of enantiofaces is frozen, freezing of a face of the pyridine ring in pyridoxal or its model compound will result in the stereoselective mimetic transamination. Kuzuhara and his co-workers have synthesized pyridoxamine analogues that have *planar* chirality and have succeeded in observing asymmetric transamination (Iwata et al. 1976, Kuzuhara et al. 1977, 1978, Sakurai et al. 1979, Ando et al. 1982, Tachibana et al. 1982a,b, Breslow et al. 1980). The reaction is shown in Scheme 31 and the results are summarized in Table 30.

Scheme 31

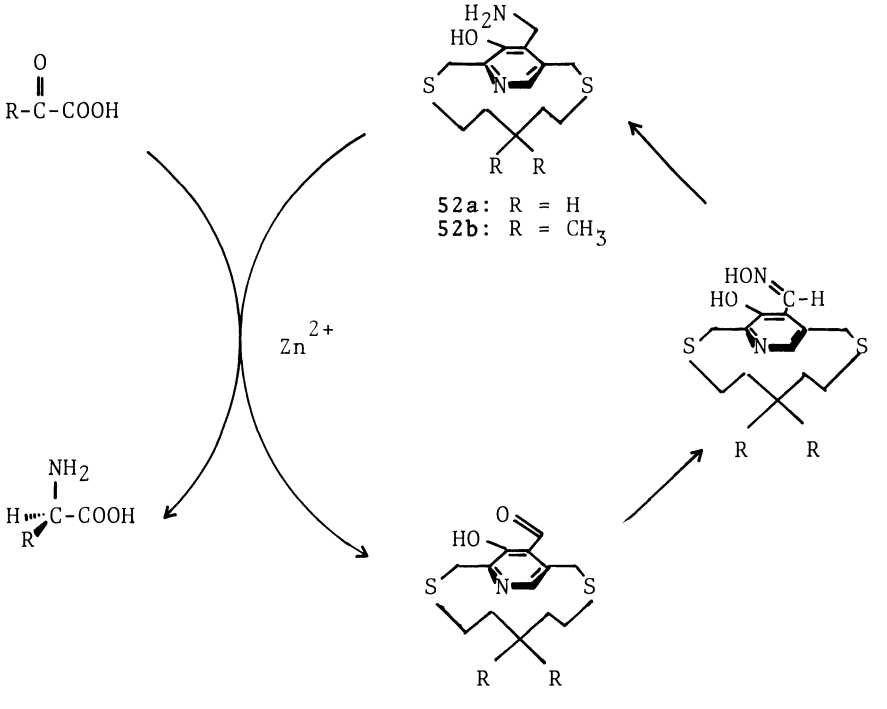


Table 30. Asymmetric syntheses of chiral α-amino acids.

R	Ana-logue	[Zn(II)]/ [52] Ratio	Solvent	Chemical yield [%]	e.e. [%]	Config. of amino acid
$(CH_3)_2CHCH_2$	*S*-52b	1/1	MeOH	65	73	*R*
	R-52b	0.5/1	MeOH	68	96	*S*
	R-52b	1/1	MeCN	33	64	*S*
	S-52a	1/1	MeOH	65	77	*R*
	S-52a	1/1	BuOH	61	43	*R*
	S-52a	1/1	MeCN	51	43	*R*
$(CH_3)_2CH$	*S*-52b	1/1	MeOH	75	52	*R*
	R-52b	0.5/1	MeOH	57	79	*S*
CH_3	*R*-52b	1/1	MeOH	75	47	*S*
	R-52b	0.5/1	MeOH	72	69	*S*
$PhCH_2$	*R*-52b	0.5/1	MeOH	60	61	*S*
	S-52b	1/1	MeOH	75	23	*R*
	S-52b	1/1	BuOH	61	12	*S*

Better yield and higher e.e. are obtained when the [Zn(II)]/[52] ratio is 0.5 than when it is 1. It is quite likely that a complex composed of two molecules of 52 and one zinc ion is formed and the open side of this complex is provided for the attack by the proton (Kuzuhara 1983).

12 RECENT PROGRESS IN ASYMMETRIC REACTIONS MEDIATED BY AN ENZYME

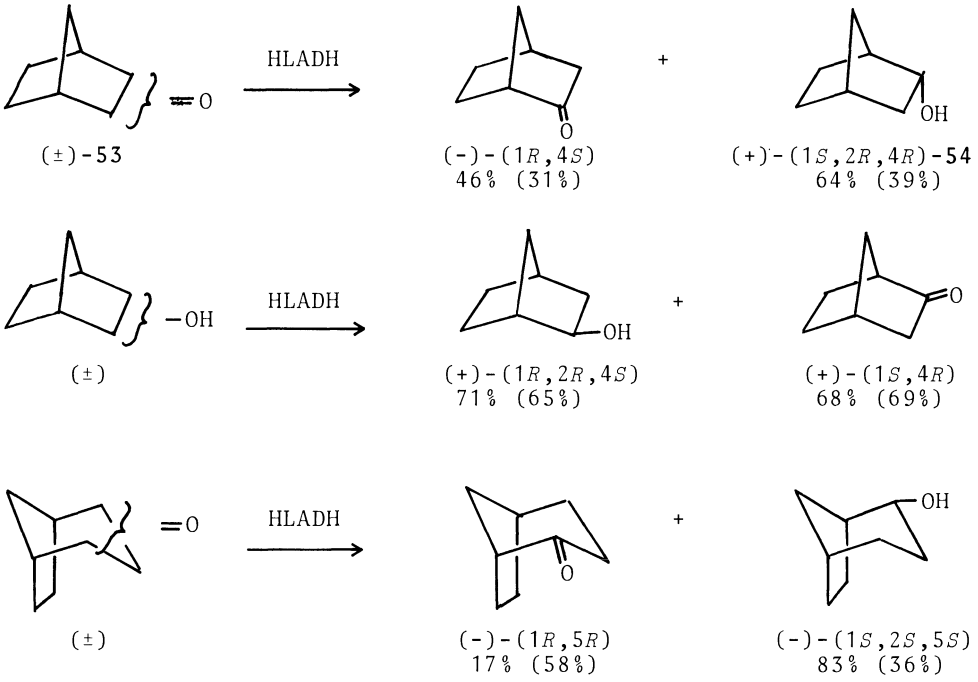
At the end of this review, it is worthwhile surveying asymmetric organic reactions mediated by an enzyme in which a biological material is used as one of the chemical reagents for an organic reaction. Oxidoreduction mediated by an microorganism was reviewed recently by Sih and Chen (1984) and the reader is recommended to consult that review for this particular topic.

Although, as has been discussed, mimetic chemistry has succeeded in constructing reaction systems that result in excellent stereoselectivity, the stereoselectivity and versatility of substrate exerted by an enzyme is still invaluable and makes it worthwhile to employ them for organic syntheses. At the same time, however, it is also true that the stereoselectivity for artificial substrates is not always satisfactory even in enzymatic reactions. For example, reduction of acyclic alkanones with a dehydrogenase is generally associated with low stereospecificity. Some substrates are eaten by the enzyme only with great difficulty. Thus, in order to use an enzyme as a chemical reagent, we have to have accumulated data to suggest what kind of substrate is converted into the desired product, what kind of effect defines the stereochemical course of the reaction predominantly, how good the stereoselectivity of the reaction is, etc.

Jones and his collaborators have studied the reduction of cyclic ketones with horse liver alcohol dehydrogenase (HLADH) extensively. This enzyme is commercially available and suitable for testing the utility in organic syntheses. When a racemic mixture of bicyclo-

[2.2.1]heptan-2-one (53) is employed as a substrate, (+)-(1*S*,4*R*)-**53** is reduced faster than the other isomer to give (+)-(1*S*,2*R*,4*R*)-**54** in 64 % optical yield. Results are summarized in Scheme 32 together with those from other interconversions (Irwin and Jones 1976).

Scheme 32[a]



[a] Percentages outside and inside the parentheses are opical and chemical yields, respectively.

Tables 31 and 32 summarize the results from the reduction of oxygen- or sulfur-containing heterocyclic ketones with HLADH (Davis and Jones 1979, Haslegrave and Jones 1982, Takemura and Jones 1983). The reduction of thianone is especially interesting because the sulfur atom is easily removed, after the reduction, by desulfurization to give the corresponding acyclic alcohol, which otherwise cannot be obtained with satisfactory stereospecificity.

It was known that HLADH oxidizes glycerol into L-glyceraldehyde stereospecifically (Hadorn et al. 1963, Bally and Leuthardt 1970). Since the stereospecific oxidation of a primary diol will afford a lactone, the synthetic utility of this oxidation is quite high. Indeed, six-membered lactones were obtained in high optical yields on oxidations of 3-substituted pentan-1,5-diol as shown in Scheme 33 (Irwin and Jones 1977a). Oxidations of diols mediated by HLADH have been further studied extensively by Jones and his collaborators. A part of a group of results is listed in Table 33 (Irwin and Jones 1977b, Jakovac et al. 1982, Ng et al. 1984, Jones and Francis 1984).

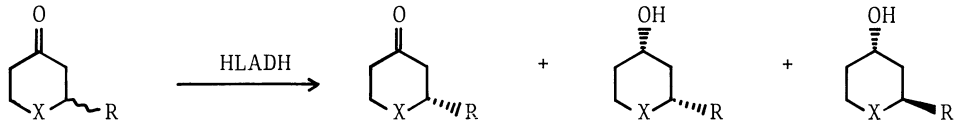
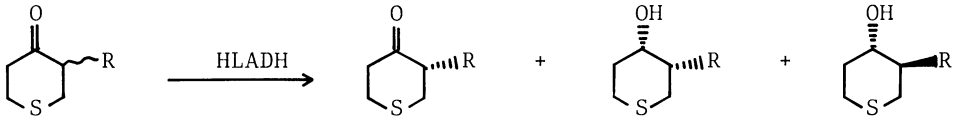


Table 31. Reduction of heterocyclic ketones with HLADH.

R	X	e.e. [%] (Chemical yield [%]) 55	56	57
CH_3	O	85 (31)	100 (33)	36 (2)
CH_3CH_2	O	88 (32)	100 (36)	83 (3)
$(CH_3)_2CH$	O	86 (41)	100 (32)	28 (2)
Ph	O	51 (44)	100 (35)	100 (8)
CH_3	S	36 (35)	100 (11)	100 (29)
CH_3CH_2	S	45 (34)	100 (24)	100 (22)
$(CH_3)_2CH$	S	39 (35)	100 (11)	100 (29)
Ph	S	9 (60)	100 (24)	100 (16)



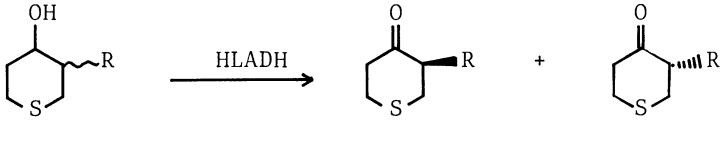
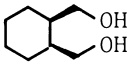
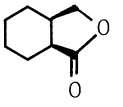
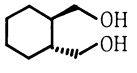
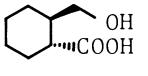
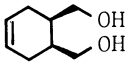
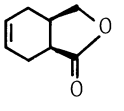
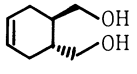
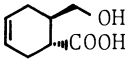
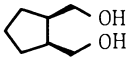
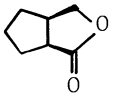
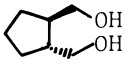
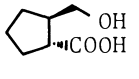
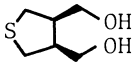
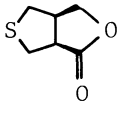
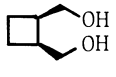
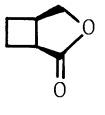
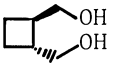
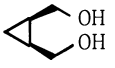
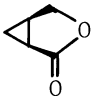
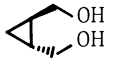
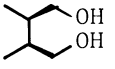
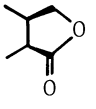
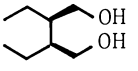
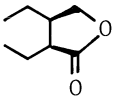
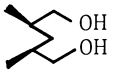
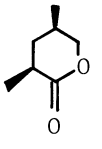
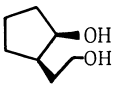
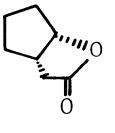
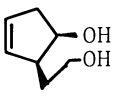
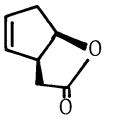
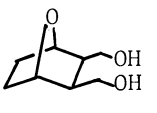
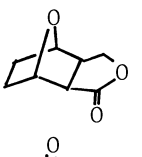
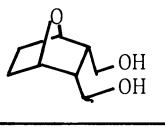
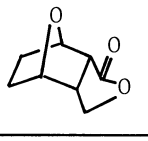


Table 32. Reduction and oxidation of 2-substituted thianones with HLADH.

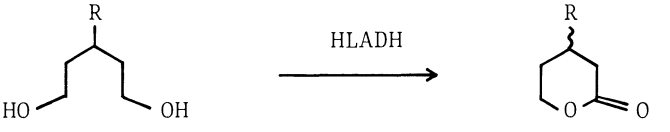
R	e.e. [%] (Chemical yield [%])				
	58	**59**	**60**	**61**	**62**
CH_3	66 (19)	60 (2)	90 (49)	20 (33)	85 (34)
CH_3CH_2	58 (32)	93 (3)	78 (47)	15 (29)	65 (38)

Table 33. Oxidation of diols mediated by HLADH.

Substrate	Product	Chemical yield [%]	e.e. [%]
		79	100
		30	8
		80	100
		50	<1
		72	100
		23	2
		81	100
		90	100

		28	2
		71	100
		44	2
		15	100
		56	100
		39	100
		90	<1
		48	49
		83	>98
		37	>98

Scheme 33



R	Optical yield [%]
CH_3	90
$(CH_3)_2CH$	25
Ph	21

Not only stereospecificity but also reactivity differs widely from one substrate to another. For example, the relative rate for the reductions of cyclohexanone, **53**, and camphor is 100 : 26 : ∿0 (Irwin and Jones 1976). After accumulation of knowledge on HLADH-mediated reactions, it was recognized that the diamond-lattice theory proposed by Prelog and illustrated in Fig. 2 was unsatisfactory in predicting the steric course and reactivity for a variety of substrate structures. Jones and Jakovac (1982), therefore, proposed a new concept (cubic-space model) as a substitute for the diamond-lattice theory. According to their proposal, the active site of HLADH is divided into 56 cubes, each, of 1.3 Å edges each. As shown in Fig. 20, the front faces of each cube are labeled alphabetically and the side faces are named numerically. In this way, each cube can be specified unambiguously by an alpha-numerical symbol such as K3. The 1.3 Å edge-length scale is suited to analyzing substrate conformations built up largely of tetrahedral carbon frameworks.

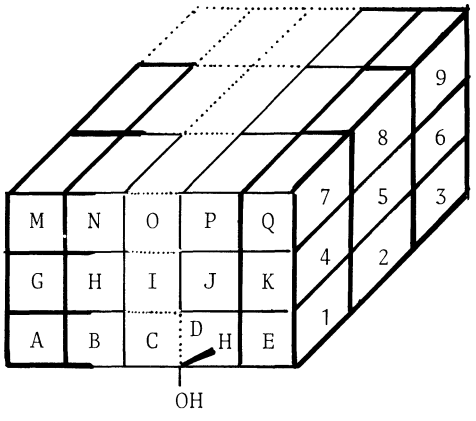


Fig. 20. Cubic-space model of the active site of HLADH. Cubes indicated by bold lines are those in prohibited regions and cubes indicated by solid lines are those in limited regions.

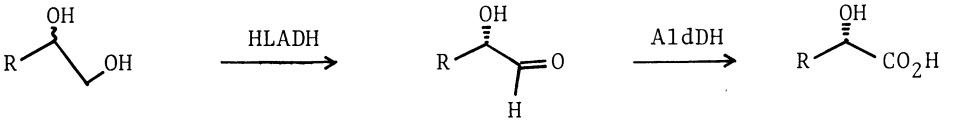
The X-ray data reveals that the A-G-M region of the cubic space is occupied by Ser-48 and Leu-57, whereas the EF-KL-QR region is occupied by Phe-93, Phe-110, Ile-116, and Ile-318. Therefore, A1, A2, G4, G5, M7, M8, E1, E2, E3, F1, F2, F3, K4, K5, K6, L4, L5, L6, Q7, Q8, Q9, R7, R8, and R9 cubes are completely forbidden for substrate occupation. The left-hand halves of B1, H4, and N7 are also a forbidden region. J4, P7, P8, and P9 and right-hand halves of B1, H4, and N7 are limitedly forbidden. Other regions are allowed and substrate binding is freely permitted. This open region corresponds to the hydrophobic pocket of the active site. The carbonyl or hydroxyl group is set downward at the border of C1 and D1 and the hydrogen comes or leaves from the front side of the cube.

With this cubic-space model, the stereochemical course of the reaction can be predicted as follows:
(1) Since the rear of the cubic space remains open and J4 and P7 are limitedly allowed locations, a large substrate will preferentially bind with the enzyme in a horizontal rather than vertical mode.
(2) An atom in a substrate cannot enter any forbidden regions, however, it may occupy a limited region, if necessary.
(3) When a limited region is occupied by a group of the substrate, a slower rate of oxidoreduction is anticipated and the effect of rate-reduction is cumulative. If two or more limited regions are occupied by a substrate, it is virtually equivalent to a forbidden interaction and essentially no reaction can be expected.
(4) Stereoisomers should be analyzed independently.
(5) When a substrate is conformationally mobile, then each major conformer should be considered independently.
(6) Of course, the stereochemical course predicted by this cubic-space model is that controlled by kinetics. The stereochemistry of a thermodynamically controlled product cannot be predicted with this model.

The cubic-space model has been used by Jones and his collaborators to analyze the reactivity and stereospecificity of many redox reactions and has proved to be quite useful.

Wong and Matos (1985) reported a stereospecific oxidation of 1,2-diols to α-hydroxy carboxylic acids *via* α-hydroxy aldehydes. The trick employed here is to coimmobilize HLADH and aldehyde dehydrogenase (AldDH) into polyacrylamide. The former enzyme oxidizes the diol to the corresponding α-hydroxy aldehyde, which is further oxidized to the α-hydroxy carboxylic acid by the latter enzyme (Scheme 34). After 40 % conversion of the substrates, enantiomer excesses in the products were found to be almost 100 %. However, a hydrophobic R group gave a relatively unsatisfactory result.

Scheme 34



R = $HOCH_2$, FCH_2, $ClCH_2$, $BrCH_2$, CH_3, C_2H_5, $(CH_3)_2CH$

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