Coenzyme Models. VIII. Reduction of the Schiff Base of Pyridoxal Analogs by 1,4-Dihydronicotinamide*

Seiji SHINKAI, Sumihiro SHIRAISHI, and Toyoki KUNITAKE

Department of Organic Synthesis, Faculty of Engineering, Kyushu University, Fukuoka, 812

(Received July 26, 1976)

Reduction of Schiff bases by 1-benzyl-1,4-dihydronicotinamide (BzlNH) was performed in refluxing methanol. Protonated imines such as *N*-(*p*-nitrobenzylidene)benzylamine hydrochloride (**1H**$^+$) and *N*-(4-nitrosalicylidene)-benzylamine hydrochloride (**1(OH)H**$^+$) were reduced to the corresponding aminomethyl derivatives. The NMR product analysis for the reaction of BzlNH and **1H**$^+$ in refluxing ethanol-*d* established that the solvent deuteron was not incorporated into the aminomethylene product. **1(OH)**, unprotonated species, was partially reducible, whereas **1** was totally unreactive. The facile reduction of **1** occurred in the presence of several amine hydrochlorides (NEt_3·HCl, imidazole hydrochloride, *N,N,N',N'*-tetramethylethylenediamine dihydrochloride). These results show that the *ortho* hydroxyl group of **1(OH)** facilitates imine reduction not only by polarization of the Schiff base *via* hydrogen bonding but also by proton transfer. The relative reactivity of the Schiff base to the BzlNH reduction is: **1H**$^+$, **1**+NEt_3·HCl≧**1(OH)H**$^+$>**1(OH)**>**1**(unreactive) This order is similar to that found in "*trans*-Schiffization" between semicarbazide and the Schiff base of pyridoxal and its analogs, as reported by Cordes and Jencks.

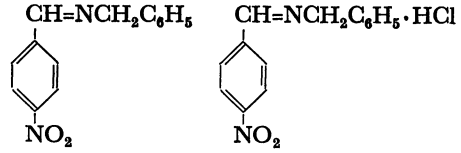
The nicotinamide adenine dinucleotide and its reduced form (NAD and NADH) serve as coenzymes in a very large number of enzymatic oxidation-reduction reactions.[1] As for example, NAD-dependent dehydrogenases catalyze the stereospecific, direct transfer of a hydride ion (or its equivalent) to aldehyde substrates.[2] Steffens and Chipman[3] and Creighton *et al.*[4] proposed a kinetically detectable complex as an intermediate along the reaction path in model systems. Contrary to this proposition, Kurz and Frieden[5] concluded that the enzyme-substrate transition state is analogous to that pictured for simple S_N2 nucleophilic substitution with "hydride" ion acting as the nucleophilic agent. In any case, it is clear that substantial charge transfer from NADH to substrate occurs in the rate-determining step, and that substrates are significantly activated by electron-withdrawing substituents.

The enzymatic reduction of pyridoxal-5'-phosphate has been investigated in detail.[6,7] Recently, it was reported that the nonenzymatic reduction of pyridoxal and its analogs by 1,4-dihydropyridines did occur at ambient conditions and that the *ortho*-hydroxyl group facilitated the "hydride" acceptability of the carbonyl group.[8-10] Intrestingly, the reduction rate for the respective pyridoxal species was parallel to the rates of other reactions which were catalyzed by these pyridoxals; imine formation, general base-catalyzed *trans*-amination, *etc.*[10]

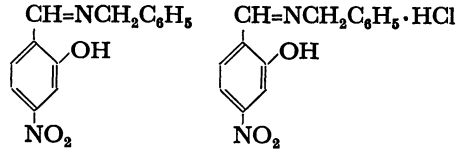
Imines show higher affinities toward the carbonyl agents than the corresponding carbonyl compounds.[11,12] A typical example would be a "*trans*-Schiffization" reaction demonstrated by Cordes and Jencks.[12] According to their detailed examination, the Schiff base is inherently more reactive than pyridoxal-5'-phosphate toward semicarbazide, and the protonated Schiff base is further activated. These results suggest that protonated imines (iminium salts) would be suitable substrates for the reduction by NADH. In fact, the enzymatic reduction of the iminium group of N^5,N^{10}-methenyl-tetrahydrofolic acid to N^5,N^{10}-methylene-tetrahydrofolic acid by NADPH,[13] and the nonenzymatic reduction of a thiazolium salt (Vitamin B_1 analogue)[14] and the iminium group of steroid derivatives to the corresponding amine[15] have been reported.

The object of the present investigation is to examine the reaction of 1-benzyl-1,4-dihydronicotinamide (BzlNH) with the Schiff base of pyridoxal analogs, *p*-nitrobenzaldehyde and 4-nitrosalicylaldehyde, and to establish the relative susceptibility of these Schiff bases toward the "hydride" reduction. In order to avoid the complication which arises from the reversible imine formation, we isolated Schiff bases, *p*-nitrobenzylidene-benzylamine (**1**), *N*-(4-nitrosalicylidene)benzylamine (**1 (OH)**), and their hydrochlorides (**1H**$^+$ and **1(OH)H**$^+$, respectively), and carried out the reaction in refluxing anhydrous methanol.

Schiff base:

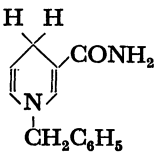


1 **1 H**$^+$



1 (OH) **1 (OH)H**$^+$

BzlNH:



Experimental

Materials. 4-Nitrosalicylaldehyde was prepared according to the method of Tsumaki *et al.*,[16] and was identified by the NMR and IR (KBr disc) methods, and by the elemental analysis. Mp 134—135 °C (lit,[16] 133—134 °C. Found: C, 50.43; H, 3.02; N, 8.40%. Calcd for $C_7H_5O_4N$: C, 50.31; H, 3.02; N, 8.38%.

* Contribution No. 404 from this Department.

The preparation of Schiff bases was conducted according to the Matsuo's method.[17] Equimolar amounts of aldehyde and benzylamine were refluxed in anhydrous methanol for 1 h. The solvent was evaporated *in vacuo*, and the residual yellow solid was recrystallized from benzene and petroleum ether. The products were identified by NMR and IR spectroscopies and elemental analysis. Elemental analysis for **1** (**OH**), Found: C, 65.48; H, 4.70; N, 10.92%. Calcd for $C_{14}H_{12}N_2O_3$: C, 65.61; H, 4.73; N, 10.93%. Other results are summarized in Table 1.

Table 1. Characterization of Schiff bases and their hydrochloride salts

	Mp (°C)	IR (KBr) $\nu_{C=N}$ (cm^{-1})	NMR ($CDCl_3$) N-CH_2- (ppm)	N=CH-
1	54—55	1635	4.84	8.35
1H$^+$	105—110			
1(OH)	88—89	1630	4.81	8.45
1(OH)H$^+$	176—179	1660		

The Schiff base was dissolved in anhydrous ether, and dry hydrogen chloride gas was introduced into the solution. White needles precipitated were collected, and recrystallized from anhydrous methanol. The analytical results are also recorded in Table 1.

N-Benzyl-4-nitro-2-hydroxybenzylamine hydrochloride (**2(OH)H**$^+$), the product expected for the BzlNH reduction of **1(OH)H**$^+$, was prepared by the sodium tetrahydroborate reduction of **1(OH)**. A solution of **1(OH)** (0.40 g, 1.6 mmoles) in 20 ml of methanol was stirred at 0—5 °C and a solution of sodium tetrahydroborate (0.06 g, 1.6 mmoles) in 10 ml of methanol was added from a dropping funnel. Subsequently, the reaction mixture was heated at 45 °C on a steam bath for 6.5 h. The solvent was evaporated *in vacuo*, the residual brown solid being extracted with water and ether. The ether layer was separated, and evaporated to dryness. The solid was again taken in anhydrous ether, from which (**2(OH)H**$^+$) was recovered by introducing dry hydrogen chloride gas. Recrystallization of the resulting precipitates from methanol–ether gave colorless powders; yield 21%, mp 210—213 °C. IR (KBr): 1525, 1340 cm^{-1} (nitro). NMR (Me_2SO-d_6): benzyl-CH_2, 4.13, 4.20 ppm, 4H; benzene rings, 7.3—7.8 ppm, 8H. Found: C, 56.93; H, 5.12; N, 9.43%. Calcd for $C_{14}H_{14}N_2O_3 \cdot HCl$: C, 57.05; H, 5.13; N, 9.45%.

The preparation of 1-benzyl-1,4-dihydronicotinamide (BzlNH) was described elsewhere.[18]

Reaction of Schiff Base and BzlNH. All the reactions were carried out in refluxing anhydrous methanol. A typical example for the reaction of **1H**$^+$ and BzlNH is stated below. A solution of **1H**$^+$ (0.64 g, 2.3 mmoles) and BzlNH (0.72 g, 3.4 mmol) in 20 ml of anhydrous methanol was refluxed for 9.5 h. The solvent was evaporated *in vacuo*, and residual oil was extracted with ether and water (pH 9 with *ca.* 0.05 M borate and 1 mM triethylamine hydrochloride). The ether layer was evaporated to dryness, again taken in ether, and dry hydrogen chloride gas was introduced. Recrystallization of the light yellow precipitates from methanol–ether gave almost colorless powders; yield 19%, mp 210—220 °C. The product was identified to be *N*-benzyl-*p*-nitrobenzylamine hydrochloride (**2H**$^+$). IR (KBr): 1510, 1350 cm^{-1} (nitro). NMR (Me_2SO-d_6): benzyl-CH_2, 4.17, 4.30 ppm, 4H; benzene ring, 7.3—7.6 ppm, 5H; *o*-proton of benzene ring, 7.87 ppm, 2H; *m*-proton of benzene ring, 8.25 ppm, 2H. Found: C, 59.55; H, 5.40; N, 9.78%. Calcd for $C_{14}H_{14}N_2O_2 \cdot HCl$: C, 60.33; H, 5.42; N, 10.05%.

Product Analysis. NMR was recorded by using a Varian A-60 instrument. The purity of the recovered products was examined by a Hitachi High Speed Liquid Chromatograph, Type 635 (Hitachi Gel 3018, methanol). UV absorption spectra were taken by a Hitachi 124 spectrophotometer at 30 °C.

Results

Spectral Study of the Reaction. The decrease in the absorption of BzlNH in refluxing methanol was followed at 354 nm (λ_{max} of BzlNH) in the absence and the presence of the Schiff base, **1** or **1(OH)** (Fig. 1).

Aliquots (3 ml) of the reaction mixture were withdrawn at appropriate intervals and scanned in the UV range at 30 °C. Figure 2 shows that the absorption of BzlNH decreases in the presence of **1(OH)**. In contrast, no significant spectral change was observed in the presence of **1** and in the absence of the Schiff base. The absorbance decrease at 354 nm in the presence of **1(OH)** was accompained by the increase in the absorption band at 250—280 nm and at 400—440 nm, but no significant increase occurred near 290 nm (Fig. 1). If the acid-catalyzed decomposition of 1,4-dihydronicotinamide occur, the product (1,4,5,6-tetrahydronicotinamide derivative) would give rise to a new, strong absorption at around 290 nm.[18-20] Therefore, the decrease in the BzlNH absorbance may be attributed to the disappearance of BzlNH due to the hydrogen transfer reaction. Apparently, the *ortho*-hydroxyl group facilitates the reaction, as in the pyridoxal-catalyzed reactions.

Reaction of BzlNH with Schiff Bases and Their Hydrochloride Salts. Introduction of hydrogen chloride gas to an ether solution obtained from the reaction of

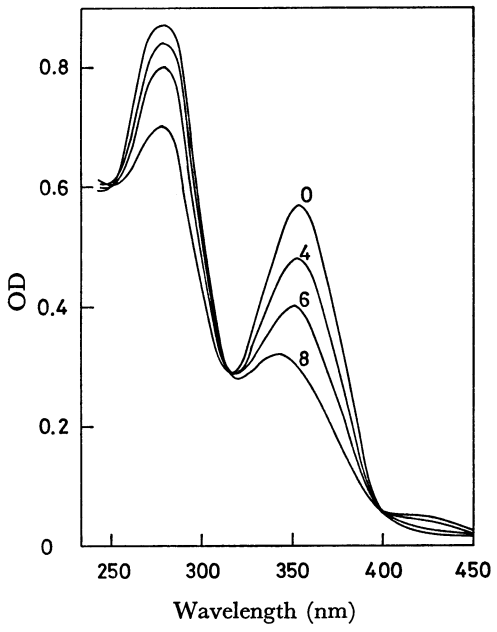


Fig. 1. Spectral changes in the reaction of 1-benzyl-1,4-dihydronicotinamide (BzlNH: 5.65×10^{-5} M) with 4-nitrosalicylidene-benzylamine (**1(OH)**: 5.34×10^{-5} M) in refluxing methanol. Numbers indicate the reaction time (h).

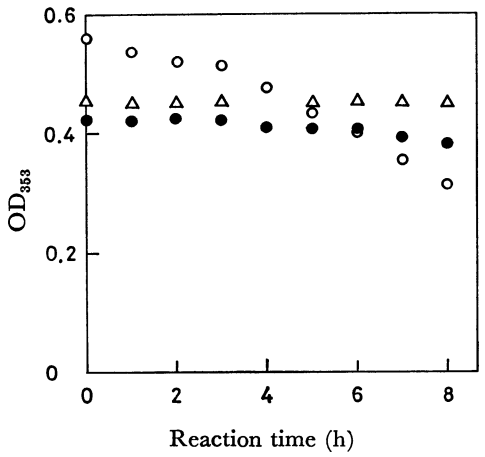


Fig. 2. Time dependence of the absorbance of BzlNH (353 nm) in the presence and the absence of the Schiff base:
○: [BzlNH]=5.65×10⁻⁵ M, [**1**(**OH**)]=5.34×10⁻⁵ M; ●: [BzlNH]=5.65×10⁻⁵ M, [**1**]=5.50×10⁻⁵ M; △: [BzlNH]=5.65×10⁻⁵ M.

1(**OH**) and BzlNH gave oily products. A TLC study (silica gel, ethyl acetate) showed that the oil is a mixture of *N*-benzyl-4-nitro-2-hydroxybenzylamine hydrochloride, **2**(**OH**)**H**$^+$ (R_f=0.64—0.65), **1**(**OH**)**H**$^+$ (R_f=0.40—0.42), and BzlNH or 1-benzylnicotinamide (R_f=0—0.23). The product analysis by liquid chromatograph (Table 2) established that it contained 38 mol% of **2**(**OH**)**H**$^+$ and 62 mol% of **1**(**OH**)**H**$^+$ (Table 2; the amount of BzlNH could not be determined because its retention time was too long).

On the other hand, the product obtained from the reaction of **1** and BzlNH displayed a broad melting point (135—156 °C), and was identified to be a mixture of benzylamine hydrochloride and BzlNH by liquid chromatography. These results, together with the spectral evidence, are explained by reaction schemes 1 and 2.

Table 2. Product analysis of the reaction of BzlNH and schiff bases

Schiff base	Recovered products[a] (%)				Yield of the reduced product (%)[c]
	$PhCH_2NH_2$·HCl	**2**H$^+$	**1**(**OH**)**H**$^+$	**2**(**OH**)**H**$^+$	
1	100	0			0
1+NEt_3·HCl[b]	0	100			26
1+Imidazole·HCl[b]	0	100			15
1+$(Me_2NCH_2)_2$·2HCl[b]	0	100			13
1H$^+$	0	100			19
1(**OH**)			62	38	
1(**OH**)+NEt_3·HCl[b]			79	21	
1(**OH**)**H**$^+$			7	93	8

a) Semi-quantitative analyses were performed by using high speed liquid chromatography: 260 nm for **2H**$^+$ and 280 nm for **1**(**OH**)**H**$^+$ (ε=18180) and **2**(**OH**)**H**$^+$ (ε=5980). b) [Amine hydrochloride]=*ca.* 10 [Schiff base]. c) Determined after recrystallization for **2H**$^+$. The low yields are due to the loss in the extraction with ether, products being poorly soluble in ether.

The hydrochloride of Schiff bases was more easily reduced by BzlNH. **1**(**OH**)**H**$^+$ was converted almost exclusively to **2**(**OH**)**H**$^+$ (93%, Table 2). Interestingly, **1H**$^+$ was also susceptible to the BzlNH reduction, though the free Schiff base was totally unreactive. The product showed a single spot (R_f=0.71) on TLC, and was confirmed to be pure *N*-benzyl-*p*-nitrobenzylamine hydrochloride, **2H**$^+$ (see Experimental). Thus, the role of the *ortho*-hydroxyl group becomes less important if the Schiff base is activated by protonation.

In order to prove direct hydrogen transfer from BzlNH to the Schiff base, the reaction of BzlNH and **1H**$^+$ was performed in ethanol-*d* (99%). The recovered **2H**$^+$ (24% yield, mp 207—222 °C) again showed a single spot on TLC. As described in Experimental, the NMR spectrum of **2H**$^+$ obtained in non-deuterated ethanol possessed the peaks of the methylene hydrogen at 4.17 and 4.30 ppm (δ) with 1:1 intensity. The NMR spectrum of **2H**$^+$ obtained in ethanol-*d* possessed similar methylene peaks. These results prove that no deuterium was incorporated to the 1-methylene group in the reaction in the deuterated medium. Therefore, the reduction of the Schiff base must occur *via* direct hydrogen transfer as in some enzymatic reductions.[2]

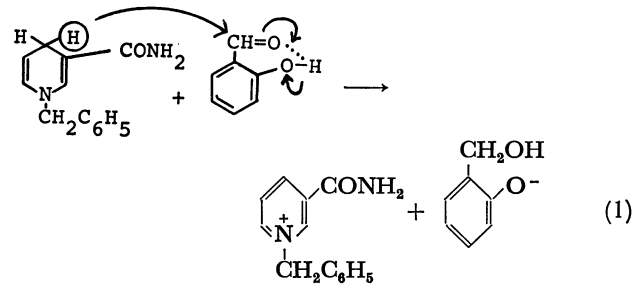
Reaction in the Presence of Amine Hydrochlorides. The susceptibility of the Schiff base to the BzlNH reduction was evaluated in the presence of amine hydrochlorides such as triethylamine hydrochloride. The reduction of **1**(**OH**) was hardly affected by the addition of triethylamine hydrochloride (10-fold of **1**(**OH**); Table 2). In contrast, **1** was readily converted to **2H**$^+$ in the presence of triethylamine hydrochloride in 23% yield. Other hydrochlorides similarly facilitated the reduction of **1**. Therefore, these hydrochlorides may replace the role played by the *ortho*-hydroxyl group of **1**(**OH**).

Discussion

Until recently, the nonenzymatic reduction by 1,4-dihydropyridines has been limited to highly electron-deficient carbonyl compounds such as hexachloroacetone,[21] trifluoroacetophenone,[3] chloranil,[22] thiobenzophenone,[23] *etc.* Some alcohol dehydrgenases contain Zn(II) at the active site,[24] and the chelation and hydrogen bonding with substrates facilitate the hydrogen transfer.[25] The use of these interactions to facilitate NADH-mediated oxidation-reduction reactions has largely escaped attention in model studies. More recently, however, the nonenzymatic transformation of the carbonyl group to the corresponding alcohol was found to be significantly catalyzed by Zn(II)[26] and Mg(II)[27] and by *ortho*-phenolic hydroxyl groups.[8-10] For example, *m*-nitrobenzaldehyde and 4-pyridinecarbaldehyde are not reduced by 1,4-dihydropyridines, whereas 5-nitrosalicylaldehyde and 3-hydroxy-4-pyridinecarbaldehyde serve as substrates.[8,10] These results clearly substantiate the importance of hydrogen bonding in lowering the activation energy for the reduction of the carbonyl function.

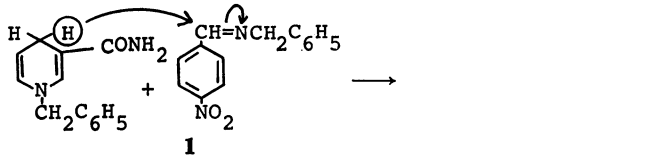
In the reaction of 1-substituted dihydronicotinamides

and carbonyl substrates, the transfer of the hydride anion or its equivalent would produce an unstable, alkoxide anion. When pK_a of this anion is considerably low due to the electron-withdrawing substituents, the reduction would be rather facile. Hexachloroacetone and trifluoroacetophenone belong to this class of the substrate. On the other hand, for the substrate with relatively high pK_a, the proton transfer to the carbonyl group prior to or at the transition state of the reaction could avoid the formation of energetically unfavorable alkoxide anions. Supposedly, the *ortho* hydroxyl groups of salicylaldehyde and 3-hydroxy-4-pyridinecarboxaldehyde can facilitate the reduction not only by polarization of the carbonyl group *via* hydrogen bonding but also by the efficient, intramolecular proton transfer (Eq. 1). This situation is similar to the ester cleavage reaction: *i.e.*, esters with good leaving groups are cleaved simply by the nucleophilic attack, while esters with poor leaving groups have to be cleaved by assistance of the general acid catalysis.[28]

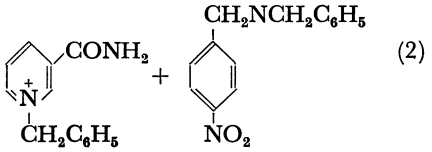


(1)

Thus, the BzlNH reduction of Schiff bases possesses a feature which is common to the amide cleavage. The simple nucleophilic cleavage of amide substrates produces the amine anion (pK_a *ca.* 27), which is so unstable that it almost certainly does not occur without the assistance of the acid catalysis.[29] Similarly, the BzlNH reduction of the Schiff base primarily produces the amine anion (Eq. 2), which would be energetically unfavorable. Therefore, appropriate proton sources are required for this reaction.



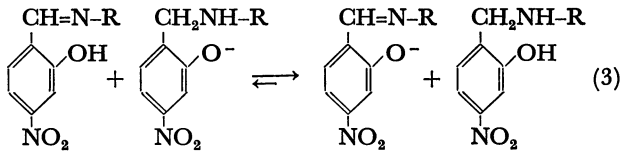
1



(2)

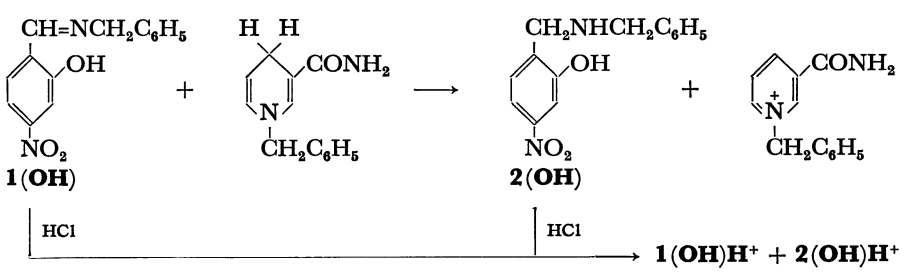
Although Schiff bases are more reactive to the nucleophilic attack than the corresponding carbonyl functions,[12] **1** did not undergo the BzlNH reduction. In contrast, **1(OH)** was reducible by BzlNH in refluxing methanol due to the presence of the *ortho* hydroxyl group as a proton source. The role of the hydroxyl group of **1(OH)** may be complicated, however. The Schiff base nitrogen is basic, so that a zwitterionic species (>C=N$^+$H–: **1(OH)-Z**) may be present in addition to the neutral species (**1(OH)-N**). Thus, two reaction paths become possible as depicted in Scheme 3; that is, one involves the concerted proton transfer from the *ortho* hydroxyl group and the other involves the preequilibrium proton transfer to the Schiff base. Fortunately, the distribution of the chemical species of pyridoxal and its Schiff base in methanol (27 °C) have been reported by Matsushima and Martell.[30] They demonstrated the presence of two species, zwitterionic (418 nm) and neutral (337 nm), for pyridoxalidenevaline. The UV spectrum of **1(OH)** recorded in dry methanol at 30 °C showed an absorption maximum at 353 nm, but not at longer wavelengths beyond 400 nm. This absorption maximum is reasonably attributed to **1(OH)-N**, and **1(OH)-Z** exists little under this condition. At refluxing temperature, however, the reaction path *via* **1(OH)-Z** may not be ruled out, since the formation of the zwitterionic form could be increased by raising the temperature. We consider both reaction paths to be plausible, though **1(OH)-N** probably being the major reacting species.

Since the reaction media were not buffered, the progress of the BzlNH reduction would produce the phenoxide anion of **2(OH)** as illustrated in Scheme 3. However, in the presence of the unreacted **1(OH)**, the prototropic equilibrium of Eq. 3 must be overwhelmingly shifted to the right because the acidity of the two phenol groups are rather different.

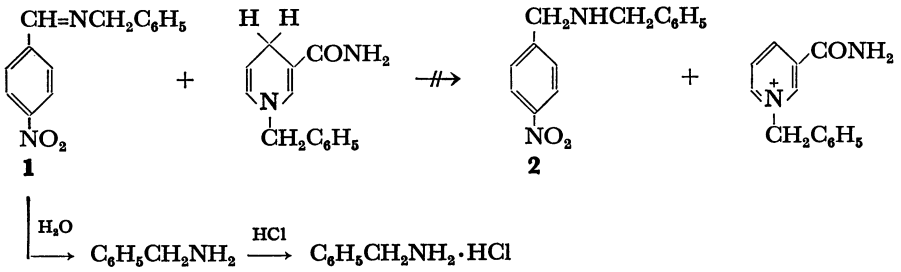


(3)

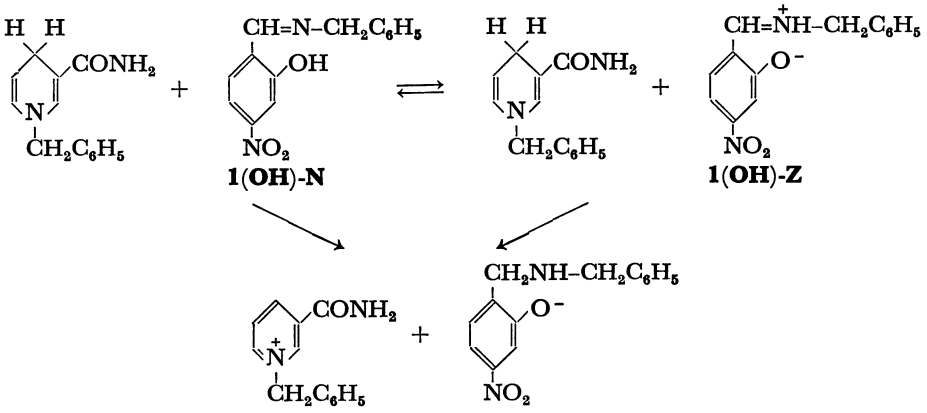
Figure 1 shows that a new, weak absorption band appears at 400—440 nm toward the end of the reaction. This peak may be ascribed to the anionic species of **1(OH)** (*cf.* λ_{max} for the 2-cyano-5-nitrophenolate anion is at 410 nm[31]). A similar spectral observation has been made for the 1,4-dihydronicotinamide reduction of 3-hydroxy-4-pyridinecarbaldehyde in refluxing methanol.[10] The anionic species of **1(OH)** will be much less



Scheme 1.



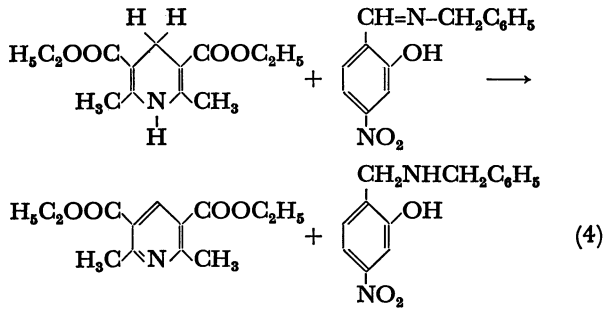
Scheme 2.



Scheme 3.

reactive in the BzlNH reduction, because of its increased electron density. This can explain why the reduction of **1(OH)** did not conclude even after refluxing for 9.5 h in methanol (Table 2).

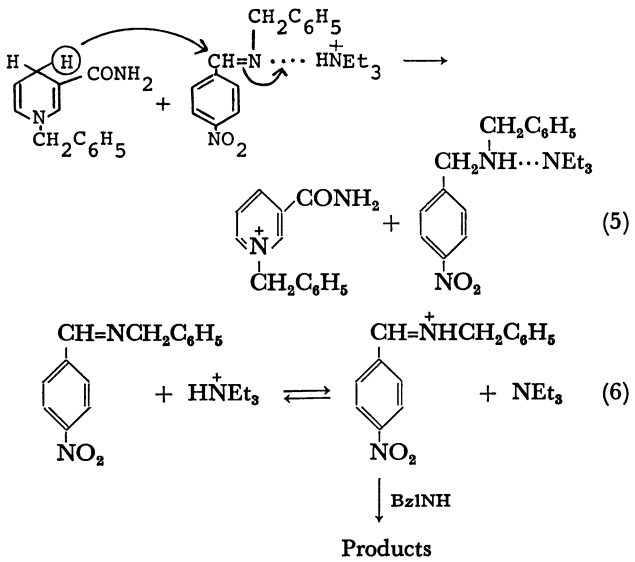
The reaction of **1(OH)** with Hantzsch ester (HE: 2,6-dimethyl-3,5-bis(ethoxycarbonyl)-1,4-dihydropyridine) did not yield an anionic species,[32] since HE can supply two hydrogenes (presumably, one as a "hydride" and the other as a proton: Eq. 4).



(4)

1(OH)H$^+$ was converted in 93% yield to **2(OH)** under the same reaction conditions (Table 2). Conceivably, **1H**$^+$ and **1(OH)H**$^+$ are quite reactive because (1) the protonated iminium group is prone to accept the "hydride" and (2) the product is not the energetically unfavorable anion.

Interestingly, addition of triethylamine hydrochloride and other amine hydrochlorides greatly improved the reactivity of **1** (Table 2). It is evident that these amine hydrochlorides act as the proton source. Here again, two proton transfer mechanisms are possible: the concerted and preequilibrium mechanisms (Eqs. 5 and 6).



(5)

(6)

Products

Equation 5 is a termolecular reaction, and entropically unfavorable. The pK_a value of *N*-(*p*-nitrobenzylidene)-*t*-butylamine was determined by Cordes and Jencks[33] to be 5.40, and is much lower than that of triethylamine (10.65).[34] Thus, the formation of **1H**$^+$ (Eq. 6) in the presence of only 10 times excesses of triethylamine hydrochloride is considered to be insignificant. It cannot be decided at present which reaction path is preferable.

In conclusion, the present study indicates the following order of the reactivity of the Schiff base toward the BzlNH reduction.

1H$^+$, **1** + NEt_3·HCl,

$\geqq$ **1 (OH)H**$^+$ $>$ **1 (OH)** $>$ **1** (unreactive).

This is in good accord with that demonstrated for the "*trans*-Schiffization,"[12] and suggests that the reduction by 1,4-dihydronicotinamide has the S_N2-type nature. Also suggested is that the reduction of the Schiff base can be acid-catalyzed, though the detailed mechanism (general acid or specific acid) was not established. These results may have important bearing with the interaction between the Schiff base and NADH in biological systems.

References

1) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanism," Vol. 2, W. A. Benjamin, New York, N. Y. (1966), Chapter 9.
2) B. Vennesland and F. H. Westheimer, "The Mechanism of Enzyme Action," ed by W. D. McElroy and B. Glass, John Hopkins, Baltimore (1954), p. 357.
3) J. J. Steffens and D. M. Chipman, *J. Am. Chem. Soc.*, **93**, 6694 (1971).
4) D. J. Creighton, J. Hajdu, G. Mooser, and D. S. Sigman, *J. Am. Chem. Soc.*, **95**, 6855 (1973).
5) L. C. Kurz and C. Frieden, *J. Am. Chem. Soc.*, **97**, 677 (1975).
6) Y. Morino and Y. Sakamoto, *J. Biochem.*, **48**, 733 (1960).
7) H. Holzer and S. Schneider, *Biochim. Biophys. Acta*, **48**, 71 (1961).
8) U. K. Pandit and F. R. Mas Cabre, *Chem. Commun.*, **1971**, 552.
9) S. Shinkai and T. C. Bruice, *J. Am. Chem. Soc.*, **94**, 8258 (1972).
10) S. Shinkai and T. C. Bruice, *Biochemistry*, **12**, 1750 (1973).
11) F. H. Westheimer and H. Cohen, *J. Am. Chem. Soc.*, **60**, 90 (1938).
12) E. H. Cordes and W. P. Jencks, *Biochemistry*, **1**, 773 (1962).
13) Ref. 1), Chapter 10.
14) S. Shinkai and T. Kunitake, *Chem. Lett.*, **1974**, 1113.
15) U. K. Pandit, R. A. Gase, F. R. Mas Cabre, and M. J. Sarink, *J. Chem. Soc., Chem. Commun.*, **1975**, 211.
16) T. Tsumaki, H. Ohta, and T. Tsutsumi, *Nippon Kagaku Kaishi*, **74**, 859 (1953).
17) Y. Matsuo, *J. Am. Chem. Soc.*, **79**, 2061 (1957).
18) S. Shinkai, R. Ando, and T. Kunitake, *Bull. Chem. Soc. Jpn.*, **48**, 1914 (1975).
19) C. C. Johnston, J. L. Gardner, C. H. Suelter, and D. E. Metzler, *Biochemistry*, **2**, 689 (1963).
20) C. S. Y. Kim and S. Chaykin, *Biochemistry*, **7**, 2339 (1968).
21) D. C. Dittmer and R. A. Fouty, *J. Am. Chem. Soc.*, **86**, 91 (1964).
22) E. A. Braude, J. Hannah, and R. Linstead, *J. Chem. Soc.*, **1960**, 3249, 3257.
23) R. H. Abeles, R. F. Hutton, and F. H. Weshimer, *J. Am. Chem. Soc.*, **79**, 712 (1957).
24) G. Popjak, *Enzymes*, **2**, 134 (1970).
25) J. H. Wang, *Science*, **161**, 328 (1968).
26) D. J. Creighton and D. S. Sigman, *J. Am. Chem. Soc.*, **93**, 6314 (1971).
27) Y. Ohnishi, M. Kagami, and A. Ohno, *Tetrahedron Lett.*, **1975**, 2437.
28) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanism," Vol. 1, W. A. Benjamin, New York (1966), Chapter 1.
29) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y. (1969), Chapter 10.
30) Y. Matsushima and A. E. Martell, *J. Am. Chem. Soc.*, **89**, 1322 (1967).
31) C. A. Bunton, M. J. Minch, J. Hidalgo, and L. Sepulveda, *J. Am. Chem. Soc.*, **95**, 3262 (1973).
32) S. Shiraishi, Senior Research Thesis of Faculty of Engineering, Kyushu University, 1976.
33) E. H. Cordes and W. P. Jencks, *J. Am. Chem. Soc.*, **85**, 2843 (1963).
34) H. K. Hall, Jr., *J. Am. Chem. Soc.*, **79**, 5441 (1957).