Mechanism of Carboxylic Acid-Catalyzed Reduction of Substituted Nitrosobenzenes by 1-Benzyl-3,5-bis(1-pyrrolidinylcarbonyl)-1,4-dihydropyridine in Acetonitrile

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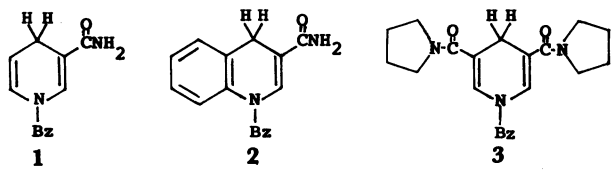
Mechanism of carboxylic acid-catalyzed reduction of substituted nitrosobenzenes by 1-benzyl-3,5-bis(1-pyrrolidinylcarbonyl)-1,4-dihydropyridine has been studied in acetonitrile at 25 °C. The major product was hydroxylamine when the dihydropyridine was used in excess over nitrosobenzene, while that was azoxybenzene in the reverse case. The observed second-order rate constant k_2^R are dependent on acid concentration in a first-order manner with the acid-catalyzed third-order rate constant k_H^R. The Hammett relationship for k_H^R for substituted nitrosobenzenes gave a ρ=−0.93. The Brönsted plot of log k_H^R vs. pK_a of acids gave a straight line with a slope of −0.34. The deuterium isotope effect for the catalysis by dichloroacetic acid was found to be k_H^R/k_D^R=2.80. These results indicate that a general acid catalysis by carboxylic acids plays an important role in the reduction of nitrosobenzene by dihydropyridine in acetonitrile.

Acid catalysis has been an interesting subject for the model studies of NAD(P)H coenzyme, since in certain dehydrogenases a protonated histidine residue is presumed to be involved as the general acid to activate substrate carbonyl group for hydride transfer from the coenzyme.[1,2] For the model studies of such acid catalysis, a problem is instability of conventional NADH analogs such as 1-benzyl-3-carbamoyl-1,4-dihydropyridine (**1**) toward acids.[3-6] This problem was solved by Shinkai et al. by the design of 1-benzyl-3-carbamoyl-1,4-dihydroquinoline (**2**) which is stable even in a strong aqueous acid solution.[7] Later, as reported previously,[8] we found that 1-benzyl-3,5-bis(1-pyrrolidinylcarbonyl)-1,4-dihydropyridine (**3**) is much more active reducing agent than **2** and more acid stable than **1** in the acid ($CHCl_2COOH$)-catalyzed reduction of substituted nitrosobenzenes in acetonitrile. In this paper, we wish to report the full accounts of the previous results[8] together with a more detailed study on the mechanism of this acid catalysis. Incidentally, it may be of interest to study the mechanisms of reduction of nitrosobenzene by NADH analogs in connection with the carcinogenesis in biological systems.[9-12]



Experimental

All melting points were uncorrected. Spectra were recorded by using the following instruments: IR, Hitachi 215; UV-visible, Hitachi 220 and 220A; NMR, JEOL JMM-PS-100 (100 MHz) and Hitachi R-20 (60 MHz). HPLC was performed by using JASCO UNIDEC-100-II, UNIFLOW-211 and Shimadzu C-R1B.

Materials. Anhydrous acetonitrile was obtained by distilling over phosphorus pentaoxide. The carboxylic acids as catalysts were commercial samples of special grade and purified by recrystallization or distillation if necessary. Substituted nitrosobenzenes were prepared by the reduction of the corresponding commercially available nitrobenzenes with zinc powder in aqueous solution containing ammonium chloride according to the literature methods and purified by recrystallization from ethanol-water: *p*-CH_3, mp 48 °C (lit,[13] 48.5°); *m*-CH_3, mp 53 °C (lit,[13] 53°); *p*-Cl, mp 97 °C (lit,[14] 90°); *p*-CF_3, mp 56—57 °C (lit,[15] 51—53°); H, commercial sample, mp 68 °C. The IR and UV spectra of these nitrosobenzenes were in accord with reported ones.[16,17]

1-Benzyl-3,5-bis(1-pyrrolidinylcarbonyl)-1,4-dihydropyridine (3) was prepared as usual by treating 3,5-pyridinedicarbonyl dichloride with an excess of pyrrolidine in chloroform. The diamide was reacted with benzyl chloride in acetonitrile under reflux to give the pyridinium chloride. The salt was then reduced by sodium dithionite in water. The reaction mixture was extracted with dichloromethane, and the usual work-up of the extract, purification by cellulose-column chromatography and recrystallization from ethanol-water gave **3**, mp 46 °C: overall yield, 65%; UV (CH_3CN), λ_{max}=355 nm (ε=6072 $M^{-1}cm^{-1}$); ^1H NMR (ppm (δ) in $CDCl_3$/TMS), 1.67—1.95 (8H, m, pyrrolidine ring CH_2), 3.25—3.65 (10H, m, dihydropyridine ring 4-CH_2 plus pyrrolidine ring NCH_2), 4.41 (2H, s, CH_2Ph), 6.55 (2H s, dihydropyridine ring 2-CH and 6-CH), 7.45 (5H, s, benzyl aromatic). Found: C, 71.51; H, 7.48; N, 11.42%. Calcd for $C_{22}H_{27}N_3O_2$: C, 72.30; H, 7.45; N, 11.50%.

1-Benzyl-3-carbamoyl-1,4-dihydropyridine (1) and 1-benzyl-3-carbamoyl-1,4-dihydroquinoline (2) were prepared according to the literature methods, mp 118 °C (decomp) (lit,[18] 120—122°) and mp 155 °C (lit,[19] 158—160°), respectively.

Dichloroacetic acid-d was prepared by the hydrolysis of dichloroacetic anhydride in D_2O. The deuterium incorporation was 82% measured by NMR method.

Product analysis was performed for unsubstituted nitrosobenzene (X=H) by HPLC method using JASCO Finepak SIL C18-column and acetonitrile/water (2:1) solvent. Examples of retention times (min) for the reaction components were: phenylhydroxylamine (**5**) (2.3), benzaldehyde (internal standard) (2.9), nitrosobenzene (**4**, X=H) (3.4), azoxybenzene (**6**) (6.6), 1-benzyl-3,5-bis(1-pyrrolidinylcarbonyl)-1,4-dihydropyridine (**3**) (8.3). The major product was **5** when **3** was used in excess over **4**, while that was **6** in

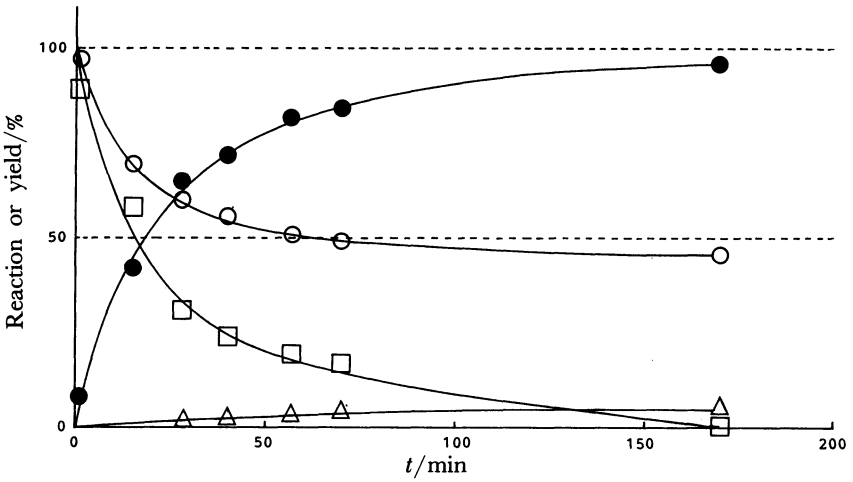


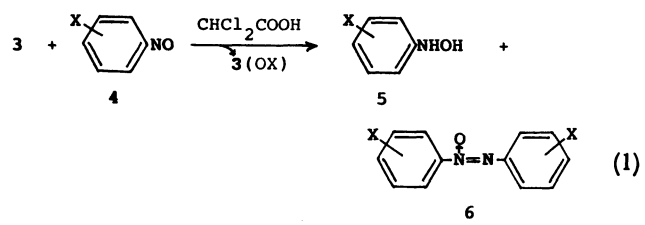
Fig. 1. Time dependent % reaction or yield: [3]=1.0×10⁻³ mol dm⁻³, [4]=0.5×10⁻³ mol dm⁻³, [$CHCl_2COOH$]=10.0×10⁻³ mol dm⁻³, in acetonitrile, 25 °C. (3) (○), (4) (□), (5) (●), and (6) (△).

the reverse case. An example of the time course of a reaction mixture is shown in Fig. 1.

Kinetics. In a 1 cm quartz UV cell stoppered with a rubber septum cover was placed a 3 ml acetonitrile solution of a dihydropyridine and the solution was well bubbled with acetonitrile saturated pure nitrogen gas through inserted needles. Stock solutions of a carboxylic acid and nitrosobenzene were treated in the same way. Then the quartz cell was mounted on a water-jacketed UV cell holder and the acid and nitrosobenzene stock solutions in water bath were kept at 25 °C for 15 min. The reaction was started by adding acid and nitrosobenzene stock solutions to the quartz cell by a Hamilton microsyringe. The rates of reduction were followed by monitoring the decrease in absorbance of either dihydropyridine at 350 nm for **1** and **2**, and at 370 nm or 400 nm for **3** or nitrosobenzene near 307 nm. In most cases, the reaction was carried out under pseudo-first-order conditions with 20 molar excess of nitrosobenzene over a dihydropyridine.

Results

Products and Kinetics. The reduction reaction was carried out in anhydrous acetonitrile under the nitrogen atmosphere. The reaction was slow in the absence of an acid. The time-dependent composition of the reaction mixture could be successfully analyzed by HPLC. The results indicated the reaction to be quantitatively described by Eq. 1. As shown in Fig. 1, nitrosobenzene (**4**, X=H) was quantitatively reduced to phenylhydroxylamine (**5**, X=H) when reacted with two molar excess of **3** in the presence of dichloroacetic acid, accompanied by the formation of a small amount of azoxybenzene (**6**). Figure 1 also shows that the disappearance of **3** and **4**, and the appearance of **5** occurred in almost the same rate and much faster than the formation of **6**. Furthermore, the curve for **3** indicates that after completion of reduction the remaining excess **3** was fairly stable without undergoing appraciable decomposition promoted by an acid. Under reversed conditions of excess **4** over **3** the major product was **6** which appeared to be formed by the acid-catalyzed condensation reaction between **5** and remaining **4**. Azoxybenzene **6** was stable without undergoing further redution by **3** under the present reaction conditions. The reduction occurred similarly in the case of



(1)

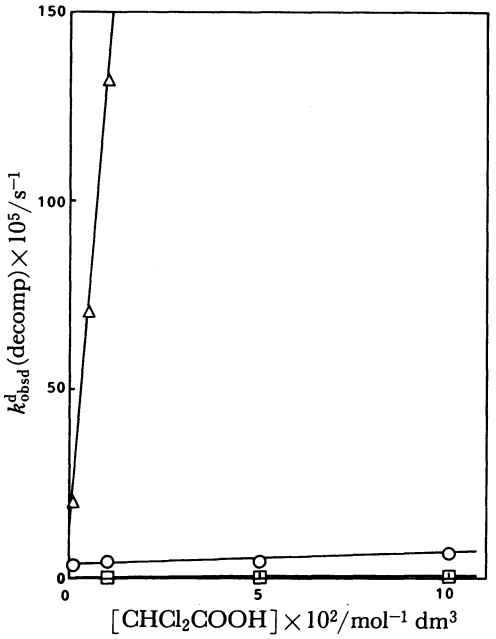


Fig. 2. Pseudo-first-order rate constants for the decomposition of (**1**) (△), (**2**) (□), and (**3**) (○) plotted as a function of the concentration of $CHCl_2COOH$ in acetonitrile at 25 °C.

Table 1. Rate Constants of the Acid-Catalyzed Decomposition of Dihydropyridines and of the Acid-Catalyzed Reduction of Nitrosobenzene by Dihydropyridines in Acetonitrile (25 °C)[a)]

Dihydropyridine	k_{un}^{d}	k_{H}^{d}	k_{un}^{R}	k_{H}^{R}
	s^{-1}	$mol^{-1}\,dm^3\,s^{-1}$	$mol^{-1}\,dm^3\,s^{-1}$	$mol^{-2}\,(dm^3)^2\,s^{-1}$
1	1.50×10^{-4}	1.09×10^{-1}	2.66	—
2	1.23×10^{-6}	7.50×10^{-6}	0.09	2.10
3	2.72×10^{-5}	3.72×10^{-4}	1.29	667

a) [**1**]=[**2**]=[**3**]=1.0×10^{-4} mol dm^{-3}, [**4**, X=H]=2.0×10^{-3} mol dm^{-3}.

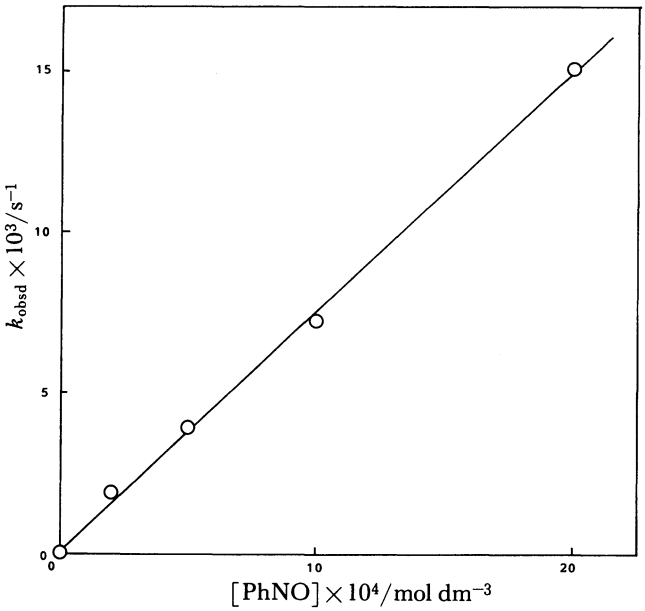


Fig. 3. A linear relationship between the observed pseudo-first-order rate constant and the concentration of nitrosobenzene in acetonitrile at 25 °C: [**3**]=1.0×10^{-4} mol dm^{-3}, [$CHCl_2COOH$]=1.0×10^{-2} mol dm^{-3}.

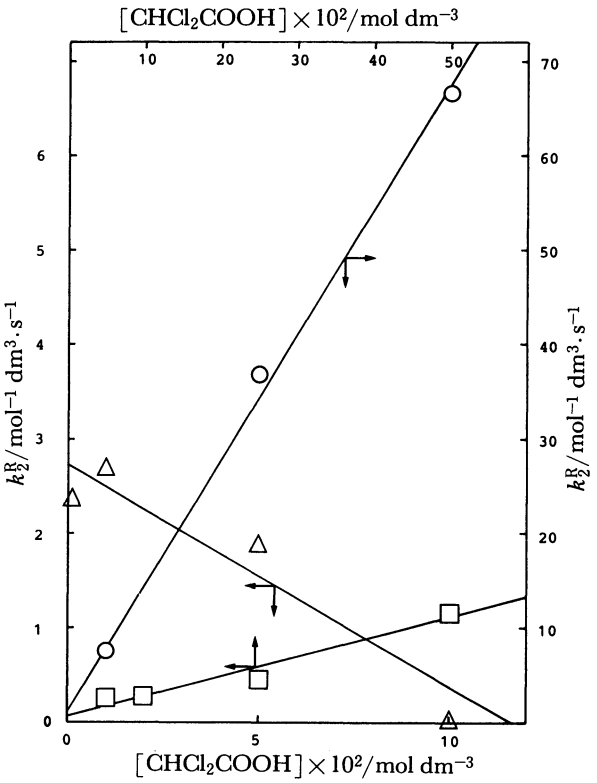


Fig. 4. Second-order rate constants for the reduction of nitrosobenzene by (**1**) (△), (**2**) (□), and (**3**) (○) plotted as a function of the concentration of $CHCl_2COOH$ in acetonitrile at 25 °C.

2, although the rate was much slower than in the case of **3**. The reduction was also observed with **1**, but the competing acid decomposition of **1** made the results complex. The decomposition of dihydropyridines by an acid were examined spectrophotometrically by measuring the rates of disappearance of dihydropyridine absorption (350 or 370 nm) in the absence of nitrosobenzene. As shown in Fig. 2, the pseudo-first-order rate constants for the decomposition (k_{obsd}^{d}) increase linearly with increasing concentration of dichloroacetic acid (AH) for all three dihydropyridines. Therefore the rate of decomposition can be represented by Eqs. 2 and 3.

$$\text{Rate(decomp)} = k_{obsd}^{d}[\text{dihydropyridine}] \quad (2)$$

$$k_{obsd}^{d} = k_{un}^{d} + k_{H}^{d}[AH] \quad (3)$$

The rate constants k_{un}^{d} obtained as the intercepts presumably arose from the air oxidation due to incomplete air extrusion from the reaction solutions. The rate constants k_{H}^{d} obtained as the slopes were those for the acid-catalyzed decomposition. These values are listed in Table 1. The Table indicates that the k_{H}^{d} value for **1** is far much larger than those for the other two. It is considered that **1** undergoes a ready acid promoted decomposition through an electrophilic addition of an acid on the enamine 5,6-double bond of dihydropyridine ring.[3-6)] Such an electrophilic addition seems to be much difficult to occur in the cases of **2** and **3** as discussed later.

The rates of reduction of nitrosobenzene were obtained similarly as the rates of disappearance of a dihydropyridine in the presence of nitrosobenzene (X=H). As shown in Fig. 3, the pseudo-first-order rate constants (k_{obsd}) were observed to increase linearly with increasing concentration of nitrosobenzene. Thus, combined with Eq. 2, the k_{obsd} value can be represented by Eq. 4 which allows to obtain a second-order rate constant for the reduction k_2^R as the slope of a straight line. Furthermore, as shown in Fig. 4, this k_2^R was observed to be linearly dependent on the acid con-

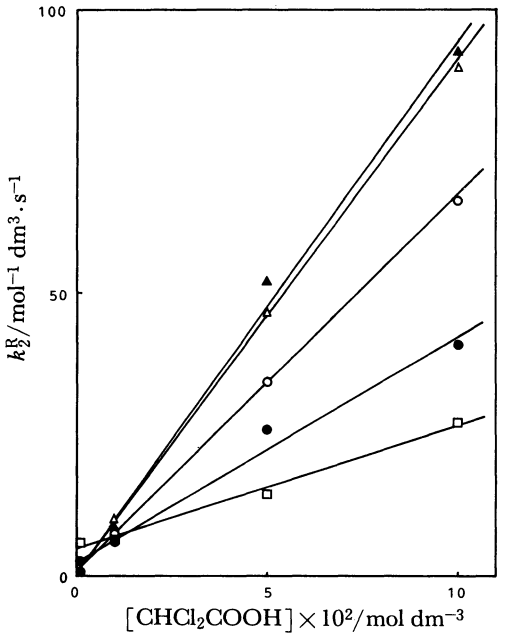


Fig. 5. Second-order rate constants for the reduction of substituted nitrosobenzenes by (**3**) as a function of the concentration of $CHCl_2COOH$ in acetonitrile at 25°C: *p*-Me (△), *m*-Me (▲), H (○), *p*-Cl (●), and *p*-CF_3 (□).

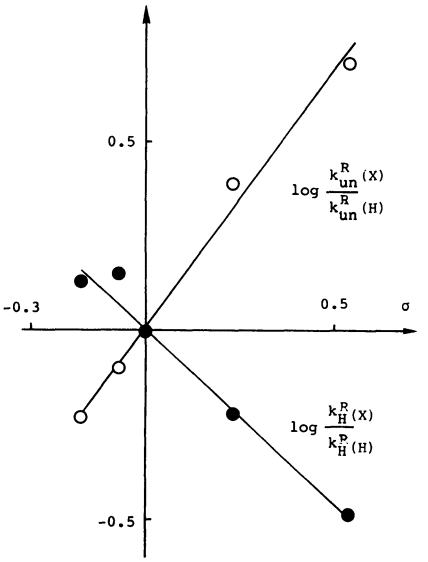


Fig. 6. Hammett plots of k^R_{un}(○) and k^R_H (●).

centration and obeyed Eq. 5. Therefore, as summarized in Eq. 6, the k_{obsd} value is composed of four kinetic terms of decomposition and reduction. The corresponding four rate constants k^d_{un}, k^d_H, k^R_{un}, and k^R_H are shown in Table 1 for three dihydropyridines. As for the former two, it was described already.

$$k_{obsd} = k^d_{obsd} + k^R_2\,[\text{nitrosobenzene}] \quad (4)$$

$$k^R_2 = k^R_{un} + k^R_H\,[AH] \quad (5)$$

$$k_{obsd} = k^d_{un} + k^d_H\,[AH] + k^R_{un}\,[\text{nitrosobenzene}] + k^R_H\,[AH][\text{nitrosobenzene}] \quad (6)$$

Of the two reduction rate constants, the uncatalyzed second-order one k^R_{un} indicates that a conventional dihydropyridine (**1**) is much more active than the other two (**2** and **3**) in the absence of an acid. However, Fig. 4 shows that the k^R_2 for **1** decreases with increasing acid concentration with a line of negative slope, in contrast to the lines of positive slopes in the cases of **2** and **3**. The reason for such an inhibitory effect of an acid for **1** is not clear at present.[20] As discussed later, it may be conceivable that for more basic **1** than the other two, protonation of dihydropyridine moiety lowers its reducing activity together with the acid decomposition. On the other hand, the acid-catalyzed third-order rate constants k^R_H could be successfully obtained for **2** and **3**. As for the reactivities of **2** and **3**, the latter is much more active than the former in both uncatalyzed and catalyzed reaction: $k^R_{un}(\mathbf{3})/k^R_{un}(\mathbf{2})=15$ and $k^R_H(\mathbf{3})/k^R_H(\mathbf{2})=318$.

Hammett Substituent Effect. The present reduction is a hydride transfer from a dihydropyridine ring to the nitroso group.[21] Consequently, it is conceivable that the uncatalyzed (k^R_{un}) process is favored by an electron-withdrawing substituent of nitrosobenzene. On the other hand, if the above acid catalysis (k^R_H) is due to the activation of a nitroso group by protonation or hydrogen bonding, such process seems to be favored by an electron-donating substituent. What actually observed is shown in Fig. 5 which indicates that the plots of k^R_2 vs. concentration of dichloroacetic acid give straight lines as seen for unsubstituted nitrosobenzene in Fig. 4. It is clear that the slopes of those straight lines (i.e. k^R_H) are larger for more electron-donating substituents. On the other hand, the intercepts (k^R_{un}) are larger for more electron-withdrawing substituent. These opposing substituent effects can be seen more clearly in the Hammett plots of Fig. 6, giving a positive $\rho=1.35$ for k^R_{un} and a negative $\rho=-0.93$ for k^R_H plot. As expected, the latter negative ρ value for k^R_H process suggests the activation of a substrate nitroso group by an acid, although its detailed mechanism, protonation or hydrogen bonding, is difficult to determine at this stage.

Brönsted Plots. The above k^R_H values for unsubstituted nitrosobenzene (X=H) were determined for eight carboxylic acids as shown in Table 2. The Table also lists the literature values of pK_a for the used carboxylic acids in water[22] and acetonitrile.[23] These values give two straight lines of Brönsted plots as shown in Fig. 7 and Eqs. 7 and 8. The α values are both negative,

$$\log k^R_H = -0.34\,pK_a(AN) + 6.88 \quad (7)$$

$$\log k^R_H = -1.17\,pK_a(\text{water}) + 4.77 \quad (8)$$

indicating a stronger acid being a more active catalyst.

Deuterium isotope effect was examined to get more insight into the mechanism of the above acid catalysis

Table 2. Acid-Catalyzed Rate Constants k_H^R with Eight Carboxylic Acids for the Reduction of Nitrosobenzene by (**3**) in Acetonitrile (25 °C)[a)]

	Carboxylic acid	$(pK_a)_{AN}$[b)]	$(pK_a)_w$[c)]	k_H^d / $mol^{-1}\,dm^3\,s^{-1}$	k_H^R / $mol^{-2}\,(dm^3)^2\,s^{-1}$
1	CF_3COOH		0.25	6.06×10^{-2}	2.95×10^{4}
2	CCl_3COOH		0.64	6.98×10^{-2}	1.76×10^{4}
3	$CHCl_2COOH$		1.30	3.72×10^{-4}	6.67×10^{2}
4	$CH_2ClCOOH$		2.86	1.03×10^{-4}	45.9
5	o-HOC_6H_4COOH	16.7	2.98	9.80×10^{-5}	22.8
6	3,5-$(NO_2)_2C_6H_3COOH$	16.9	2.82	3.13×10^{-5}	6.90
7	m-BrC_6H_4COOH	19.5	3.81	2.92×10^{-5}	1.20
8	CH_3COOH	22.3	4.76	6.31×10^{-6}	0.21

a) $[\mathbf{3}]=1.0\times10^{-4}$ mol dm^{-3}, [**4**, X=H]$=2.0\times10^{-3}$ mol dm^{-3}. The concentration of acids were varied from 2.0×10^{-4} mol dm^{-3} for CF_3COOH up to 2.0 mol dm^{-3} for CH_3COOH. b) pK_a values in acetonitrile.[22)] c) pK_a values in water.[21)]

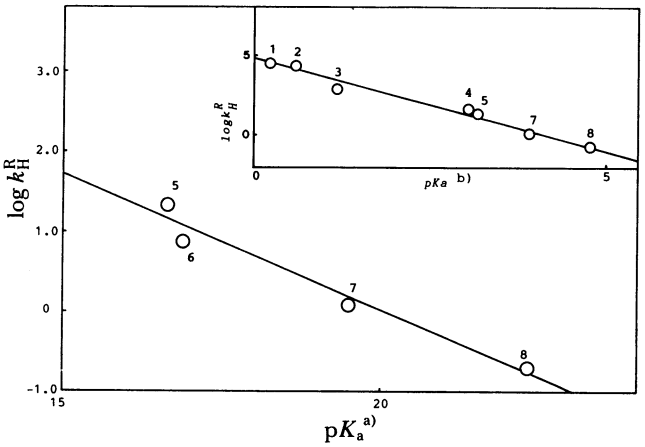


Fig. 7. Brönsted plots of the data in Table 2. Numbers in the figure correspond to the carboxylic acids in Table 2.
a) pK_a values in acetonitrile,[22)] b) pK_a values in water.[21)]

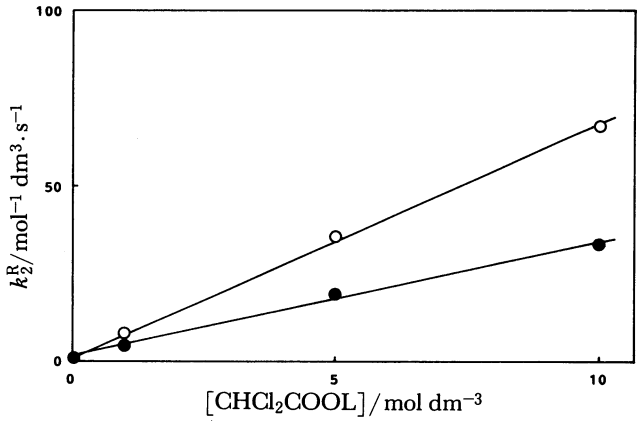


Fig. 8. Second-order rate constants for the reduction plotted as a function of the concentration of $CHCl_2COOH$ (○) and $CHCl_2COOD$ (●) in acetonitrile at 25 °C. $[\mathbf{3}]=1.0\times10^{-4}$ mol dm^{-3}.

by using dichloroacetic acid-*d* (82% atom D-content), unsubstituted nitrosobenzene, and (**3**). The results are shown in Fig. 8. The slopes of the two straight lines are $k_D'(R)=315$ and $k_H^R=618$ in the unit of $mol^{-2}\,(dm^3)^2\cdot s^{-1}$ to give an isotope effect of $k_H^R/k_D'(R)=2.1$. This value can be corrected for the true isotope effect (k_H^R/k_D^R) based on Eqs. 9 and 10,

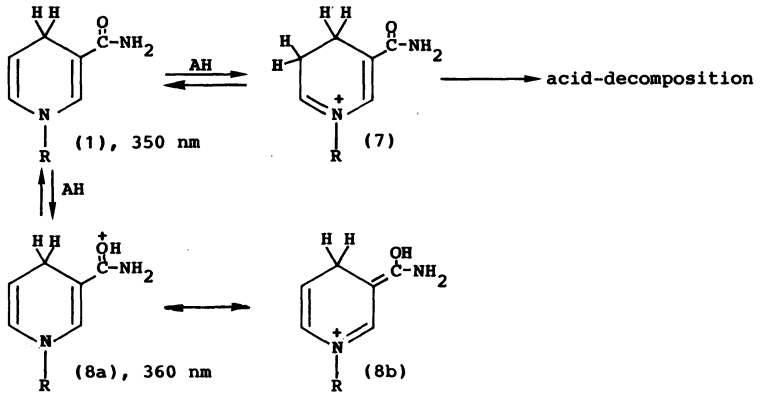
$$k'_D(R)([AD]+[AH]) = k_{DL}^R[AD] + k_{HL}^R[AH] \quad (9)$$

$$\frac{k_H^R}{k_D^R} = \frac{\gamma\cdot(k_H^R/k'_D(R))}{(1+\gamma)-(k_H^R/k'_D(R))} = 2.80 \quad (10)$$

where [AD] and [AH] are the concentrations of deuterium-labelled and unlabelled dichloroacetic acids, respectively, and $\gamma=[AD]/[AH]=82/18=4.56$ is used based on the above isotope content (82% D). In the same procedure, an isotope effect of $k_H^R/k_D^R=1.73$ was obtained for the reduction of *m*-nitrosotoluene.

Discussion

In the catalysis of lactate dehydrogenase and of related enzymes, the substrate carbonyl group is presumed to be activated by an acidic group toward the hydride transfer from NAD(P)H coenzyme.[1,2)] There have been known some model studies which support such acid catalysis. Salicylaldehyde[24)] or 3-hydroxy-4-pyridinecarbaldehyde[25)] undergoes a ready reduction by NADH analogs, in which the *o*-hydroxyl group is presumed to act as the acid to activate the neighboring carbonyl group through a hydrogen bonding. Intermolecular general acid catalysis was examined by using protonated amines as the acids.[26)] However, since protonated amines are usually very weak acids, they can only activate already activated substrates like hexachloroacetone[26a)] or Schiff base.[26b)] In order to examine the acid catalysis of stronger acids, one must make some device, such as to avoid a direct contact of a dihydropyridine moiety with an acid or to design a specially acid-stable NADH analog, since a conventional NADH analog like **1** is acid-unstable.[3-6)] Indeed Shinkai et al. was able to study the acid catalysis of a strong acid by using a specially designed acid-stable **2**[7)] Meanwhile we reported in the previous communications[8,27,28)] that **3** is also an acid-stable NADH analog having comparable reducing activity with that of **1**. A

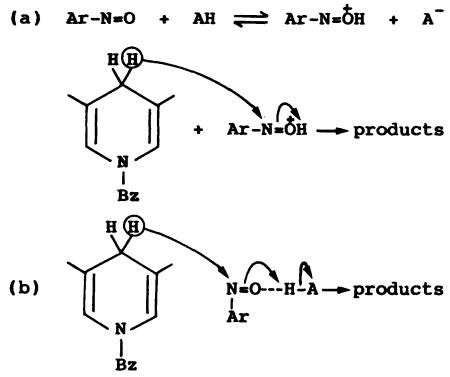


Scheme 1. Acid-promoted protonation and decomposition of (1).

more detailed features of acid catalysis of **3** are now reported for the reduction of nitrosobenzenes. Interests in the reduction of this substrate come from the following two reasons. One is from the mechanistic interests in that nitrosobenzene is much more reactive than carbonyl compounds and hence its reduction would allow to see more detailed features of acid catalysis by using acids of wide range of activity. The other is from the biochemical interests in the carcinogenesis promoted by the nitrosobenzene metabolism.[9-12]

As for the acid-stability, the data in Fig. 2 and Table 1 (k_H^d) indicate **1** to be the most acid-unstable among the three dihydropyridines examined. According to the literatures,[3-6] **1** undergoes a ready protonation to the 5,6-enamine double bond (Scheme 1, **7**). Such an electrophilic addition must be more difficult to occur in the cases of **2** and **3** because the 5,6-double bond is a part of aromatic ring in **2** and in conjugation with the carbonyl group at the 5-position in **3**. It is shown in Table 2 that the rates of decomposition (k_H^d) increase with increasing acidity (pK_a) of the acids. In a parallel way, the acid-catalyzed rates of reduction (k_H^R) also increase with increasing acid-acidity. However, it should be noticed that the rate of decomposition of **3** is negligibly small as compared to that of reduction, i.e. the oxidation of **3** by nitrosobenzene. For example, it can be calculated by using the rate data in Table 1 that the k_{obsd}^d of **3** is $6.44\times10^{-5}\,s^{-1}$ in the presence of 0.1 mol dm^{-3} dichloroacetic acid, while the corresponding k_{obsd} for reduction is $1.40\times10^{-1}\,s^{-1}$ in the presence of 2.0×10^{-3} mol dm^{-3} nitrosobenzene.

As for the reduction, the results in Fig. 4 may be the first to be discussed. The redution is inhibited by adding an acid in the case of **1** in contrast to the acceleration in the other two cases. In the case of **1**, concomitantly to the inhibition, a red-shift of dihydropyridine absorption occurs, even in a mixture of low concentration of [**1**]=[dichloroacetic acid]$=1.0\times10^{-4}$ mol dm^{-3}. Similar red shift is also observed for **3** (355 to 370 nm), but in the presence of a much higher acid concentration. In the case of **2**, the red shift is small or negligible. These red shifts are likely due to the protonation at the 3-carbamoyl group (Scheme 1, **8**).[3]



Scheme 2. Two possible mechanisms of acid catalysis.

Another protonation site is the 5-position **7**. Both of these protonation may deactivate the dihydropyridine ring as a hydride donor. Thus deactivation overcomes the catalysis in the case of **1**. On the other hand, since the protonation at 5-position is negligible in **2** or small in **3**, the total balance is over the catalysis rather than inhibition in these two cases.

The next to be discussed is the data in Figs. 6, 7, and 8, which were obtained by using **3**. In Fig. 6 are shown the two opposing Hammett plots. A positive $\rho=1.35$ for the uncatalyzed k_{un}^R indicates that, in the absence of an acid, an electron-withdrawing substituent helps the substrate to accept the hydride. On the other hand, a negative $\rho=-0.93$ for the acid-catalyzed k_H^R suggests that an electron-donating substituent helps the nitroso group to accept a proton from an acid and to become a better electrophile toward a hydride. Two mechanisms of acid catalysis are conceivable (Scheme 2): (a) Preequilibrium formation of protonated nitrosobenzene followed by a rate-limiting hydride transfer, and (b) a one-step concerted hydride transfer assisted by general acid-catalyzed proton transfer. These two mechanisms may be discernible by examining a Brönsted relationship and the H-D isotope effect. The Brönsted plots in Fig. 7 show a negative slope of $\alpha=-0.34$, based on the data in Table 2 and the pK_a of an acid in acetonitrile.[23] This result strongly suggests a mechanism of general acid catalysis,[29-31] i.e. the second mechanism (b) in Scheme 2. Namely the value 0.34 is

much smaller than 1.0, but rather close to 0.5, suggesting that the proton transfer is not complete, rather in a halfway at the transition state. The deuterium isotope effect shown in Fig. 8 also supports this general acid mechanism. A corrected value of $k_H^R/k_D^R=2.80$ is well in a range of primary isotope effect for general acid catalysis.[29-32]

The above general acid mechanism is of minor importance in aqueous buffer solutions, according to our previous observation.[27] The major mechanism observed at low pH is the hydronium ion (H_3O^+) catalysis, presumably similar to the first mechanism (a) in Scheme 2. Near neutral pH, the rates are independent of pH in aqueous media. These results indicate that the proton transfer is not important at the transition state in aqueous media. It seems to be reasonable that a general acid mechanism becomes important on changing the solvent from more polar water to less polar acetonitrile.

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