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ACID CATALYZED REDUCTION OF NITROSOBENZENE IN WATER BY 1-BENZYL-3,5-DIPYRROLIDINOCARBAMOYL-1,4-DIHYDROPYRIDINE AS A NADH ANALOG

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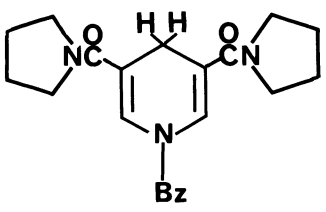
Acid catalysis has been observed for the reduction of substituted nitrosobenzenes in water by an acid stable NADH analog, 1-benzyl-3,5-dipyrrolidinocarbamoyl-1,4-dihydropyridine.

Acid catalysis in the reaction of coenzyme NAD(P)H and its analogs is an interesting subject as the model catalysis of certain dehydrogenase enzyme[1,2] as well as for the synthetic application of NADH analogs.[3] Yet such study has been hampered due to instability of conventional NADH analogs toward acids with a few exceptions.[3-7] Recently we found that 1-benzyl-3,5-dipyrrolidinocarbamoyl-1,4-dihydropyridine 1 is an acid stable NADH analog and the reduction of substituted nitrosobenzenes 3 by 1 in dry acetonitrile undergoes acid catalysis.[7] It is interesting to know further whether such acid catalysis occurs also in water as in dry acetonitrile, since the two solvents behave quite differently in many NADH model reactions.[8-10] It is also interesting to study the mechanism of reduction of nitrosobenzene by NADH analogs for the understanding of arylamine mediated carcinogenesis in biological systems.[11,12] We now wish to report that the acid catalyzed reduction of 3 can be observed in water by using 1, whereas a conventional NADH analog 2 undergoes a rapid hydration to lose reducing activity under acidic



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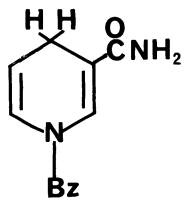

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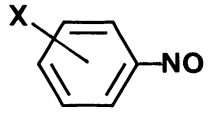

3

conditions.

The reduction of 3 by 1 was carried out in aqueous buffer solutions under nitrogen atmosphere. The products were phenylhydroxylamine and azoxybenzene as observed previously in dry acetonitrile.[7] The rates of reduction were measured spectrophotometrically by observing the decrease of absorption of 1 and they were found to be first order with respect to each concentration of 3 and 1 to give the second order rate constants k_2. The pH-rate profiles for the k_2 values are shown in Fig. 1a. These k_2 values are very large as compared to those obtained previously in dry acetonitrile,[7] i.e. there is roughly 100 fold rate difference between the two solvent systems under neutral conditions.[13] It is noteworthy that such reactivity comparison is usually difficult for other carbonyl substrates because of difficulty in carrying out the reduction in aqueous media. Figure 1a indi-

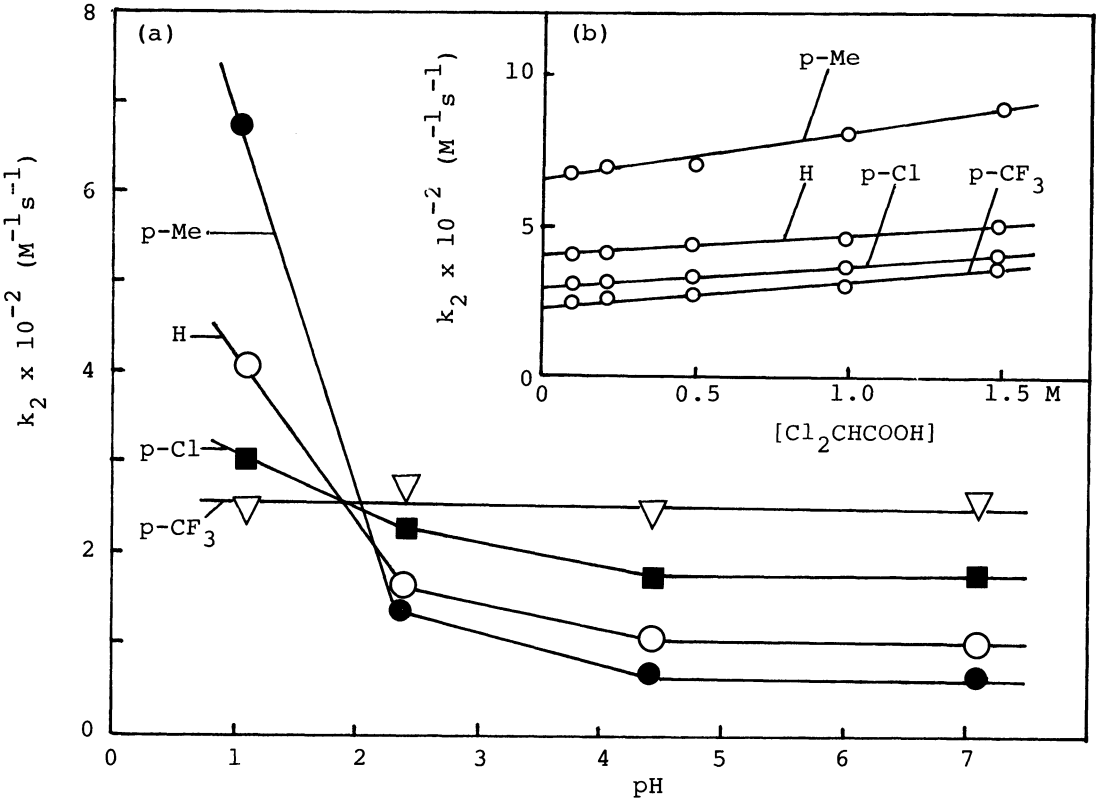

Fig. 1. (a) pH-rate profile for the second order rate constants (k_2) in the reduction of nitrosobenzenes 3 by a NADH model 1 at 25 °C: buffers(0.1 M, μ=1.0 M (NaCl)) were 2,6-lutidine, acetate, monochloroacetate, and dichloroacetate at pH 7.10, 4.42, 2.43, and 1.13, respectively. (b) Plots of k_2 vs. dichloroacetate concentration at pH 1.13.

cates that the rates are almost independent of pH in a range of 4-7 and they are in the order of p-CF_3 > p-Cl > H > p-Me to give a ρ=0.85 in the Hammett-σ plot. At a lower pH of 1.13, the rates are reversed as in the order of p-Me > H > p-Cl > p-CF_3. Thus the substrate is activated by an electron-withdrawing substituent at neutral pH, while by an electron-donating one at acidic pH. Under neutral or weakly acidic conditions, there was observed essentially no buffer catalysis indicating neither general acid nor base catalysis being important in accordance with the report of Becker and Sternson.[11] However, as shown in Fig. 1b, the rates at pH 1.13 increase with increasing the concentration of buffer acid($Cl_2CHCOOH$) in a first order manner for each substrate to give a straight line of Eq. 1. In Eq. 1, the first water catalyzed rate($k_o[H_2O]$) is approximately equal to the k_2 value at neutrality(pH 4-7), and then the second hydronium ion catalyzed rate can be calculated as $k_H{}^+[H_3O^+]=(k_2-k_o[H_2O])$ at zero buffer concentration. The Hammett-σ plots of these k_2([AH]=0) and $k_H{}^+[H_3O^+]$ values give ρ= -0.56 and -1.8, respectively. The slopes of straight lines of Fig. 1b(k_{AH} in Eq. 1) are due to the general acid (AH) catalyzed rates, although they are small and difficult to obtain a good correlation with the Hammett substituent constants. The hydronium ion catalysis which seems to be more important in this reduction suggests the protonated nitrosonium ion 4 as the activated intermediate to undergo the reduction. Although less important, the general acid catalysis does occur, presumably by involving hydrogen-bonded intermediate or transition state 5. Substituent effects seem to support these 4 and 5 since an electron-donating group favors the formation of them to facilitate the reduction.

$$k_2 = k_o[H_2O] + k_H{}^+[H_3O^+] + k_{AH}[AH] \quad (1)$$

$$\underset{\underline{4}}{Ar\text{-}\overset{+}{N}\text{=}OH} \qquad \underset{\underline{5}}{Ar\text{-}N\text{=}O\cdots H\text{-}A}$$

Much remain to be clarified for more detailed mechanism of the above reduction.[14] Nevertheless the observation of acid catalysis in aqueous system seems to be important for further understanding of the mechanism of enzymatic acid catalyzed reaction of NAD(P)H coenzyme.

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13) A stopped flow spectrophotometer(Union-Giken) was used to measure fast rates.

14) For example, we must know the pK_a values of both 1 and 3 which are not easy to determine at present.

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