Coenzyme Models. Part 16. Studies of General-acid Catalysis in the NADH Model Reduction

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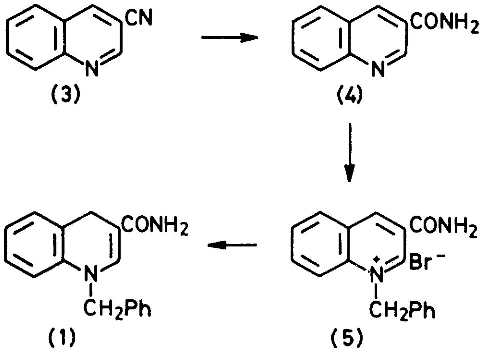
The reduction of trifluoroacetophenone was performed in an aqueous acetate (and analogues) buffer solution with an acid-stable NADH model compound, 1-benzyl-3-carbamoyl-1,4-dihydroquinoline. The resultant Brönsted plots had a slope of −0.20, indicating that the reduction process is general-acid catalysed. This is the first example of general-acid catalysis observed in an NADH model reduction performed in an aqueous system.

THE chemical background for NADH coenzyme has recently been extended in several laboratories and it has led to information relevant to biological NADH-dependent oxidation–reduction.[1–7] However, much of the research effort has been spent on understanding the metal catalysis from a viewpoint of model studies of alcohol dehydrogenases. Recent *X*-ray crystallographic studies of D-glyceraldehyde-3-phosphate dehydrogenase suggest that the protonated imidazole of the histidine residue may act as a general-acid during the reduction process,[8,9] but the corresponding model study has escaped attention. van Eikeren and Grier[10] and Shinkai *et al.*[11,12] have found that the nonenzymatic dihydronicotinamide reduction of carbonyl substrates in nonaqueous solvents is accelerated by added acids. The results may support, though indirectly, the occurrence of general-acid catalysis. To the best of our knowledge, however, the direct evidence for general-acid catalysis in an aqueous system has not been reported.



SCHEME 1

It is well-known that the conventional NADH model compounds (*i.e.* 1,4-dihydronicotinamides) decompose rapidly in the presence of acids,[13,14] so that the rate of the dihydronicotinamide reduction cannot be determined accurately in acidic pH region where general-acid catalysis may occur. We thus prepared an acid-stable NADH model compound, 1-benzyl-3-carbamoyl-1,4-dihydroquinoline (1) (see Scheme 1) in which the acid-sensitive 5,6-double bond is involved in the aromatic ring, and investigated the effect of added acids (HA) on the reduction of trifluoroacetophenone (2) (see Scheme 2).

EXPERIMENTAL

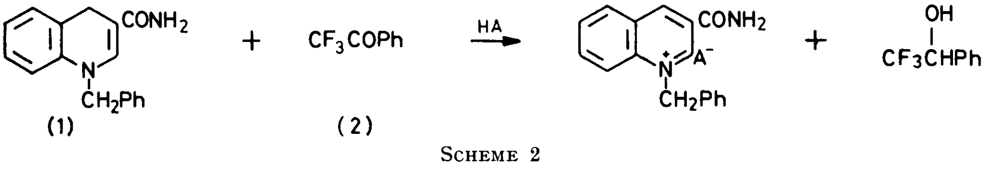
Materials.—Compound (1) was synthesised as follows.

3-*Carbamoylquinoline* (4). To a solution of 3-cyanoquinoline (3) (10.0 g, 0.065 mol) in water (150 ml) and ethanol (100 ml) was added 5N-NaOH (5 ml) and the reaction mixture was heated at 75—85 °C. The progress of the reaction was monitored by t.l.c. (silica gel–ethyl acetate). After 2.5 h, (3) was hydrolysed to give (4) and a trace of quinoline-3-carboxylic acid. Ethanol was evaporated under reduced pressure and the precipitate formed was collected by suction. Recrystallisation from ethanol–benzene gave (4) (10 g, 95%), m.p. 193—194 °C (lit.,[15] 198—199 °C).

1-*Benzyl*-3-*carbamoylquinolinium Bromide* (5).—Benzyl bromide (25 g, 0.146 mol) and (4) (8.0 g, 0.047 mol) were dissolved in dimethylformamide (100 ml) and the reaction mixture was heated at 85—95 °C for 30 h. After cooling of the reaction mixture the colourless precipitate was collected and recrystallized from ethanol; the *product* had m.p. 224—225 °C; yield 45% (Found: C, 58.2; H, 4.3; N, 8.2. $C_{17}H_{15}BrN_2O$ requires C, 59.49; H, 4.41; N, 8.16%).

1-*Benzyl*-3-*carbamoyl*-1,4-*dihydroquinoline* (1).—Compound (5) (2.0 g, 0.005 8 mol) in freshly distilled methanol (50 ml) was allowed to react with *N*-propyl-1,4-dihydronicotinamide (1.2 g, 0.007 2 mol) in methanol (15 ml) for 3 h at room temperature under a stream of N_2. The solvent was evaporated and the residue was taken up in chloroform–water. The chloroform layer was separated and dried (Na_2SO_4). After evaporation of the chloroform under reduced pressure, the residue was recrystallised from ethanol–water; the *product* had m.p. 158—160 °C (decomp.); yield 84% (Found: C, 77.25; H, 6.1; N, 10.4. $C_{17}H_{16}N_2O$ requires C, 77.25; H, 6.10; N, 10.60%); δ(Me_2SO) 3.42 (s, NH_2), 3.82 (s, 4-CH_2), 5.00 (s, *N*-CH_2), 7.52 (s, C_6H_5), and 6.9—7.9 (m, C_6H_4 and 2-CH).

Kinetics.—Kinetic measurements were carried out at 50 ± 0.1 °C in 25% (v/v) aqueous isopropyl alcohol with an ionic strength of 1.5. A typical example of the reaction procedure was as follows. A cuvette containing 3.0 ml of a



SCHEME 2

buffer solution was placed in a thermostatted cell-holder and after equilibration of the cell at 50 °C (15 min) an isopropyl alcohol solution of (2) (50 μl) was added. The u.v. absorption band near 255 nm increased gradually, which is attributable to the hydration equilibrium of (2). After the absorbance had reached a constant value (30—40 min), an isopropyl alcohol solution of (1) (50 μl) was added and the progress of the reaction was followed by monitoring the decrease of (1) at $\lambda_{max.}$ (334 nm). Since an excess of (2) was present in all the cases, the pseudo-first-order behaviour was observed for up to 3.5 half-lives. The

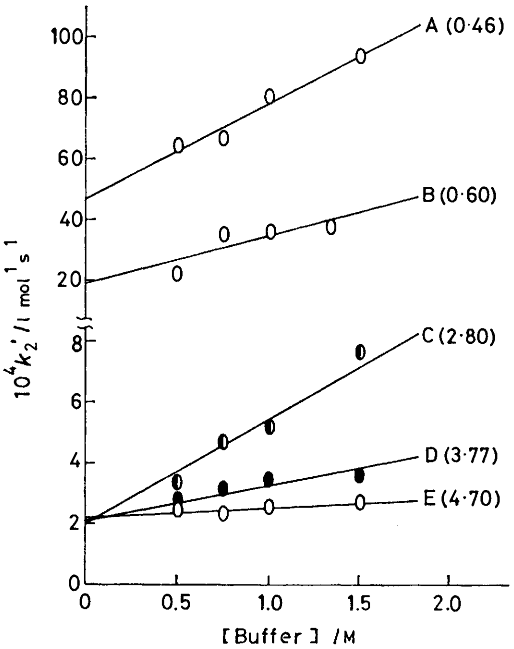


FIGURE 1 Apparent second-order rate constants plotted as a function of total buffer concentration. $[(1)] = 5.0 \times 10^{-5}$M, $[(2)] = 3.00 \times 10^{-2}$M; A = CF_3CO_2H, B = CCl_3CO_2H, C = $ClCH_2CO_2H$, D = CH_3CO_2H, and E = CH_3CO_2H

$$k_{obs} = k_d + k_2' [(2)] \tag{2}$$

pseudo-first-order rate constant (k_{obs}) is given by equation (2), where k_d denotes the acid-catalysed decomposition of (1) and k_2' is the apparent second-order rate constant for the reduction reaction. The first term was estimated separately in the absence of (2), and k_2' [(2)] was determined by subtracting the value from k_{obs}. It was corroborated that the k_2' [(2)] term is linearly correlated to the concentration of (2). Under the experimental conditions, the k_d term was smaller by more than 10-fold than the k_2' [(2)] term.

RESULTS

The apparent second-order rate constants (k_2') are plotted as a function of the total concentration of buffer in Figure 1. Examination of Figure 1 reveals that (i) the rate increases linearly with increasing buffer concentration and (ii) the intercept is enhanced drastically at pH region lower than 1. The results indicate that the reaction is catalysed by buffer acid as well as by oxonium ion. Hence, the rate constants can be described by equation (3), in which k_{H_2O} is the water-catalysed term, and k_H and k_{HA} are the catalytic constants

$$k_2' = k_{H_2O}[H_2O] + k_H[H^+] + k_{HA}[HA] \tag{3}$$

for oxonium ion and buffer acid. One can separately estimate $k_{H_2O}[H_2O] + k_H[H^+]$ and k_{HA} from the intercept and the slope in Figure 1. The results are summarized in Table 1. The k_{HA} values are calculated based on the undissociated species of buffer acids.

Here, one should take into account that trifluoroacetophenone is extensively hydrated in an aqueous solution [16,17] and the observed rate is dependent on the concentration of free, unhydrated ketone, (2). We estimated the fraction of free ketone, (2), by the u.v. spectral method [17] and corrected each rate term based on the concentration of free ketone (Table 2).

The pH dependence of $\log(k_{H_2O}[H_2O] + k_H[H^+])$ (corrected) is shown in Figure 2. The logarithm of the rate term is almost constant at pH values higher than 3, while it is approximated with a linear relation of slope —1 at pH values lower than 1. Clearly, the oxonium ion catalysis contributes to the total rate term only in the very acidic pH region. The k_{H_2O} and k_H values were determined from the least-squares computation of a linear plot of $[H^+]$ *vs.* $k_{H_2O}[H_2O] + k_H[H^+]$: k_{H_2O} * $= 2.2 \times 10^{-5}$ l^2 mol^{-2} s^{-1} and $k_H = 4.75 \times 10^{-2}$ l^2 mol^{-2} s^{-1}. The solid curve in Figure 2

TABLE 1

The rate constants for the reduction of (2) [uncorrected for hydration of (2)]

	pK_a	Reaction pH	$k_{HA} \times 10^4$ / l^2 mol^{-2} s^{-1}	$(k_{H_2O}[H_2O] + k_H[H^+]) \times 10^4$ / l mol^{-1} s^{-1}
CF_3CO_2H	0.46	0.46	59.4	47
CCl_3CO_2H	0.60	0.60	28.8	19
$ClCH_2CO_2H$	2.80	2.80	6.80	2.0
CH_3CO_2H	4.70	3.77	1.14	2.2
CH_3CO_2H	4.70	4.70	0.76	2.1

TABLE 2

The rate constants for the reduction of (2) [corrected for hydration of (2)]

	Reaction pH	Fraction of free ketone	$k_{HA} \times 10^4$ / l^2 mol^{-2} s^{-1}	$(k_{H_2O}[H_2O] + k_H[H^+]) \times 10^4$ / l mol^{-1} s^{-1}
CF_3CO_2H	0.46	0.243	244	193
CCl_3CO_2H	0.60	0.223	129	85
$ClCH_2CO_2H$	2.80	0.225	30.2	8.9
CH_3CO_2H	3.77	0.202	5.64	10.9
CH_3CO_2H	4.70	0.196	3.88	10.7
H_2O			0.22	
H_3O^+			475	

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* The water concentration $[H_2O]$ in 25% (v/v) aqueous isopropyl alcohol is 41.7M.

was depicted by using these values. Thus, the third-order rate constants (k_3) for acetic acid and derivatives, oxonium ion, and water were obtained. Figure 3 gives the Brönsted plot for the k_3 term. The least-squares computation of seven data sets provided equation (4) with a correlation

$$\log k_3 = -0.20\ (\pm 0.03)\mathrm{p}K_a - 1.90 \qquad (4)$$

coefficient of 0.948.* In the general-catalysed system, the points for water and hydroxide ion are frequently found to deviate from Brönsted correlations.[19] It seems from Figure 3 that the point for water deviates from the linearity to the upper area and that the slope may be determined by the point for water which has a quite different pK_a value from others. If this point was excluded from the least-squares computation, equation (5) with a better correlation coefficient of 0.982 resulted.

$$\log k_3 = -0.33\ (\pm 0.03)\ \mathrm{p}K_a - 2.30 \qquad (5)$$

DISCUSSION

The dihydronicotinamide reduction produces an anionic charge on the substrate. Taking the reduction of a carbonyl substrate, for instance, the hydrogen transfer results in the alkoxide ion. According to Jencks,[19] concerted general acid-base catalysis of complex reactions can occur at sites that undergo a large change in pK_a and in cases when such a change in pK_a converts an unfavourable into a favourable proton transfer with respect to the catalyst. Thus, in case the pK_a of the product alkoxide is very high relative to that of the catalyst acid, the transition state of the dihydronicotinamide reduction may undergo general-acid catalysis. However, the concerted reaction path requires the additional loss of entropy for the inclusion of a properly located catalyst molecule in the transition state.[19] We speculate, therefore, that general-acid catalysis in the dihydronicotinamide reduction, if it occurs, would become possible (i) when the intramolecular acid source is properly located or (ii) when relatively strong acids are present in the reaction medium.

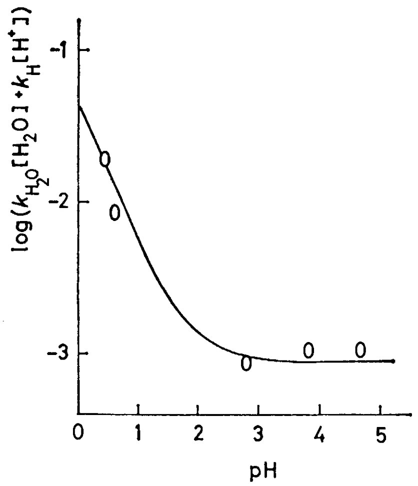

FIGURE 2 The logarithm of $k_{H_2O}[H_2O] + k_H[H^+]$ plotted against pH

It has been known that the *ortho*-hydroxy-group plays an important role in the NADH model reduction of C=X (X = O, N, and S) double bonds attached to the aromatic ring.[20-23] However, the role was left ambiguous because of two undistinguishable effects: [3] that is, the polarization of C=X double bonds through hydrogen bonding in the initial state and the stabilization of the transition state by specific solvation. The latter effect may correspond to the intramolecular general-acid catalysis. In 1973, Wallenfels *et al.*[5] reported that the addition of acetic acid greatly improves the yield of product in the dihydronicotinamide reduction in ethanol of electron-deficient C=C double bonds. This result seems to indicate that the reduction of C=C double bonds might be general-acid catalysed. However, a detailed kinetic investigation showed that the reaction mechanism is a complex one [24] in which general-acid catalysis is not substantiated.

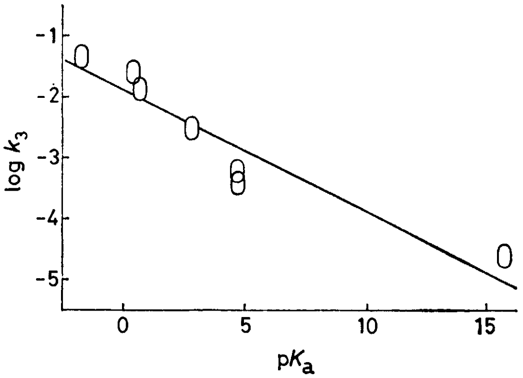

FIGURE 3 Brönsted plots for general-acid catalysis

Recently, van Eikeren and Grier [10] reported on the solvent effect of dihydronicotinamide reductions in connection with the environmental effect of enzymes. They found that the addition of dimethyl sulphoxide to aqueous reaction media led to a remarkable suppression in the rate of the dihydronicotinamide reduction. They emphasized the importance of the protic nature of the solvent during the reduction: that is, the protic solvent stabilises the negative charge developed on atom X in the transition state. This result stimulated us to investigate general-acid catalysis in aqueous acidic media, since acid species would stabilise the developing negative charge more efficiently. However, conventional NADH analogues are so sensitive to acid-catalysed decomposition [13,14] that the NADH model reduction in aqueous acidic media has not been investigated.

Since compound (1) was found to be 10^3—10^4 times more stable to acids than conventional NADH analogues,[12] an accurate determination of the rate constants in the acidic pH region was possible. The results of the kinetic studies are given in Figure 3. It is presumed that the oxonium ion also acts as a general acid since (i) the plot in Figure 3 is approximated by equation (4) and (ii) the hypothetical second-order rate constant for the reduction of protonated (2) would have to be

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* The pK_a values of oxonium ion and water were approximated by −1.75 and 15.7.[18]

10^8—10^9 l mol^{-1} s^{-1} [calculated based on the assumption that pK_a of (2) is *ca.* -10 [25,26]], which is almost comparable with the diffusion-controlled reaction rate. Therefore, specific acid catalysis of the oxonium ion may be disregarded. On the other hand, the catalytic behaviour of water is still ambiguous. The libido rule of Jencks [19] requires that when the pK_a change for proton transfer becomes small or positive, the general-catalysed path should become insignificant and be replaced by a different mechanism (*e.g.* by the stepwise path). The proton transfer from water (pK_a 15.7) [18] to the alkoxide ion is such a case.* It is generally accepted that the water molecule seldom acts as a simple general-acid in the addition reaction toward a carbonyl group.[19] The slope of Brönsted plots was, therefore, calculated by two ways: -0.20 for seven points including water and -0.33 for six points excluding water. In both cases, the slope is exactly compatible with that proposed for general-acid catalysis:[27,28] for example, -0.25 for semicarbazone formation with benzaldehyde derivatives [29] and -0.13 to -0.26 for the addition of thiol anions to acetaldehyde.[30]

In summary our conclusions based on these results show that the NADH model reduction in an aqueous system is general-acid catalysed. The relatively small Brönsted slope suggests that the proton transfer from the acid is involved to a smaller extent in the rate-determining step [27] and the strength of the acid is a less significant factor for the overall reaction rate. Although our conclusions are based on results for the reduction of (2), it should be more generally applicable to the reduction of inactivated carbonyl substrates since for substrates which produce alkoxides with higher pK_a values general-acid catalysis is more necessary.[19] Hopefully, the findings reported here will lead to a more detailed understanding of the mechanism of hydrogen transfer in NADH-dependent dehydrogenases.

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* The pK_a of trifluoromethylphenylcarbinol is presumed to be *ca.* 12 (ref. pK_a of trifluoroethanol, 12.4).[18]

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