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Reduction by a Model of NAD(P)H. XVII. Isotope Effects and Magnesium Ion Catalysis

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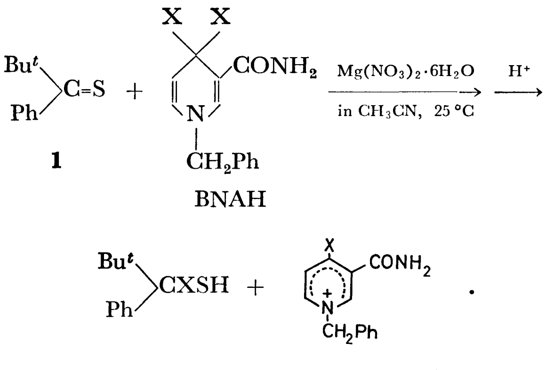
The reduction of thiopivalophenone (**1**) with 1-benzyl-1,4-dihydronicotinamide (BNAH) gives the same value for kinetic deuterium isotope effect (k_H/k_D) and isotopic distribution in the product (Y_H/Y_D) regardless of the presence or absence of magnesium ion. The reduction of methyl benzoylformate (**2**) with a BNAH-analog in the presence of magnesium ion also gives similar values for k_H/k_D and Y_H/Y_D. It has been concluded that magnesium ion stabilizes the transition state in the reduction of **2**, whereas an intermediate is stabilized by magnesium ion in the reduction of **1**.

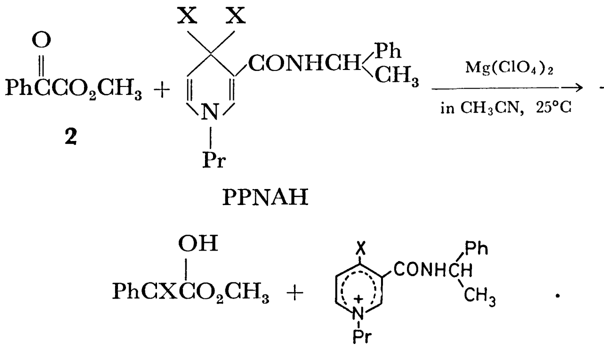
In the preceeding paper, we reported that magnesium ion decelerates the reduction of thiopivalophenone (**1**) with 1-benzyl-1,4-dihydronicotinamide (BNAH).¹⁾ The deceleration was accounted for by the formation of non-reactive complexes, $\mathbf{1}\cdot Mg^{2+}$ and $BNAH\cdot Mg^{2+}$. On the other hand, the reduction of α-keto esters is accelerated by magnesium ion and the acceleration was ascribed to the formation of the reactive complex, $BNAH\cdot Mg^{2+}$.²⁾

This paper describes, based on the results from the study on isotope effects, that such an apparently contradictory phenomenon as $BNAH\cdot Mg^{2+}$ being non-reactive in one reaction but reactive in another is not a real contradiction.



(1)

X=H or D



(2)

X=H or D

TABLE 1. ISOTOPE EFFECTS IN THE REDUCTION OF THIOPIVALOPHENONE

Reductant	$10^3[Mg^{2+}]$, M	10^3k_2, $M^{-1}s^{-1}$ a)	k_H/k_D	Y_H/Y_D
BNAH	0	5.80 ±0.18		
BNAH-d_1	0	3.49 ±0.03	4.9±0.2	5.0±0.4
BNAH-d_2	0	1.16 ±0.03	5.0±0.2	
BNAH	6.00	3.74 ±0.11 b)		
BNAH-d_1	6.00	2.22 ±0.07 b)	5.3±0.2	4.9±0.3
BNAH-d_2	6.00	0.779±0.02 b)	4.8±0.2	

a) $k_2=k_{obsd}/[BNAH\text{-}d_x]$. b) Values obtained under the condition of $[\mathbf{1}]=2.73\times10^{-3}$ M and $[BNAH\text{-}d_x]=5.27\times10^{-2}$ M.

TABLE 2. ISOTOPE EFFECTS IN THE REDUCTION OF METHYL BENZOYLFORMATE IN THE PRESENCE OF MAGNESIUM ION a)

Reductant	$10k_2$, $M^{-1}s^{-1}$ b)	k_H/k_D	Y_H/Y_D
PPNAH	1.46 ±0.04		
PPNAH-d_1	0.900±0.03	4.3±0.2	3.7±0.5 c)
PPNAH-d_2	0.340±0.01	4.3±0.2	

a) Under the condition of $[\mathbf{2}]=1.0\times10^{-2}$ M, $[PPNAH\text{-}d_x]=2.0\times10^{-4}$ M, and $[Mg^{2+}]=1.0\times10^{-2}$ M. b) $k_2=k_{obsd}/[\mathbf{2}]$. c) Under the condition of $[\mathbf{2}]=1.0\times10^{-2}$ M, $[PPNAH]=1.0\times10^{-3}$ M, and $[Mg^{2+}]=2.0\times10^{-3}$ M. $Y_H/Y_D=3.1\pm0.3$ was obtained when each 6.8×10^{-2} M of reagent was used.

Results

All reactions were carried out in acetonitrile at 25 °C. Pseudo-first-roder kinetics for the reduction of methyl benzoylformate (**2**) with *N*-(α-methylbenzyl)-1-propyl-1,4-dihydronicotinamide (PPNAH) was followed spectrophotometrically by observing the decrease in intensity of absorption maximum at 354 nm.²ᵇ⁾ Kinetics for the reduction of **1** with BNAH was followed vapor-phase-chromatographically by observing the decrease in concentration of **1**.¹⁾ It was confirmed that spectrophotometric and vapor-phase-chromatographic methods gave the identical result.¹⁾ It was also confirmed that magnesium nitrate used for the reduction of **1** behaved similarly to magnesium perchlorate.¹⁾ Since the rate of the reduction of **1** is a complex function of the concentration of magnesium ion,¹⁾ the kinetics was followed under a constant concentration of magnesium ion. The rate of the reduction of **2** followed zero-order in magnesium ion under its existence in large excess.²ᵇ⁾ In Tables 1 and 2 are summarized kinetic results together with isotopic ratio (Y_H/Y_D) in the product, 1-phenyl-2,2-dimethyl-1-propanethiol and methyl mandelate, from the reduction with BNAH-4-*d* and PPNAH-4-*d*, respectively. Kinetic isotope effect (k_H/k_D) was calculated after Steffens and Chipman.3) The values for k_H/k_D and Y_H/Y_D were corrected for isotopic contents of deuterated BNAH and PPNAH used.

Discussion

Steffens and Chipman suggested elegantly that there is at least one intermediate in the reduction of α,α,α-trifluoroacetophenone with BNAH or its analogs.3) Their argument based on the fact that kinetic deuterium isotope effect, k_H/k_D, is much smaller than the ratio of the amounts of isotopes incorporated in the product, Y_H/Y_D. Later, Creighton and his coworkers reported similar result in the reduction of 1,10-phenanthroline-2-carbaldehyde.4) We confirmed that the intermediate proposed by these authors is indeed a charge-transfer-type complex.5)

The large discrepancy between the values of k_H/k_D and Y_H/Y_D appears only when the free energy of the transition state for k_1-step in Eq. 3 is slightly larger than that for the k_2-step as shown by a solid line in Fig. 1.

$$S+BH \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} S\cdot BH \xrightarrow{k_2} SH^- + B^+. \qquad (3)$$

The values of k_H/k_D and Y_H/Y_D for the reduction of **1** or hexachloroacetone6) are equal, respectively, which indicates that the free energy of the transition state for the k_2-step is much larger than that for the k_1-step in this reduction as shown in Fig. 1 by a dotted line. Note that both **1** and hexachloroacetone are substrates that can be reduced without the assistance of metal ion. When metal ion is added to the reaction system, the metal ion may form a stable ternary complex, say, **1**$\cdot$Mg$^{2+}\cdot$BNAH.1) If the free energy of the complex becomes small enough so that the activation energy for the k_2-step becomes larger than that for the k_1-step, the net reduction (more precisely, the transfer of a proton) through the ternary complex becomes difficult to proceed (dashed line in Fig. 1). This argument is partly supported by the following observation:7) 4,4′-Dichlorobenzil is reduced by BNAH in the presence of magnesium ion in acetonitrile. However, no ESR-signal was detected from the reaction solution. When acetonitrile was substituted by dimethyl sulfoxide, no reduction took place, whereas strong ESR-signal from 4,4′-dichlorobenzil anion radical paired by magnesium ion was recorded. Even in the absence of magnesium ion, weak ESR-signal of the anion radical could be recorded from the mixture in dimethyl sulfoxide. This observation reveals that the charge-transfer intermediate is too unstable in the reacting system to be detected by ESR-spectroscopy. When the solvent is changed to the one which stabilizes the intermediate, the net reduction is prohibited because the k_2-step becomes unfavorable and the intermediate is accumulated. Magnesium ion increases the stability of the intermediate.8)

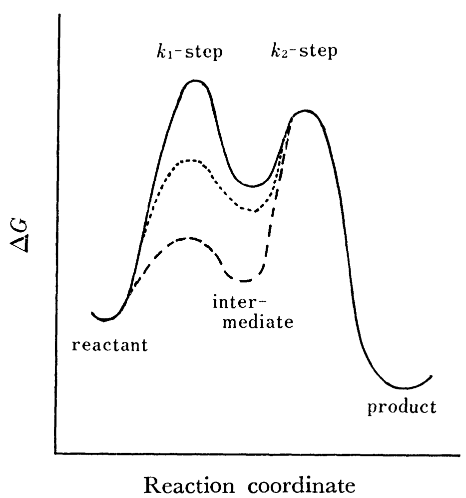


Fig. 1. Schematic energy diagram.

Reduction of **2** in acetonitrile does not take place without magnesium ion. Therefore, there is no doubt that magnesium ion plays a role to reduce the free energy of a transition state for either of k_1- or k_2-step, or both of this reaction. However, it is highly unlikely to expect that the process of electron-transfer is associated with such a large kinetic isotope effect as 4.3, the value which is comparable to that of **1**. This leads to a conclusion that magnesium ion catalyzes the k_1-step, or an electron-transfer process, and the energy diagram for this reaction may be represented by the dotted line in Fig. 1. The similarity in the values of Y_H/Y_D for the reductions of **1** and **2** suggests that the positions of transition states for these reactions may be similar.

It is noteworthy that the ternary complex, **2**$\cdot$Mg$^{2+}\cdot$PPNAH, has been proposed to be the transition state,2) whereas the ternary complex, **1**$\cdot$Mg$^{2+}\cdot$BNAH, is in the ground state.1) The metal ion complex must stabilize the transition state of the reaction in order to assist the reaction effectively, and metal ion complex formation in the ground state depresses the reaction rate. In true catalyses such as enzymic catalysis the strongest coordination must be to the transition state, and not to the reactant or product.9)

Klinman argued on the transition state of enzymic reductions.10) Kinetic isotope effects and substituent effects10,11) reconcile with the energy diagram schematically shown by the dotted line in Fig. 1 provided general-acid catalysis is assumed for the reduction with alcohol dehydrogenases.12–15) Furthermore, it is interesting to point out that the energy diagrams for the present model reactions with **1** and **2** without magnesium ion correspond fortuitously with those for enzymic reductions of ketones and α-keto esters, respectively. The rate-determining step for the reaction with an alcohol dehydrogenase is the process of a proton-transfer, whereas that with a lactate dehydrogenase is the process that takes place prior to the proton-transfer.16)

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Experimental

Preparation, purification, and identification of materials were described previously.1,2,17) Procedures for the kinetics and the analyses of isotopic contents were also described in previous papers.[1,2b)]

The deuterium contents in BNAH-4-*d* or PPNAH-4-*d* were 99.0±1%, and those in BNAH-4,4-d_2 or PPNAH-4,4-d_2 were 91.5±1—94.5±1% depending on the runs of preparation. For the calculation of the purity of dideuterated compound, no contamination of undeuterated compound was assumed.

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