290 BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 51 (1), 290—293 (1978) [Vol. 51, No. 1

Reduction by a Model of NAD(P)H. XVI. Effect of Magnesium Ion for the Reduction of Thiopivalophenone

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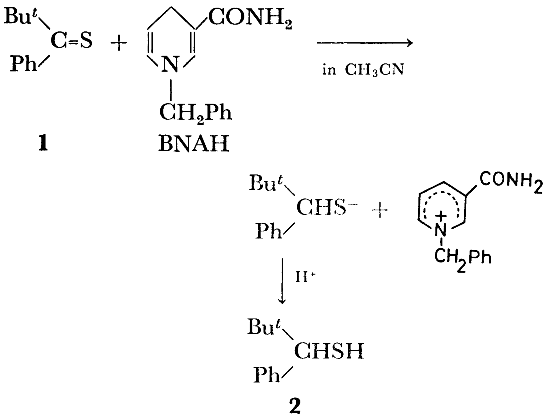
(Received July 11, 1977)

Magnesium ion retards the reduction of thiopivalophenone with 1-benzyl-1,4-dihydronicotinamide (BNAH) in acetonitrile. Kinetics and other investigation have revealed that the retardation is due to the formation of non-reactive complexes, thiopivalophenone·Mg^{2+} and BNAH·Mg^{2+}.

It has been reported that the reduction of certain substrates, namely those that contain a pyridine moiety, with a derivative of 1,4-dihydronicotinamide is accelerated by a divalent metal ion such as magnesium or zinc ion.[1-4] The acceleration has been interpreted in terms of bidentate chelation of a metal ion onto the substrate to polarize the carbonyl group to be reduced. We reported that magnesium or zinc ion also promotes the reduction of α-keto esters, and the chelation of a metal ion onto a 1,4-dihydronicotinamide derivative was attributed to the promotion of reduction.[5] The polarization of the carbonyl group in an α-keto ester takes place only at the transition state of the reduction.[6]

Hexachloroacetone can be appointed for a third type substrate. The reduction of this compound is decelerated by the addition of a metal ion.[7] These authors proposed that the deceleration is caused by the formation of a complex between a 1,4-dihydronicotinamide derivative and a metal ion.[7]

In order to obtain a universal insight into the role of a metal ion, we studied the reduction of thiopivalophenone (**1**), a substrate in the third type, with 1-benzyl-1,4-dihydronicotinamide (BNAH) in the presence or absence of magnesium ion.



Results

The reaction of **1** with an equivalent molar amount of BNAH in dry acetonitrile at a room temperature afforded 1-phenyl-2,2-dimethyl-1-propanethiol (**2**) in quantitative yield. The direct transfer of a hydrogen at the C_4-position of BNAH onto the C_1-position of the product was confirmed by an experiment with BNAH-4,4-d_2.

As a source of magnesium ion, magnesium nitrate was employed for most reactions. It was confirmed that the substitution of the salt for magnesium perchlorate did not affect the kinetics. Magnesium nitrate contains six molecules of water of crystallization. However, that the water of crystallization did not affect the kinetics was tested with a run with more than equivalent amount of water in the absence of magnesium nitrate.

Kinetics was followed vapor-phase-chromatographically by observing the decrease in the amount of **1**, or,

TABLE 1. RATE CONSTANT FOR THE REACTION OF THIOPIVALOPHENONE (**1**) WITH 1-BENZYL-1,4-DIHYDRONICOTINAMIDE (BNAH) IN ACETONITRILE AT 25 °C

10^3[**1**], M	10^2[BNAH], M	10^4k_{obsd}, s^{-1}	$r^{a)}$	10^3k_2, M^{-1} s^{-1} $^{b)}$
2.80	5.80	3.37	0.9996	5.82
2.80	5.83	3.38	0.9995	5.79
2.80	2.95	1.73	0.9993	5.86
3.34	3.69	2.13	0.9990	5.77
41.3$^{c)}$	0.175	2.18	0.9987	6.05
2.78$^{d)}$	5.81	3.25	0.9997	5.59
2.86$^{e)}$	5.90	3.93	0.9995	6.66

a) Correlation coefficient. b) $k_2=k_{obsd}$/[BNAH]. Estimated error in k_2 is ±3%. c) Followed spectrophotometrically. d) The solution contains 2.78×10^{-2} M water. e) The solution contains 1.18×10^{-2} M ammonium perchlorate.

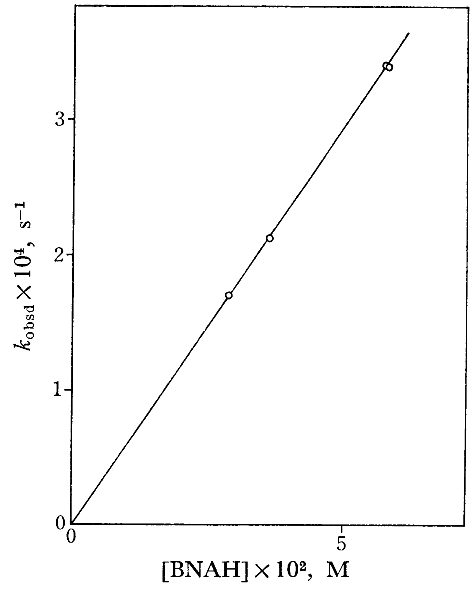


Fig. 1. A linear relationship between observed pseudo-first-order rate constant and the concentration of BNAH, from which the second-order rate constant at 25 °C was calculated.

Table 2. Rate constant for the reaction of thiopivalophenone (**1**) with 1-benzyl-1,4-dihydronicotinamide (BNAH) in the presence of magnesium ion in acetonitrile at 25 °C

10^3[**1**], M	10^2[BNAH], M	10^4[Mg^{2+}], M	$10^4 k_{obsd}$, s^{-1}	r[b)]
2.80	5.81	3.76	3.29	0.9998
2.80	5.87	7.57	3.31	0.9996
2.80	5.82	9.44	3.37	0.9997
1.96	5.84	11.27	3.29	0.9995
3.34	2.74	11.90	1.26	0.9994
2.82	4.90	11.93	2.53	0.9998
2.84	6.00	11.93	3.34	0.9997
2.82	13.71	12.00	1.74	0.9998
2.80	2.91	18.80	1.25	0.9998
2.80	5.85	18.86	3.05	0.9995
1.40	2.92	18.92	1.36	0.9997
2.69	5.80	20.44	2.97	0.9993
2.80	5.81	27.55[c)]	2.92	0.9999
2.80	5.85	28.28	2.94	0.9999
2.78	2.81	29.76	1.01	0.9992
2.82	4.91	29.84	2.14	0.9994
2.84	6.15	29.84	2.85	0.9996
3.34	3.80	30.00	1.64	0.9993
2.80	5.83	37.83	2.73	0.9997
2.89	5.86	40.01	2.55	0.9999
2.80	5.80	40.87	2.46	0.9998
2.80	5.78	41.77	2.78	0.9997
2.89	5.85	46.95[c)]	2.41	0.9995
2.80	5.71	58.48	2.20	0.9996
2·78	2.97	59.51	0.717	0.9994
2.82	4.88	59.67	1.61	0.9992
2.84	6.04	59.67	2.28	0.9998
3.34	3.72	59.98	1.09	0.9996
2.80	5.82	75.47	2.07	0.9992
2.78	4.46	80.34	1.14	0.9998
2.79	3.82	80.34	0.934	0.9994
2.83	5.91	80.34	1.91	0.9996
2.88	8.63	80.34	4.06	0.9996
2.80	5.82	83.46	1.75	0.9993
43.3[d)]	0.175	13.10	2.03	0.9989
32.8	0.165	49.90	0.494	0.9983

a) $Mg(NO_3)_2 \cdot 6H_2O$ was used unless otherwise indicated. b) Correlation coefficient. c) $Mg(ClO_4)_2$ was used. d) Followed by spectrophotometry.

in part, spectrophotometrically by observing the decrease in the intensity of absorption at 350 nm (from BNAH). Each run gave a good pseudo-first-order correlation ($r > 0.999$). From the result listed in Table 1, the second-order rate constant, k_2, at 25 °C was calculated to be 5.80×10^{-3} M^{-1} s^{-1} (see Fig. 1). Table 2 summarizes kinetic result in the presence of magnesium ion.

Discussion

The fact that two different methods gave the same pseudo-first-order rate constant indicates that the decreases of the concentration of BNAH corresponds directly to that of **1**.[8)]

In contrast to the reduction of α-keto esters, the reduction of **1** is retarded by the presence of magnesium ion. On the other hand, ammonium perchlorate results in slight increase in the rate, probably due to a salt effect. In the presence of magnesium ion, the second-order rate constants, k_2, tentatively calculated by dividing k_{obsd} by the concentration of BNAH gave neither a constant value nor a simple dependence on the concentration of magnesium ion. In previous papers we reported that magnesium ion forms a complex with BNAH.[6)] However, the complex-formation alone does not explain the present result. That is, one cannot expect the change in the concentration of BNAH.

In analogy with a treatment for kinetics of fully and noncompetitively inhibited enzymic reactions,[9)] plots were made for $1/k_{obsd}$ against 1/[BNAH] for each constant concentration of magnesium ion. The result is illustrated in Fig. 2, from which it is obvious that the substrate, **1**, forms a non-reactive (noncompetitive) complex with magnesium ion. Moreover, the plots give curved lines instead of linear relationships. The result can be interpreted with the following reaction scheme:

$$\mathbf{1} + \mathrm{BNAH} \xrightleftharpoons{K_1} \mathbf{1}\cdot\mathrm{BNAH} \xrightarrow{k_2} \text{Products}$$

$$\mathbf{1} + \mathrm{Mg^{2+}} \xrightleftharpoons{K_3} \mathbf{1}\cdot\mathrm{Mg^{2+}}$$

$$\mathrm{BNAH} + \mathrm{Mg^{2+}} \xrightleftharpoons{K_4} \mathrm{BNAH}\cdot\mathrm{Mg^{2+}}$$

$$\mathbf{1}\cdot\mathrm{Mg^{2+}} + \mathrm{BNAH} \xrightleftharpoons{K_5} \mathbf{1}\cdot\mathrm{Mg^{2+}}\cdot\mathrm{BNAH}$$

$$\mathrm{BNAH}\cdot\mathrm{Mg^{2+}} + \mathbf{1} \xrightleftharpoons{K_6} \mathbf{1}\cdot\mathrm{Mg^{++}}\cdot\mathrm{BNAH}$$

$$\mathbf{1}\cdot\mathrm{BNAH} + \mathrm{Mg^{2+}} \xrightleftharpoons{K_7} \mathbf{1}\cdot\mathrm{Mg^{2+}}\cdot\mathrm{BNAH}.$$

Based on the above reaction scheme, the concentrations of free **1** and BNAH are expressed by Eqs. 1 and 2, respectively.[10)]

$$[\mathbf{1}] = \frac{[\mathbf{1}]_0}{1+[\mathrm{BNAH}]/K_1+[\mathrm{Mg^{2+}}]/K_3+[\mathrm{BNAH}][\mathrm{Mg^{2+}}]/K_1K_7}, \tag{1}$$

$$[\mathrm{BNAH}] = \frac{[\mathrm{BNAH}]_0}{1+[\mathbf{1}]/K_1+[\mathrm{Mg^{2+}}]/K_4+[\mathbf{1}][\mathrm{Mg^{2+}}]/K_1K_7}, \tag{2}$$

where the subscript 0 denotes a stoichiometric concentration. Since

$$v = k_2[\mathbf{1}\cdot\mathrm{BNAH}] = k_2[\mathbf{1}][\mathrm{BNAH}]/K_1, \tag{3}$$

when A, B, C, and D are defined by Eqs. 4—7, the rate expression shown in Eq. 8 is obtained.

$$A = K_1K_7(K_3+[\mathrm{Mg^{2+}}])(1+[\mathrm{Mg^{2+}}]/\mathrm{K}_4), \tag{4}$$

$$B = K_3\ (K_7+[\mathrm{Mg^{2+}}]), \tag{5}$$

$$C = (K_3+[\mathrm{Mg^{2+}}])(K_7+[\mathrm{Mg^{2+}}]), \tag{6}$$

$$D = \frac{1}{2C}\left(\sqrt{\{A+B([\mathrm{BNAH}]_0-[\mathbf{1}]_0)\}^2+4AB[\mathbf{1}]_0} - \{A+B([\mathrm{BNAH}]_0-[\mathbf{1}]_0)\}\right), \tag{7}$$

$$\frac{1}{v} = \frac{K_1}{k_2[\mathrm{BNAH}]_0}\left\{\frac{1}{K_1}\left(1+\frac{[\mathrm{Mg^{2+}}]}{K_7}\right)+\frac{1}{D}\left(1+\frac{[\mathrm{Mg^{2+}}]}{K_4}\right)\right\}. \tag{8}$$

The denominator of the right-hand side in Eq. 8 is composed of the second-, three-halves-, and first-order terms of the stoichiometric concentration of BNAH in accord with the observed result. The result leads to a conclusion that both BNAH-magnesium ion and **1**-magnesium ion complexes are non-reactive, which is a marked contrast with the reaction of α-keto esters. It is known that thioketones form complexes with various metal salts[11] as well as with BNAH.[12]

The validity of the proposed reaction scheme can be tested in several ways. In the range of BNAH-concentrations where the curves in Fig. 2 can be approximated by straight lines, slopes of the lines, m, are expressed by Eq. 9.

$$m = \frac{1}{k_2}\left(1+\frac{[\mathrm{Mg}^{2+}]}{K_7}\right). \tag{9}$$

A plot of m against the concentration of magnesium ion indeed gave a straight line as shown in Fig. 3. From the intercept and slope the values of k_2 and K_7 were calculated to be $5.81\times10^{-3}\,\mathrm{M}^{-1}\,\mathrm{s}^{-1}$ and $3.77\times10^{-3}\,\mathrm{M}$, respectively. Thus, calculated rate constant is identical to the observed one.[13]

The dependence of the rate constants in the presence and absence of magnesium ion on the reaction temperature is listed in Table 3, from which the energy of activations in the presence and absence of magnesium ion were calculated to be identical (8.9±0.5 and 8.0±0.3 kcal/mol for reactions with and without magnesium ion, respectively).[14]

The reduction of **1** with optically active N-(α-methylbenzyl)-1-propyl-1,4-dihydronicotinamide (**3**) affords optically active **2**. The reductions with R-**3** in the presence and absence of magnesium ion resulted in the formation of **2** of $[\alpha]_D = -5.8$ and -6.2, respectively. Although we do not know the degree of rotation for optically pure **2**, there is no doubt that both reactions gave the identical stereochemical result.

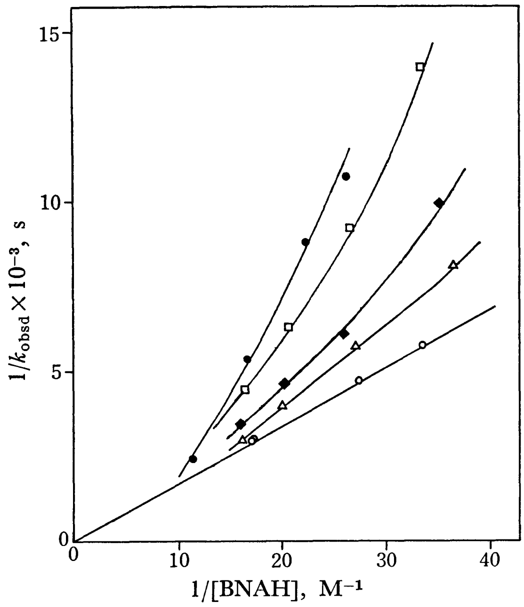


Fig. 2. Correlation of $1/k_{\mathrm{obsd}}$ against 1/[BNAH] for constant concentrations of magnesium ion: ○ $[\mathrm{Mg}^{2+}]=0$; △ $[\mathrm{Mg}^{2+}]=1.2\times10^{-3}$ M; ◆ $[\mathrm{Mg}^{2+}]=3.0\times10^{-3}$ M; □ $[\mathrm{Mg}^{2+}]=6.0\times10^{-3}$ M; ● $[\mathrm{Mg}^{2+}]=8.0\times10^{-3}$ M.

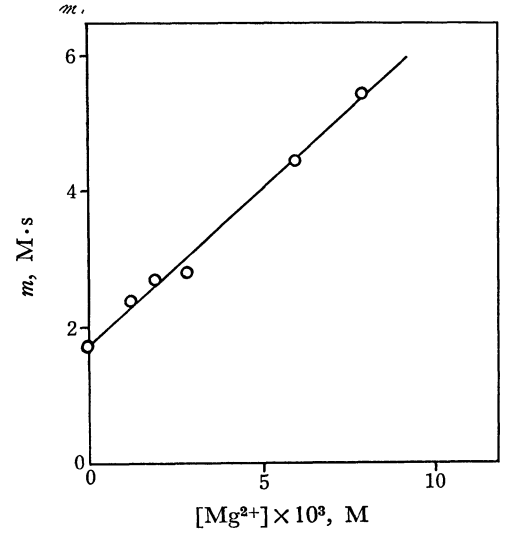


Fig. 3. Dependence of m on the concentration of magnesium ion.

TABLE 3. TEMPERATURE-DEPENDENCE OF RATE CONSTANTS IN PRESENCE AND ABSENCE OF MAGNESIUM ION[a)]

$10^3[\mathrm{Mg}^{2+}]$, M[b)]	Temp, °C[c)]	k
6.00	14	0.672[d)]
6.00	25	1.09[d)]
6.00	30	1.58[d)]
0	10	2.83[e)]
0	17	3.98[e)]
0	25	5.80[e)]
0	30	7.12[e)]

a) Under the condition of $[\mathbf{1}]=2.80\times10^{-3}$ M and $[\mathrm{BNAH}]=3.72\times10^{-2}$ M. b) $\mathrm{Mg(NO_3)_2\cdot6H_2O}$ was used. c) ±0.05 °C. d) 10^4k_{obsd}, s^{-1}. e) 10^3k_2 $(=10^3\,k_{\mathrm{obs}}/[\mathrm{BNAH}]$, M^{-1} s^{-1}.

The final diagnosis was made by kinetic deuterium isotope effect using BNAH-4,4-d_2. The isotope effects, k_H/k_D, for both reactions were found to be identical (5.0±0.2).

All of above results indicate that magnesium ion is not involved in the product-forming process and that the polarization of a substrate does not necessarily result in the acceleration of the reduction. Detailed discussion on the difference in reactivities of **1** and α-keto esters will appear in the following paper.[15]

Partial support in the form of a Scientific Research Grant from the Ministry of Education, Japan, is acknowledged.

Experimental

Materials. Thiopivalophenone[16,17] and 1-benzyl-1,4-dihydronicotinamide[18] were prepared according to literatures. 1-Benzyl-1,4-dihydronicotinamide-4,4-d_2 was prepared from BNA$^+$Cl$^-$ by three times repetition of $\mathrm{D_2O}$–$\mathrm{Na_2S_2O_4}$-reduction—malachite green-oxidation.[18] Magnesium nitrate and perchlorate were commercially available (Wako Chem. Co.) and purified prior to use.[6b)] Acetonitrile was distilled once on phosphorus pentaoxide and kept over molecular sieves 4A.

Procedure. Preparation of solutions for kinetic studies and the method to follow the kinetics by spectrophotometry were described previously.[6)] For kinetic observations under the conditions of excess BNAH, 1 ml aliquots were taken out from a 20 ml reaction solution after appropriate time intervals and poured into 5 ml of dilute hydrochloric acid. The organic materials were extracted with 5 ml hexane. The hexane-layer (20 μl) was subjected to VPC (Yanagimoto G-18000, FID, 15% BDS, 1 m, 135 °C). 1,4-Dipropoxybenzene was used as an internal standard. At least 10 points were obtained for a run. The reaction temperatures were kept constant within the accuracy of ±0.05 °C. Least-squares method was employed to evaluate linear relashioships.

Deuterium contents were analyzed on a Varian T-60 NMR Spectrometer and Shimadzu LKB-9000S GC-MS Spectrometer.[19,20)] The optical activity of the thiol was measured on a Perkin-Elmer 241 Polarimeter (30 mg/2 ml EtOH).

The method for isolation and identification of 1-phenyl-2,2-dimethyl-1-propanethiol was the same as described previously for the case of 2,2,4,4-tetramethyl-3-pentanethiol.[21)]

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13) K_4 was observed to be 5.56×10^{-4} M. See Ref. 6b.

14) Since the observed rate constant in the presence of magnesium ion is a complex function of the concentration of magnesium ion, the intercept of the line obtained by plotting $\ln k_{\mathrm{obsd}}$ against $1/T$ has no physical meaning. From runs without magnesium ion, $\Delta H^{*} = 8.56 \pm 0.3$ kcal/mol and $\Delta S^{*} = -56.3 \pm 2$ eu were calculated. The values are comparable to those of a similar reaction[7)] and reasonable for the proposed mechanism.

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