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Kinetic Studies on the Formation of Dimethylnitrosamine

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The formation of dimethylnitrosamine was studied kinetically in aqueous acetate and nitrite buffers of pH 4.0, using the initial rate method. The reaction rate was proportional to the dimethylamine concentration and to 1.8 power of the nitrite concentration. It was found that first- and second-order reactions of nitrite occurred concurrently, and that both of them were catalyzed by acetate ion. It is concluded that essentially the same factors govern the formation of dimethylnitrosamine as those found in diazotization.

The nitrosation of primary aromatic amines, which represents slow steps in diazotization, has been studied extensively by many workers, and its mechanism seems to be well established.[2-6] However, there have been few kinetic studies on the nitrosation of aliphatic secondary amines, probably because the rate of this reaction is slow and nitrous acid is unstable at elevated temperatures. Taylor and Price reported kinetic studies on the reaction of aliphatic amines with nitrite,[7] and recently Mirvish reported kinetic studies on dimethylamine formation in buffered aqueous solutions of various pH values.[8] In both reports only one kinetic term was demonstrated, which was first-order for amine and second-order for nitrite. Thinking that essentially the same kinetic terms should be observed for nitrite and other catalyzing nucleophiles as those observed in diazotization, we studied the formation of dimethylnitrosamine kinetically. According to Mirvish[8] the rate of dimethylnitrosamine formation was maximal between pH 3 and 4 at 25°, so we examined the reaction in acetate and nitrite buffers of pH 4. Dialkylnitrosamines in foods and biochemical systems have received considerable attention because of their carcinogenicities.

Experimental

Materials——Reagent grade sodium nitrite was dried over phosphorous pentoxide. Aqueous dimethylamine solutions were standardized with hydrochloric acid before use. Dimethylnitrosamine used to make the calibration curve (see below) was prepared by the method of Vogel.[9] Sodium perchlorate was recrystallized from 90% aqueous ethanol. The other chemicals used were reagent grade. Deionized water was used throughout.

Apparatus——Polarographic measurements were made with a Shimadzu, type RP-50, polarograph. A Toadempa, model HM-5A, pH-meter with glass and saturated calomel electrodes was used for pH-measurements; the saturated calomel electrode was connected by an agar bridge containing 1 N sodium chloride.

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1) Location: *Toneyama, Toyonaka, Osaka.*
2) *a*) E.D. Hughes, C.K. Ingold, and J.H. Ridd, *J. Chem. Soc.*, **1958**, 58; *b*) *Idem, ibid.*, **1958**, 65; *c*) *Idem, ibid.*, **1958**, 70; *d*) *Idem, ibid.*, **1958**, 77; *e*) *Idem, ibid.*, **1958**, 82; *f*) *Idem, ibid.*, **1958**, 88.
3) *a*) G. Stedman, *J. Chem. Soc.*, **1959**, 2943; *b*) *Idem, ibid.*, **1959**, 2949; *c*) C.A. Bunton and G. Stedman, *ibid.*, **1959**, 3466; *d*) G. Stedman, *ibid.*, **1960**, 1702.
4) T.A. Turney and G.A. Wright, *Chem. Rev.*, **59**, 497 (1959).
5) J.H. Ridd, *Quart. Rev.*, **15**, 418 (1961).
6) A.T. Austin, *Sci. Progr.* (London), **49**, 619 (1961).
7) T.W.J. Taylor and L.S. Price, *J. Chem. Soc.*, **1929**, 1897.
8) S.S. Mirvish, *J. Nat. Cancer Inst.*, **44**, 633 (1970).
9) A.I. Vogel, *J. Chem. Soc.*, **1948**, 1846.

Kinetic Measurements——All reactions were carried out at 25±0.05° in a 20 ml volumetric flask. The reaction in an acetate buffer of pH 4.0 was initiated by adding an aqueous solution of freshly prepared sodium nitrite to a solution of dimethylamine in the buffer. The reaction in a nitrite buffer of pH 4.0 was initiated by adding an aqueous solution of dimethylamine, adjusted to pH 4.0 with perchloric acid, to the freshly prepared nitrite buffer. When necessary, the ionic strength was adjusted by addition of a calculated amount of sodium perchlorate. At appropriate intervals 0.5 or 1.0 ml of the reaction solution was pippetted into 2 ml of 0.5N sulfamic acid in a 10 ml volumetric flask. The mixture was subjected to polarographic measurements after adding 0.05 ml of 1% gelatine solution and a necessary amount of 0.13N sodium chloride solution. The amount of dimethylnitrosamine was determined by comparing the polarographic wave height with values on a calibration curve prepared with authentic samples.

When the reaction rate was analyzed using integrated rate equations, none of the following three kinetics[2] gave satisfactory results, since in all cases rate constants gradually decreased with time (Table I);

a. Third-Order (k_3): Second-order for nitrite and first-order for amine.
b. Second-Order (k_2): First-order for nitrite and first-order for amine.
c. Second-Order (k_2'): Second-order for nitrite and zero-order for amine.

TABLE I. Values of Various Rate Constants Calculated from Integrated Rate Equations (see text)[a]

Time (min)	10^2k_3 ($M^{-2}min^{-1}$)	10^3k_2 ($M^{-1}min^{-1}$)	$10^3k_2'$ ($M^{-1}min^{-1}$)
30	5.97	7.67	3.02
60	5.91	7.58	2.89
90	6.11	7.52	2.83
160	5.77	7.46	2.59
210	5.66	6.91	2.51
280	5.23	6.36	2.30
370	5.10	6.13	2.19
450	5.08	6.07	2.12

a) pH 4.13 (1.00M acetate); 25°; μ=0.3;
initial total dimethylamine concentration, 4.89×10^{-2}M;
initial total nitrite concentration, 1.26×10^{-1}M

The results in Table I are mainly correlated with the decomposition of nitrite under the experimental conditions.[10] The decomposition of nitrite was examined and the results are summarized in Table II. The concentration of nitrite was determined by iodometric titration. As seen in Table II, *ca.* 7% of nitrite was decomposed in 6 hr. These preliminary results indicate that the reaction could not be analyzed using integrated rate equations, so the initial rate method was used for its analysis. The initial rate was obtained from the slope of concentration-time curve at time zero.

TABLE II. Decomposition of Nitrite at pH 4.03 (1.00M acetate) and 25°

Time (min)	10^2[nitrite] (M)	Time (min)	10^2[nitrite] (M)	Time (min)	10^2[nitrite] (M)
5	9.96	6	4.90	3	2.65
95	9.88	60	4.89	60	2.58
158	9.71	120	4.80	120	2.55
215	9.53	180	4.77	180	2.54
275	9.53	240	4.71	240	2.46
335	9.31	300	4.62	300	2.46
		360	4.50	360	2.39

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[10] The results in Table I suggest that the order of the reaction is more than third, if the decrease in the rate constants is due to erroneous assumptions on the order of the reaction.

Results and Discussion

Reaction Order with Respect to Amine and Nitrite in Acetate Buffer

The initial rate of formation of dimethylnitrosamine showed strictly first-order dependence on the concentration of amine, as illustrated in Fig. 1. On the other hand, for nitrite the order was 1.82 (Fig. 1). A non-integral order has also been reported for the diazotization of aniline, and explained by the concurrent contributions of first- and second-order terms.[2c,3d,11] When the value of the hypothetical third-order rate constant, $k_{3\text{obs}}$, defined as in equation (1), was plotted against the reciprocal of the initial stoichiometric nitrite concentration, a straight line was obtained (Fig. 2 line A);

$$(dp/dt)_{t=0} = k_{3\,\text{obs}}[\text{nitrite}]_0^2[\text{amine}]_0 \tag{1}$$

where $(dp/dt)_{t=0}$ represents the initial rate of formation of dimethylnitrosamine, and $[\text{nitrite}]_0$ and $[\text{amine}]_0$ indicate the initial stoichiometric concentrations of nitrite and dimethylamine, respectively. If nitrite is assumed to contribute first- and second-order terms, equation (1) will be modified to equation (2).

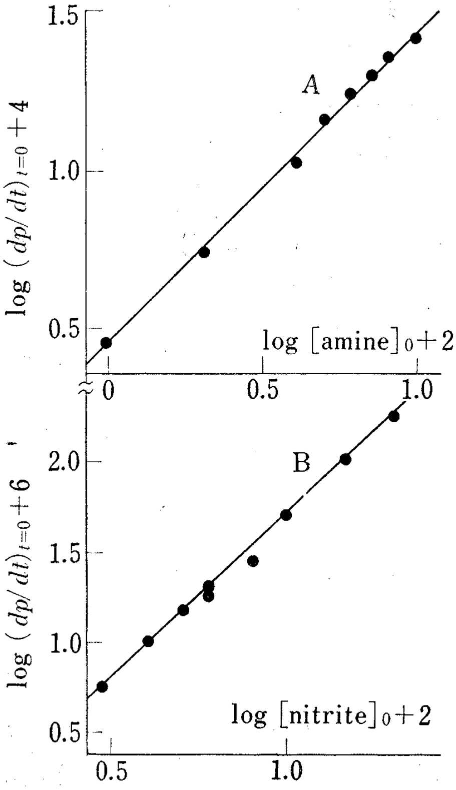

Fig. 1. Dependence of the Initial Rate on the Concentrations of Dimethylamine (line A)[a] and Nitrite (line B)[b]; pH 4.0 (4.5M acetate), 25°, $\mu=0.8$

a) total nitrite concentration, 2.02×10^{-1}M; slope=1.00

b) total dimethylamine concentration, 5.32×10^{-1}M; slope=1.82

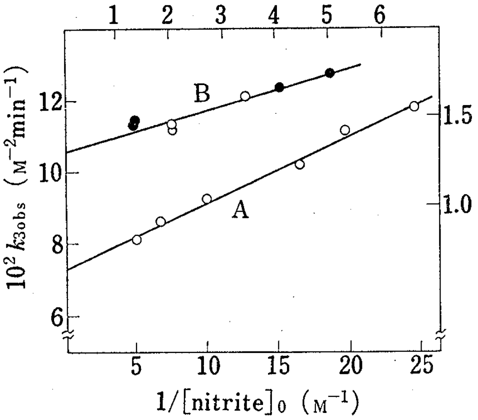

Fig. 2. Variation of the Hypothetical Third-order Rate Constant with Concentration of Nitrite, at 25,° $\mu=0.8$

line A (left ordinate and lower abscissa): in 4.5M acetate buffer, pH 4.0; total dimethylamine concentration, 5.32×10^{-1}M; slope=1.83×10^{-3}; intercept=7.33×10^{-2}; $r=0.996$. line B (right ordinate and upper abscissa): in nitrite buffer, pH 4.0; total dimethylamine concentration, ○ 2.03×10^{-2}M; ● 4.05×10^{-2}M; slope=8.19×10^{-4}; intercept=1.32×10^{-2}; $r=0.957$.

$$(dp/dt)_{t=0} = k_3[\text{nitrite}]_0^2[\text{amine}]_0 + k_2[\text{nitrite}]_0[\text{amine}]_0 \tag{2}$$

where k_3 and k_2 are third- and second-order rate constants, respectively.

From equations (1) and (2),

$$k_{3\,\text{obs}} = k_3 + k_2/[\text{nitrite}]_0 \tag{3}$$

The values of k_3 and k_2 were estimated from the slope and the intercept of the straight line, respectively, as $k_3=7.33\times10^{-2}$ $\text{M}^{-2}\,\text{min}^{-1}$, and $k_2=1.83\times10^{-3}$ $\text{M}^{-1}\,\text{min}^{-1}$.

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11) C.A. Bunton and M. Masui, *J. Chem. Soc.*, **1960**, 304.

Acetate Ion Catalysis

Fig. 3 shows the effect of acetate ion on $k_{3\text{obs}}$, the hypothetical third-order rate constant, at constant nitrite (1.5×10^{-1} M) and amine concentrations. The straight line conforms to equation (4) with $k_0=1.73\times10^{-2}$ M^{-2} min^{-1} and $k_{\text{OAc}}=1.01\times10^{-1}$ M^{-3} min^{-1},

$$k_{3\,\text{obs}} = k_0 + k_{\text{OAc}}[\text{AcO}^-] \qquad (4)$$

where $[\text{AcO}^-]$ represents the acetate ion concentration.

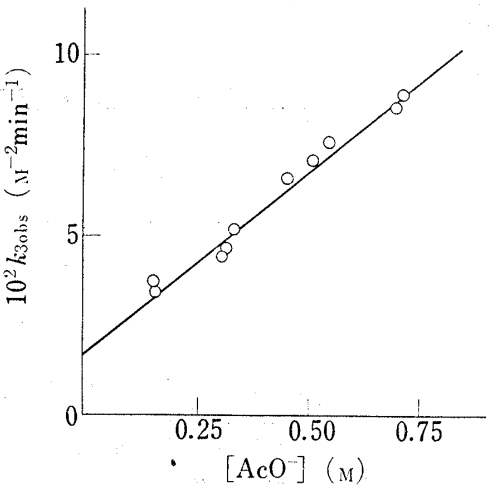

Fig. 3. Effect of Concentration of Acetate Buffer on Hypothetical Third-order Rate Constant, pH 4.0, 25°, $\mu=0.8$

total nitrite concentration, 1.50×10^{-1}M; total dimethylamine concentration, 1.62×10^{-1}M; slope$=1.01\times10^{-1}$; intercept$=1.73\times10^{-2}$; $r=0.983$.

Reaction in Nitrite Buffer

The hypothetical third-order rate constant, $k_{3\text{obs}}$, was plotted against the reciprocal of the nitrite concentration in the same way as for the reaction in the acetate buffer (Fig. 2 line B). Values of $k^0{}_3=1.32\times10^{-2}$ M^{-2} min^{-1} and $k^0{}_2=8.19\times10^{-4}$ M^{-1} min^{-1} were estimated form the slope and the intercept of the line, respectively. In Fig. 2 line B, the value of $k_{3\text{obs}}$ extrapolated to $[\text{nitrite}]_0=1.5\times10^{-1}$M is 1.87×10^{-2}M^{-2}min^{-1}, which is in good agreement with the value obtained by extrapolation to $[\text{AcO}^-]=0$ in Fig. 3 ($k_{3\text{obs}}=k_0=1.73\times10^{-2}$M^{-2}min^{-1}).

The results described so far indicate that both first- and second-order reactions of nitrite occur concurrently. Similar first- and second-order contributions of nitrite have been demonstrated in the nitrosation of N-methylaniline in solutions of fairly high acidities.[12] In an acetate buffer the over-all nitrosation was catalyzed by acetate ion (Fig. 3).[13] Three possible mechanisms of catalysis of the over-all reaction by acetate may be considered. Namely, acetate ion may catalyze both the first- and second-order reactions of nitrite, or either of them. Only the first mechanism is possible because in the latter two cases either $k_2=k^0{}_2$ or $k_3=k^0{}_3$ should be observed, whereas the results show that they are not. For the first mechanism the following equations should be valid;

$$k_{\text{OAc}} = k_{3\,\text{OAc}} + k_{2\,\text{OAc}}/[\text{nitrite}]_0 \qquad (5)$$

$$k_2 = k_2{}^0 + k_{2\,\text{OAc}}[\text{AcO}^-] \qquad (6)$$

$$k_3 = k_3{}^0 + k_{3\,\text{OAc}}[\text{AcO}^-] \qquad (7)$$

where $k_{3\text{OAc}}$ and $k_{2\text{OAc}}$ are fourth- and third-order catalytic constants, respectively. Using the values of k_2 and k_3 obtained at $[\text{AcO}^-]=0.67$M (*cf.* Fig. 2 line A), $k_2{}^0$ and $k_3{}^0$ obtained in the nitrite buffer, and equations (6) and (7), $k_{2\text{OAc}}=1.51\times10^{-3}$M^{-2}min^{-1} and $k_{3\text{OAc}}=8.97\times10^{-2}$ M^{-3}min^{-1}. Then from equation (5), $k_{\text{OAc}}=1.00\times10^{-1}$M^{-3}min^{-1} at $[\text{nitrite}]_0=1.5\times10^{-1}$M, which is in good agreement with the value of k_{OAc} (1.01×10^{-1}M^{-3}min^{-1}) obtained experimentally. The rate of over-all nitrosation in an acetate buffer will then be represented as in equation (8).

$$(dp/dt)_{t=0} = (k_3{}^0[\text{nitrite}]_0{}^2 + k_{3\,\text{OAc}}[\text{nitrite}]_0{}^2[\text{AcO}^-] + k_2{}^0[\text{nitrite}]_0 + k_{2\,\text{OAc}}[\text{nitrite}]_0[\text{AcO}^-])[\text{amine}]_0 \qquad (8)$$

Thus essentially the same kinetic terms with respect to nitrite and acetate contribute to the nitrosation of dimethylamine as those observed in diazotization. The present results only involve a limited range of possible factors which will affect the reaction, but from the results it seems reasonable to conclude that the mechanism of nitrosation of aliphatic secondary amines is the same as that of primary aromatic amines.

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[12] E. Kalatzis and J.H. Ridd, *J. Chem. Soc.* (*B*), **1966**, 529.

[13] Catalysis by molecular acetic acid is improbable from mechanistic point of view.[2–6]