A Model for Metabolic Activation of Dialkylnitrosamines. Oxidative Dealkylation of 2-(*N*-Nitrosoalkylamino)acetonitriles by a Flavin Mimic in Aqueous Solution[1]

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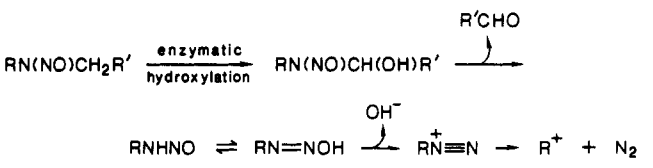
It is found for the first time that a flavin mimic, benzo[1,2-*g*:5,4-*g'*]dipteridine (BDP), reacts with 2-(*N*-nitrosoalkylamino)acetonitriles [$RN(NO)CH_2CN$] via oxidative dealkylation to yield the corresponding alcohols (ROH) and the 2-e-reduced BDP in aqueous acetonitrile. Kinetic studies reveal that the rates are first order with respect to [$RN(NO)CH_2CN$] and [OH^-], respectively. Kinetic isotope effects (k_H/k_D) for $RN(NO)CD_2CN$ (R = Me, *n*-Bu, Ph, and $PhCH_2$) are found to be 2.2–4.2, indicating that deprotonation is involved in the rate-determining step. The mechanism of the oxidative dealkylation of the nitrosamines by the flavin mimic is discussed.

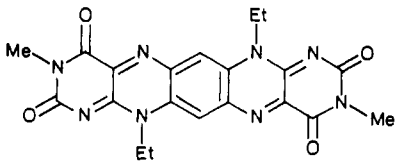
Nitrosamines are known to be carcinogens which usually require metabolic activation in hepatic microsomal mixed function oxidase systems.[2] For example, a dialkylnitrosamine is oxidatively dealkylated through α-hydroxylation to afford the corresponding aldehyde and the highly unstable monoalkylnitrosamine, which decomposes spontaneously to the alkyl cation through the alkyldiazonium ion as shown in Scheme I. The alkyl cation thus formed is believed to cause an initial process of carcinogenesis by alkylating cellular components and bases of DNA.[2,3] The enzymatic α-hydroxylation of dialkylnitrosamines is generally considered to involve a cytochrome P-450 dependent monooxygenase,[4] although other enzymes are implicated.[5]

In model systems for the P-450 monooxygenase, Smith et al. have shown that dibenzylnitrosamine is oxidatively dealkylated to afford benzaldehyde and benzyl alcohol by tetraphenylporphinate, iron(III) or manganese(III) chloride, and oxidants such as iodosobenzene, *m*-chloroperbenzoic acid, and *tert*-butyl hydroperoxide in benzene.[6] On the other hand, Lake et al. have proposed that a monoamine oxidase (MAO) is responsible in part for metabolic activation of the nitrosamines, on the basis of the observations that the substrates and inhibitors of the MAO repress the metabolism of the nitrosamine.[7] Arcus et al., however, have reported that the purified MAO has no demethylase activity for dimethylnitrosamine.[4d] Thus it would be of interest to examine the reaction of *N*-nitrosamines with an oxidation-active flavin model compound.

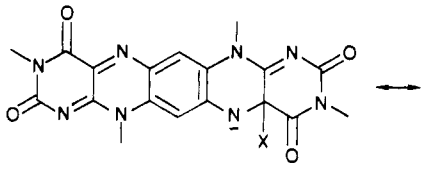
Scheme I



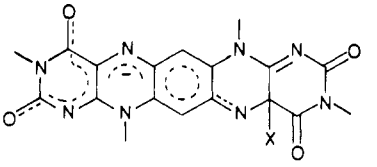
Scheme II



BDP



C(4a)-adduct



Recently we have shown that 7,14-diethyl-3,10-dimethylbenzo[1,2-*g*:5,4-*g'*]dipteridine-2,4,9,11-(3*H*,7*H*,10*H*,14*H*)-tetrone (BDP) functions as a flavin mimic and exhibits a remarkably high oxidizing activity toward oxidation reactions proceeding via C(4a)-adduct formation due to stabilization of the negative charge generated on the N(5) atom by the long conjugative system (Scheme II). For example, BDP is ca. 10^7-fold more reactive than 3,10-dimethylisoalloxazine for the oxidations of thiols and phenylhydrazine.[8]

(1) A portion of this work was reported in a preliminary comunication: Yano, Y.; Yokoyama, T.; Yoshida, T. *Tetrahedron Lett.* **1986**, *27*, 5121.

(2) (a) Magee, P. N.; Monesano, R.; Preussman, R. In *Chemical Carcinogens*; Searle, C. E., Ed.; American Chemical Society: Washington, DC, 1976; Chapter 11. (b) Michejida, C. J.; Kroeger-Koepke, M. B.; Koepke, S. R.; Kupper, J. In *N-Nitrosamines*; Anselme, J. P., Ed.; American Chemical Society: Washington, DC 1979; Chapter 5. (c) Mochizuki, M.; Tanaka, T. *Eisei Kagaku* **1978**, *24*, 271.

(3) (a) Magee, P. N.; Barnes, J. M. *Cancer Res.* **1967**, *10*, 163. (b) Ioannides, C.; Parke, D. V. *Chem. Ind. (London)* **1980**, 854.

(4) (a) Czygan, P.; Grein, H.; Gano, A. J.; Hutterer, F.; Schaffner, F.; Poper, H.; Rosenthal, O.; Cooper, D. Y. *Cancer Res.* **1973**, *33*, 2983. (d) Lotlikar, P. D.; Baldy, M. J.; Duyer, E. N. *Biochem. J.* **1975**, *152*, 705. (e) Argus, M. F.; Arcos, J. C.; Paster, K. M.; Nu, N. C.; Ventakesan, N. *Chem. Biol. Interact.* **1976**, *13*, 127. (f) Lai, D. Y.; Meyers, S. C.; Woo, Y.-T.; Greene, E. J.; Friedman, M. A.; Argus, M. F.; Arcos, J. C. *Ibid.* **1979**, *28*, 107. (g) Yang, C. S.; Tu, Y. Y.; Koop, D. R.; Coon, M. J. *Cancer Res.* **1985**, *45*, 1140.

(5) Kroeger, M. B.; Michejida, C. J. *Cancer Res.* 1979, *39*, 1587.

(6) Smith, J. R. L.; Nee, M. W.; Noar, J. B.; Bruice, T. C. *J. Chem. Soc., Perkin Trans 2* **1984**, 225.

(7) Rowland, I. R.; Lake, B. G.; Phillips, J. C.; Gangolli, S. D. *Mutat. Res.* **1980**, *72*, 63 and references are cited therein.

(8) (a) Yano, Y.; Nakazato, M.; Vasquez, R. E. *J. Chem. Soc., Chem. Commun.* **1985**, 226. (b) Yano, Y.; Nakazato, M.; Sutoh, S.; Vasquez, R. E.; Kitani, A.; Sasaki, K. *J. Chem. Res. Synop.* **1985**, 404.

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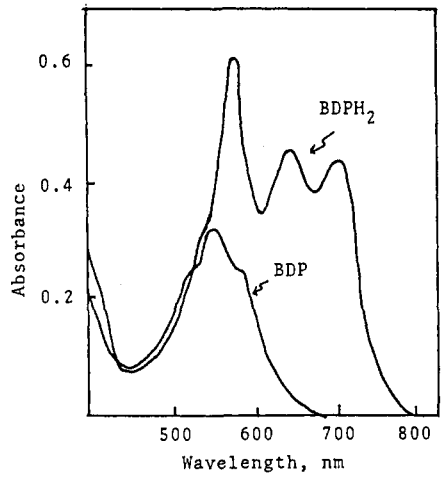
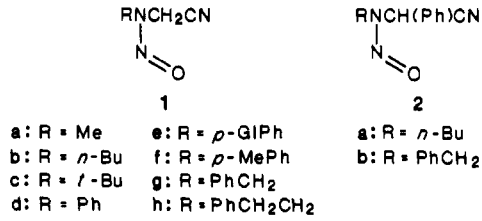


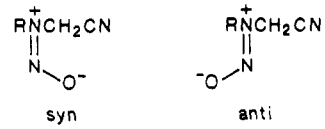
Figure 1. Absorption spectra of BDP and the final solution of the reaction of BDP with **1a** in aqueous acetonitrile (0.02 M borate buffer/MeCN = 1:1, pH 8.62), 25 °C, N_2.

In this paper, we describe kinetic studies for the oxidative dealkylation of 2-(*N*-nitrosoalkylamino)- or 2-(*N*-nitrosoarylamino)acetonitriles (**1** and **2**) by BDP in aqueous acetonitrile under anaerobic conditions and discuss the mechanism of the oxidative dealkylation of nitrosamines.



Results and Discussion

The nitrosamines **1** and **2** were prepared by nitrosation of the corresponding 2-(alkylamino)- or 2-(arylamino)-acetonitriles.[9] Ratios of syn and anti isomers were determined by ^1H NMR spectra:[10] **1a**, 53/47; **1b**, 82/18; **1c**, 100/0; **1d**, 100/0; **1f**, 100/0; **1g**, 75/25; **1h**, 71/29; **2a**, 62/38; **2b**, 50/50. The existence of the syn and anti isomers of the *N*-nitrosamines indicates that the *N*–*N* bond possesses a double bond character as shown.[10] Thus, the



syn preference may be explained by a repulsive effect between the negatively charged atom and the bulky substituent or/and π-electrons of the phenyl group.

Product Analysis. The reaction of BDP with **1a** was first examined spectrophotometrically in aqueous acetonitrile under an aerobic conditions. The absorption of BDP and the product are shown in Figure 1. The spectrum of the product was completely consistent with that of the 2-e-reduced BDP($BDPH_2$) obtained by EDTA-photoreduction of BDP or by the oxidation of 2-mercaptoethanol by BDP under the same conditions. Introduction of O_2 regenerated quantitatively the starting

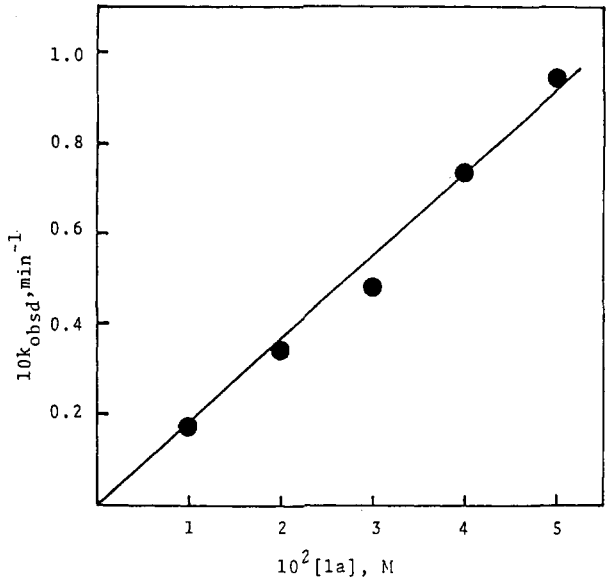
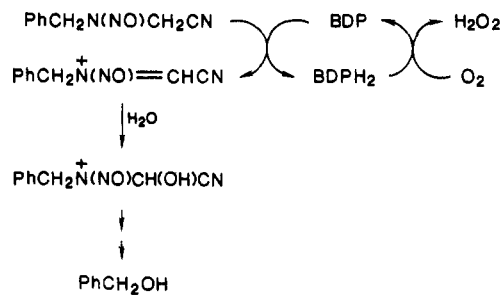


Figure 2. Plot of k_{obsd} vs [**1a**]: [BDP] = 1.0×10^{-5} M, pH 8.20 (0.1 borate, μ = 0.3), 25 °C, N_2.

Scheme III



spectrum of BDP. The oxidation product of the *N*-nitrosamine was examined in a preparative scale by employing **1g** (17-fold) over BDP in aqueous acetonitrile under air atmosphere. After workup, benzyl alcohol was isolated in 64% yield based on **1g**, indicating that BDP functions as a turnover catalyst as shown in Scheme III. No formation of benzyl alcohol was confirmed in the control experiment in the absence of BDP.

It should be noted that BDP is unable to effect oxidation of dimethylnitrosamine under the same conditions as the oxidation of **1**, implying that acidity of α-hydrogens of *N*-nitrosamines is a crucial factor for the oxidative dealkylation of *N*-nitrosamines.

Kinetics. Rate measurements were performed spectrophotometrically by following the absorption increase of the reduced BDP at 700 nm in aqueous or aqueous acetonitrile under anaerobic conditions. Acetonitrile was employed to dissolve the nitrosamines except for **1a**. The pseudo-first-order rate constants for **1** were obtained from the initial slopes of the first-order plots, since the plots deviate downward after ca. 50% of the reaction owing to formation of a charge-transfer complex between BDP and the reduced BDP formed during the reactions.[11] The rate for **2**, however, followed first-order kinetics up to more than two half-lives. It has been generally observed that the rates for the absorption increase of the reduced BDP follow first-order kinetics up to more than two half lives for the faster reactions.[11]

Effects of the substrate concentration and pH on the rates were examined in aqueous solution by employing **1a**

(9) (a) Knoevengal, E. *Ber.* **1904**, *37*, 4073. (b) Daeniker, H. U.; Druey, J. *Helv. Chim. Acta* **1962**, *46*, 2426.

(10) (a) Looney, C. E.; Phillips, W. D.; Reilly, E. L. *J. Am. Chem. Soc.* **1957**, *79*, 6136. (b) Korabatsos, G. J.; Taller, R. A. *Ibid.* **1964**, *86*, 4373. (c) Szymanski, S.; Stefaniak, L.; Witanowski, M.; Ejchart, A. *Org. Magn. Reson.* **1977**, *9*, 699.

(11) Yano, Y.; Nakazato, M., to be published.

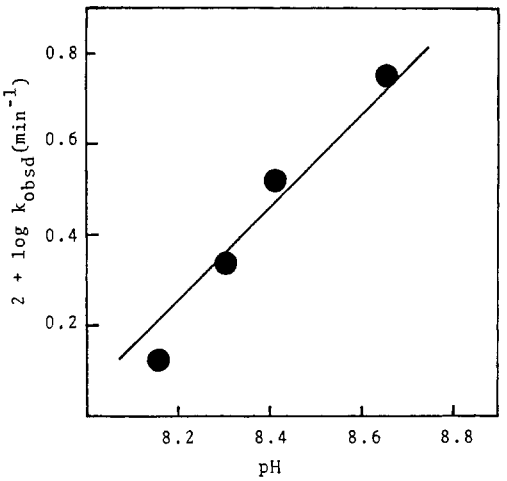


Figure 3. Plot of log k_{obsd} vs pH for the reaction of BDP with **1a**: [BDP] = 1.5×10^{-5} M, [**1a**] = 3.00×10^{-2} M, 0.1 M borate buffer, μ = 0.3, 25 °C, N_2.

Table I. Pseudo-First-Order Rate Constants and Relative Rates for the Reaction of *N*-Nitrosamines with BDP

N-nitrosamine[a]	k_{obsd}, min^{-1} [b]	rel rate
1a	$(6.22 \pm 0.20) \times 10^{-2}$	1.0
1b	$(4.42 \pm 0.15) \times 10^{-2}$	0.71
1c	$(3.17 \pm 0.08) \times 10^{-3}$	0.051
1d	$(6.92 \pm 0.15) \times 10^{-2}$	1.1
1e	$(8.21 \pm 0.25) \times 10^{-2}$	1.3
1f	$(5.90 \pm 0.20) \times 10^{-2}$	0.94
1g	$(9.20 \pm 0.12) \times 10^{-2}$	1.5
1h	$(7.93 \pm 0.20) \times 10^{-2}$	1.3
2a[c]	3.63 ± 0.10	3.5×10^{2} [d]
2b[c]	4.42 ± 0.15	4.3×10^{2} [d]

[a] [BDP] = 1.0×10^{-5} M, [1] = 3.00×10^{-2} M, pH 8.76 (0.02 M borate buffer/MeCN = 1:1), 25 °C, N_2. [b] Average of three or four runs. [c] [2] = 5.00×10^{-3} M. [d] Calculated from apparent second-order rate constants, k_{obsd}/[1] and k_{obsd}/[2].

(Figures 2 and 3), indicating that the rates are first order with respect to [**1a**] and [OH^-], respectively. Further it was found that the rate is not affected by buffer concentration in a range of 0.025–0.1 M of borate buffer (pH 8.40, μ = 0.3) under the conditions of Figure 3, suggesting that the present oxidation proceeds via specific base catalysis. The rate constants and the relative rates are presented in Table I.

As can be seen in Table I, the electronic effect of the substituents (R) in $RN(NO)CH_2CN$ seems to be small. Namely, the Hammett ρ value for **1d–f** was calculated to be +0.36, indicating that the electron-withdrawing group slightly accelerates the rates of the oxidation. However, the phenyl substituent at the cyanomethyl carbon (**2**) considerably accelerates the rate. This may be explained by facile proton removal from the cyanomethyl group due to conjugative effect of phenyl group.[12] This also suggests that the proton removal is involved in the rate-determining step. A relatively large steric effect of R is observed (**1c**).

Primary kinetic isotope effects were determined by employing $RN(NO)CD_2CN$ (R = Me, *n*-Bu, Ph, and $PhCH_2$) (Table II). The results clearly indicate that deprotonation is involved in the rate-determining step.

Mechanism of the Oxidative Dealkylation of N-Nitrosamines. The oxidative dealkylation of *N*-nitrosamines by a flavin mimic may be considered to proceed via one of the following mechanisms: (a) one-electron transfer mechanism, (b) carbanion mechanism, and (c) two-electron transfer mechanism via nucleophilic addition of the nitroso oxygen.

Table II. Rate Constants for $RN(NO)CD_2CN$[a] and Kinetic Isotope Effects

R	$10^2 k_{obsd}$, min^{-1} [b]	k_H/k_D[c]
Me	2.29 ± 0.08	2.7
n-Bu	1.26 ± 0.04	3.5
Ph	3.15 ± 0.15	2.2
$PhCH_2$	2.21 ± 0.14	4.2

[a] [BDP] = 1.0×10^{-5} M, [$RN(NO)CD_2CN$] = 3.00×10^{-2} M, pH 8.76 (0.02 M borate buffer/MeCN = 1:1), 25 °C, N_2. [b] Average of three or four runs. [c] The rate constants for $RN(NO)CH_2CN$ (k_H) are given in Table I.

Silverman et al. have reported that mitochondrial monoamine oxidase, which is a flavin-dependent enzyme, catalyzes the oxidative dealkylation of monoamines via two one-electron transfers from the substrates to the flavin.[13] Such a radical mechanism is also shown in chemical,[14] photochemical,[15] and electrochemical[16] oxidations of amines. Masui et al. also showed that the radical cation of *N*-nitrosamines is produced by electrochemical oxidation.[17]

The radical mechanism for the oxidation of the *N*-nitrosamines could be depicted as shown in Scheme IV. The initial electron transfer from the *N*-nitrosamine is considered to be unfavorable compared with that of conventional amines, and the subsequent proton removal from the nitrosamine cation radical is much easier than it is from the simple amine cation radical due to the electron-withdrawing nature of the nitroso and cyano groups. Thus,

Scheme IV

$$RN(NO)CH_2CN \overset{-e^-}{\rightleftharpoons} RN^{\bullet+}(NO)CH_2CN \overset{-H^+}{\rightleftharpoons} RN(NO)C^{\bullet}HCN \xrightarrow{-e^-} RN^+(NO)=CHCN \xrightarrow{+H_2O} RN(NO)CH(OH)CN$$

if the present oxidation proceeds via mechanism a, the rate-determining step might be the initial electron transfer. In this case, the rate would be accelerated by the electron-donating substituent, and k_H/k_D would be near unity because of the secondary isotope effect. These are not consistent with the kinetic observations. Thus mechanism a would be excluded.

The carbanion mechanism b represents the oxidation of the carbanion from the *N*-nitrosamine by BDP (Scheme V), which is similar to that for the oxidation of a nitroalkane with D-amino acid oxidase,[18] in which the nitroethane anion is the reactive species.

Scheme V

$$RN(NO)CH_2CN \underset{k_{-1}}{\overset{k_1[OH^-]}{\rightleftharpoons}} RN(NO)C^-HCN \xrightarrow{k_2[BDP]} RN^+(NO)=CHCN \xrightarrow[\text{fast}]{H_2O} RN(NO)CH(OH)CN$$

(12) Matthews, W. S.; Bares, J. E.; Bartness, J. E.; Bordwell, F. G.; Conforth, F. J.; Drucher, G. G.; Margolin, Z.; McCollum, G. J.; Vanier, N. R. *J. Am. Chem. Soc.* **1975**, *97*, 7006.

(13) (a) Silverman, R. S.; Hoffman, S. J. *J. Am. Chem. Soc.* **1980**, *102*, 884. (b) Silverman, R. B.; Hoffman, S. J.; Catus, W. B., III. *Ibid.* **1980**, *102*, 7126.

(14) (a) Huli, L. A.; Davis, G. T.; Rosenblatt, D. H.; Williams, H. K. R. Weglein, R. C. *J. Am. Chem. Soc.* **1967**, *89*, 1163. (b) Rosenblatt, D. H.; Davis, G. T.; Huli, L. A.; Forberg, G. D. *J. Org. Chem.* **1968**, *33*, 1649.

(15) Cohen, S. G.; Parola, A.; Parsons, G. H. *Chem. Rev.* **1973**, *73*, 141.

(16) (a) Smith, P. J.; Mann, C. K. *J. Org. Chem.* **1969**, *34*, 1821. (b) Smith, J. R. L.; Mashder, D. *J. Chem. Soc., Perkin Trans.* 2 1976, 47.

(17) Ohmori, H.; Maeda, H.; Ueda, C.; Sayo, H.; Masui, M. *Chem. Pharm. Bull.* **1986**, *34*, 3086.

(18) (a) Porter, D. J.; Voet, J. G.; Bright, H. *J. Biol. Chem.* **1972**, *247*, 1951; (b) **1973**, *248*, 4400. (c) Bruice, T. C. In *Progress in Bioorganic Chemistry*; Kaiser, E. T., Kezdy, F. J., Eds.; Wiley, New York, 1976; Vol. 4, p 38.

Table III. Rate Constants of H–D Exchange of $RN(NO)CH_2CN$[a]

$RN(NO)CH_2CN$	10^2k_{obsd}, min^{-1}
1a	3.67 ± 0.66
1d	2.32 ± 0.45

[a] $[RN(NO)CH_2CN]$ = 0.25 M, pD 9.32 (0.1 M borate buffer/CD_3CN = 3:7), 35.1 °C.

The rate equation is expressed as eq 1 by assuming the steady state to the carbanion concentration, where $[RN(NO)CH_2CN]_0$ and $[BDP]_0$ stand for the initial concentrations of $RN(NO)CH_2CN$ and BDP, respectively. As

$$\text{rate} = \frac{k_1k_2[RN(NO)CH_2CN]_0[OH^-][BDP]_0}{k_{-1} + k_1[OH^-] + k_2[BDP]_0} \quad (1)$$

already described, the pH–rate profile exhibits that the rate is first order with respect to $[OH^-]$, and the kinetic order of BDP for the oxidation of **1** is reasonably assumed to be first order, since the rates for **2** are first order with respect to [BDP], thus requiring the assumption of $k_{-1} \gg k_1[OH^-] + k_2[BDP]_0$. Meanwhile this assumption requires that the rate of H–D exchange of the cyanomethyl hydrogens of the *N*-nitrosamines is faster than that of the oxidation and also requires no kinetic isotope effect. To confirm the former, we determined rates of the H–D exchange of **1a** and **1d** in deuteriated solvent by an NMR technique. The results are shown in Table III. The table shows that the rates for **1a** and **1d** are slower by one order of magnitude than those of the oxidative dealkylation on the consideration of the differences of the pH and temperature. These results together with the values of k_H/k_D clearly indicate the assumption of $k_{-1} \gg k_1[OH^-] + k_2[BDP]_0$ to be incorrect. Therefore mechanism b is also excluded.

The nitroso oxygen of *N*-nitrosamines is known to act as a nucleophile,[19] and BDP remarkably enhances the oxidation reactions proceeding through C(4a)-adduct formation. This allows us to formulate mechanism c as shown in Scheme VI. Namely, the nitroso oxygen attacks at the C(4a)-position of BDP to form a transient adduct followed by base-catalyzed 1,4-elimination to give $RN^+(NO)$=CHCN and the reduced BDP. The rate equation is expressed as eq 2 by assuming the steady state to [4a-adduct].

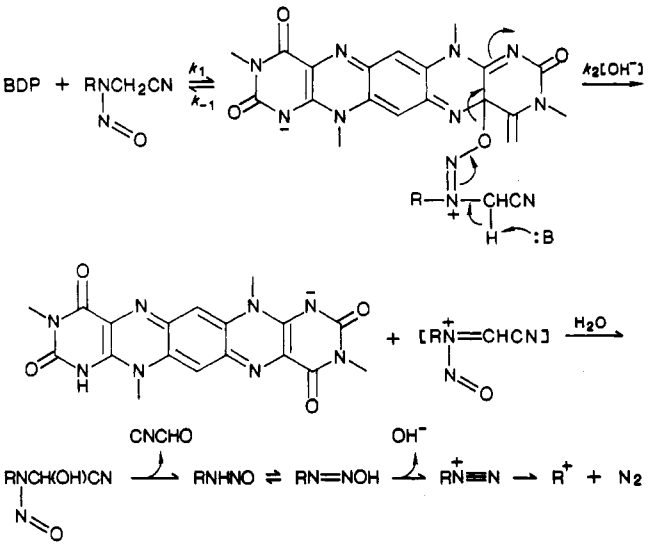
$$\text{rate} = \frac{k_1k_2[RN(NO)CH_2CN]_0[OH^-][BDP]_0}{k_{-1} + k_1[RN(NO)CH_2CN]_0 + k_2[OH^-]} \quad (2)$$

The first-order dependence of the rates on $[RN(NO)CH_2CN]_0$ and $[OH^-]$ allows one to assume $k_{-1} \gg k_1[RN(NO)CH_2CN]_0 + k_2[OH^-]$, giving eq 3. All the kinetic data

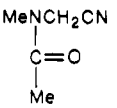
$$k_{obsd} = \frac{k_1k_2}{k_{-1}}[RN(NO)CH_2CN]_0[OH^-] \quad (3)$$

obtained seem to be compatible with mechanism c. The rate retardation caused by the bulky substituent could be explained by steric hindrance in either the nucleophilic attack (k_1) or the base-catalyzed 1,4-elimination (k_2). In the latter step, the base-catalyzed double bond forming elimination is considered to proceed favorably in the coplanar transition state in which the partially forming double bonds are able to conjugate with each other. The values of k_H/k_D (2.2–4.2) imply that the elimination proceeds via "nearly carbanionic transition state" in which C–H bond breaking is fairly advanced owing to the acidic hydrogens.[20]

Scheme VI



Furthermore, it should be noted that 2-(*N*-acetylmethylamino)acetonitrile (**3**), not possessing a nucleophilic group, does not react with BDP under the conditions similar to those for the oxidation of the *N*-nitrosamines **1**.



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The present study demonstrates that the acidity of α-hydrogens of the *N*-nitrosamines is crucial for the oxidative dealkylation. A question, whether the nitrosamines **1** and **2** activated by cyano group could be model compounds of dialkylnitrosamines may arise. It is well established that the acidity of hydrophobic carbon acids is remarkably strengthened by cationic micelles.[21] This implies that the deprotonation of dialkylnitrosamines could be a possible process in special circumstances such as biological systems. To the best of our knowledge, this is the first example of a flavin mimic that reacts with nitrosamine derivatives via oxidative dealkylation similar to the metabolic activation of dialkylnitrosamines in vivo.

Experimental Section

Melting points are uncorrected. ^1H NMR spectra were recorded on a Hitachi R-24 spectrometer. The chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane as the internal standard. Absorption spectra were recorded on a Hitachi UV-200 spectrophotometer. IR spectral data are reported in cm^{-1}, and UV spectral data are reported in nm (with ε given in parentheses). Distilled water was used for buffer solutions. Acetonitrile was purified by distillation from P_2O_5. BDP was supplied from our previous study.[8]

2-(*N*-Nitrosoalkylamino)- or 2-(*N*-nitrosoarylamino)acetonitriles were prepared by nitrosation of the corresponding 2-(alkylamino)- or 2-(arylamino)acetonitriles, which were prepared from the

(19) (a) Chang, S. K.; Harrington, G. W.; Vearle, H. S.; Swern, D. *J. Org. Chem.* **1976**, *41*, 3752. (b) Michejida, C. J.; Koepke, S. R.; Mahaffy, J. *Tetrahedron Lett.* **1976**, 2573.

(20) (a) Bunnett, J. F. *Angew. Chem., Int. Ed. Engl.* **1962**, *1*, 225. (b) Oae, S. *Elimination Reactions*; Tokyo Kagaku Dojin: Tokyo, 1965.

(21) (a) Yano, Y.; Okonogi, T.; Tagaki, W. *J. Org. Chem.* **1973**, *38*, 392. (b) Okonogi, T.; Umezawa, T.; Tagaki, W. *J. Chem. Soc., Chem. Commun.* **1974**, 363. (c) Yano, Y.; Kawada, S.; Tagaki, W. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 493. (d) Minch, M. J.; Giaccio, M.; Wolff, R. *J. Am. Chem. Soc.* **1975**, *97*, 3766.

corresponding amines, formaldehyde and NaCN according to the procedures of the literature.[9,19a]

2-(*N*-Nitrosomethylamino)acetonitrile (**1a**) is known to be carcinogenic in experimental animals.[22] Thus all the nitrosamines were carefully treated by using disposal gloves.

2-(Alkylamino)- or 2-(Arylamino)acetonitriles. To a stirred solution of the amine (0.055 mol) in H_2O (20 mL) containing concentrated HCl (6.9 g) were added NaCN (2.84 g, 0.055 mol), portionwise, and HCHO (4.46 g of formalin, 0.055 mol) in MeOH (20 mL), dropwise, at 0 °C over a period of 0.5 h. Stirring was continued at 20 °C for 3 h. The solution was extracted with diethyl ether (3 × 80 mL), washed with saturated brine, and dried ($MgSO_4$). After evaporation of the ether, the residual product was nitrosated without purification. (Methylamino)acetonitrile hydrochloride is commercially available.

2-(*N*-Nitrosoalkylamino)- and 2-(*N*-Nitrosoarylamino)-acetonitrile (1 and 2). To a stirred solution of 2-(alkylamino)- or 2-(arylamino)acetonitrile hydrochloride (0.14 mol) in H_2O (150 mL) was added $NaNO_2$ (10.4 g, 0.15 mol) in H_2O (23 mL) dropwise over a period of 0.5 h, with adjustment of the pH of the solution to 3 by adding concentrated HCl. The mixture was stirred at room temperature for 17 h in the dark. After evaporation of H_2O, the residual oil was extracted with CH_2Cl_2 (3 × 50 mL). The CH_2Cl_2 layer was washed with saturated brine and dried ($MgSO_4$). After evaporation of CH_2Cl_2, the product was purified by distillation recrystallization, or column chromatography (silica gel, 50% Et_2O–hexane).

1a: 93% yield; bp 74–75 °C (0.5 mm) [lit.[9b] bp 72–73 °C (0.4 mm)]; IR ($CHCl_3$) 2250 (C≡N), 1460 (N=O), 1010 (N–N); UV (H_2O) λ_{max} 229 (6600); ^1H NMR ($CDCl_3$) δ 3.07 (s, anti CH_3), 3.86 (s, syn CH_3), 4.36 (s, syn CH_2CN), 5.05 (s, anti CH_2CN).

1b: 88% yield; bp 90 °C (0.5 mm) [lit.[9b] bp 70 °C (0.1 mm)]; IR ($CHCl_3$) 2250 (C≡N), 1460 (N=O), 1060 (N–N); UV (H_2O) λ_{max} 231 (6600); ^1H NMR ($CDCl_3$) δ 0.76–2.06 (m, $CH_3CH_2CH_2$), 3.59 (t, J = 7 Hz, anti $C_3H_7CH_2$) 4.26 (t, J = 7 Hz, syn $C_3H_7CH_2$), 4.31 (s, syn CH_2CN), 5.01 (s, anti CH_2CN).

1c: 53% yield; mp 73–74 °C (Et_2O–hexane); IR ($CHCl_3$) 2250 (C≡N), 1460 (N=O), 1140 (N–N); UV (H_2O) λ_{max} 228 (6900); ^1H NMR ($CDCl_3$) δ 1.60 (s, $C(CH_3)_3$), 4.20 (s, CH_2CN). Anal. Calcd for $C_6H_{11}N_3O$: C, 51.16; H, 7.84; N, 29.69. Found: C, 51.10; H, 7.85; N, 29.70.

1d: 30% yield; mp 56–57 °C (Et_2O–hexane); IR ($CHCl_3$) 2250 (C≡N), 1470 (N=O), 1110 (N–N); UV (H_2O) λ_{max} 271 (5900); ^1H NMR ($CDCl_3$) δ 4.68 (s, CH_2CN), 7.50 (s, C_6H_5). Anal. Calcd for $C_8H_7N_3O$: C, 59.62; H, 4.38; N, 26.07. Found: C, 59.60; H, 4.34; N, 26.16.

1e: 30% yield; mp 97–98 °C (Et_2O–hexane) (lit.[9b] mp 97–98 °C); IR ($CHCl_3$) 2250 (C≡N), 1480 (N=O), 1090 (N–N); UV (H_2O) λ_{max} 276 (7000); ^1H NMR ($CDCl_3$) δ 4.72 (s, CH_2CN), 7.45 (s, C_6H_4).

1f: 39% yield; mp 32 °C (lit.[9b] mp 32–33 °C); IR ($CHCl_3$) 2250 (C≡N), 1470 (N=O), 1110 (N–N); UV (H_2O) λ_{max} 276 (6000); ^1H NMR ($CDCl_3$) δ 2.41 (s, CH_3), 4.73 (s, CH_2CN), 7.43 (s, C_6H_4).

1g: 47% yield; oil (column chromatographed) [lit.[9b] bp 98 °C (0.05 mm)]; IR ($CHCl_3$) 2250 (C≡N), 1460 (N=O), 1120 (N–N); UV (H_2O) λ_{max} 234 (7600); ^1H NMR ($CDCl_3$) δ 4.12 (s, syn CH_2CN), 4.75 (s, anti CH_2CN), 4.82 (s, anti CH_2CN), 5.37 (s, CH_2Ph), 7.00–7.57 (m, C_6H_5). Anal. Calcd for $C_9H_9N_3O$: C, 61.70; H, 5.18; N, 23.99. Found: C, 61.98; H, 5.17; N, 24.12.

1h: 65% yield: oil (column chromatographed) [lit.[9b] bp 127 °C (0.05 mm)]; IR ($CHCl_3$) 2250 (C≡N), 1460 (N=O), 1120 (N–N); UV (H_2O) λ_{max} 233 (6600); ^1H NMR ($CDCl_3$) δ 2.75 (t, anti CH_2CH_2Ph), 3.06 (t, syn CH_2CH_2Ph), 3.81 (t, anti CH_2CH_2Ph), 4.12 (s, syn CH_2CH), 4.46 (t, syn CH_2CH_2Ph), 4.66 (s, anti CH_2CN), 6.91–7.38 (m, C_6H_5).

2a: 82% yield; oil (column chromatographed) [lit.[9b] bp 115 °C (0.1 mm)]; IR ($CHCl_3$) 2250 (C≡N), 1460 (N=O), 1110 (N–N); UV (H_2O) λ_{max} 239 (6800); ^1H NMR ($CDCl_3$) δ 0.57–1.98 (m, n-C_3H_7), 3.35 (t, anti $CH_2C_3H_7$) 4.05 (t, syn $CH_2C_3H_7$), 6.87 (s, anti *CHPhCN*), 7.10 (s, syn *CH*PhCN), 7.19–7.53 (m, C_6H_5).

2b: 74% yield; oil (column chromatographed); IR($CHCl_3$) 2250 (C≡N), 1460 (N=O), 1110 (N–N); UV (H_2O) λ_{max} 240 (6500); ^1H NMR ($CDCl_3$) δ 4.56 (s, anti CH_2Ph), 5.14 (s, syn CH_2Ph), 6.74 (s, anti *CH*PhCN) 6.85 (s, syn *CH*PhCN), 6.70–7.44 (m, 2 C_6H_5). Anal. Calcd for $C_{15}H_{13}N_3O$: C, 71.70; H, 5.21; N, 16.72. Found: C, 71.50; H, 5.20; N, 16.68.

The deuteriated nitrosamines were obtained by H–D exchange of the corresponding nitrosamines (**1a,b,d,g**, 500 mg) in D_2O (99.75%, 8 mL)–CD_3COCD_3 (99.5%, 8 mL) at 40–50 °C for 2 h. The H–D exchange of **1g** was performed at 100 °C for 3 h. The crude deuteriated nitrosamines were obtained by evaporating the solvents. The exchange was repeated three times, and finally the deuteriated nitrosamines were purified as described above. Disappearance of CH_2CN protons was confirmed by ^1H NMR spectra.

Product Analysis of the Reaction of BDP with 1g. A mixture of BDP (40 mg, 0.092 mmol) and **1g** (268 mg, 1.59 mmol) in aqueous acetonitrile (200 mL; 0.02 M borate buffer/MeCN, 1/1; pH 8.7) was stirred at room temperature for 17 h and at 75 °C for 4 h under aerobic conditions. The mixture was extracted with Et_2O (3 × 100 mL). The etheral solution was washed with saturated brine (2 × 50 mL) and dried ($MgSO_4$). After evaporation of Et_2O, $PhCH_2OH$ was obtained by distillation (110 mg, 64% based on **1g**).

Kinetics. a. Oxidative Dealkylation of *N*-Nitrosamines by BDP. In a Thunberg cuvette, BDP (30 μL of (1.0–1.7) × 10^{-3} M in dimethylacetoamide) was placed in the cell part with the buffer solution (2.67 mL), and nitrosamine (0.3 mL of 3.00 × 10^{-1} M in MeCN) was placed in the upper section. Both the solutions were degassed by bubbling with vanadous ions–scrubbed N_2 for 20 min. After equilibration at 25 °C, the reaction was initiated by mixing. The rate constants were determined by following the absorption increase of the reduced BDP at 700 nm.[8]

b. H–D Exchange of $RN(NO)CH_2CN$. An NMR tube containing 0.2 mL of the solvent was placed in the NMR probe set at 35.1 °C for 30 min, and the *N*-nitrosamine (0.05 mmol) was added quickly into the tube ([$RN(NO)CH_2CN$] = 0.25 M). Disappearance of the cyanomethyl protons was recorded with nonexchangeable protons (methyl protons for **1a** and phenyl protons for **1d**). The rate constants were calculated from the ratios of the peak areas (CH_2CN/nonexchangeable protons) with time as described previously.[21a]

Registry No. **1a**, 3684-97-7; **1b**, 3422-21-7; **1c**, 10242-50-9; **1d**, 827-51-0; **1e**, 829-29-8; **1f**, 829-28-7; **1g**, 1202-33-1; **1h**, 62736-81-6; **2a**, 96651-53-5; **2b**, 65551-49-7; BDP, 96994-47-7.

(22) Druckrey, H.; Preussman, R.; Schmahl, D.; Ivankovic, S. *Z. Krebsforsch.* **1967**, *69*, 103.