N-Nitroso Compounds. Part 5.[1] Hydrogen–Deuterium Exchange of *N*-Nitroso-2-(alkyl or arylamino)acetonitriles in Aqueous Solution

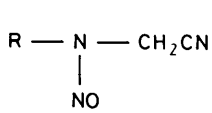
Kitaro Yoshida *
Department of Chemistry, Saitama Medical School, 981 Kawakado, Moroyama, Saitama 350-04, Japan
Yumihiko Yano
Department of Chemistry, Gunma University, Kiryu, Gunma 376, Japan

The hydrogen–deuterium exchange of the cyanomethyl protons of a series of nitrosamines, $RN(N{=}O)CH_2CN$ (R = alkyl, benzyl, *p*-substituted phenyl, and cyanomethyl), has been investigated kinetically in CD_3COCD_3–D_2O or CD_3CN–deuteriated phosphate buffer at 35.1 °C. The order of the ring substituents of the arylnitrosamines is found to be *p*-MeO > H > *p*-Cl. The electronic effect of the R group on the relative stability of the nitrosamino carbanion is discussed in relation to the resonance structure of the nitrosamines in aqueous solution.

In 1970, Keefer and Fodor found that the α-hydrogens of aliphatic nitrosamines readily undergo base-catalysed hydrogen–deuterium exchange in alkaline D_2O.[2] The facile exchange of the α-hydrogens was attributed to the stabilization of the intermediate nitrosamino carbanion by the adjacent positively charged amino nitrogen. This finding led to two stimulating discussions on the reactivity of nitrosamines. First, Seebach's group recognized that the lability of the α-hydrogens could be utilized to introduce various electrophiles onto the α-carbon atom of nitrosamines.[3] After substitution of the α-position by the electrophile, the nitroso group can be removed by denitrosation in the presence of acids to provide the desired α-substituted secondary amines. The abstraction of the α-proton is not normally possible for secondary amines themselves. For this reason, Seebach coined the term 'umpolung' which means 'polarity reversal' of secondary amines by nitrosation. Secondly, it is now widely believed that biological activation of nitrosamines involves enzymatic α-hydroxylation.[4] Koepke and Michejda reported the oxidation of deuteriated and non-deuteriated dimethylnitrosamine or methylphenylnitrosamine by rat liver microsome.[5] The kinetic isotope effect suggested that the breakage of the α-C–H bond is the rate-determining step of enzymatic oxidation. Koepke and Michejda also suggested that this step involves a nitrosamino carbanion as intermediate, although the possibility of homolytic C–H bond cleavage could not be excluded.

In model systems for P-450-dependent mono-oxygenase, Lindsay Smith *et al.* have shown that dibenzylnitrosamine is oxidized to benzaldehyde and benzyl alcohol by iodosylbenzene, 3-chloroperbenzoic acid, or t-butyl hydroperoxide catalysed by tetraphenylporphyrinato-iron(III) or -manganese(III) chloride.[6] On the other hand, we have recently shown that a series of *N*-nitroso-2-(alkyl or arylamino)acetonitriles (**1**) is oxidatively dealkylated by benzo[1,2-*g*:5,4′-*g*]dipteridine, an oxidation-active flavin mimic, in a similar manner to the metabolic degradation of nitrosamines in biological systems.[7] The kinetic study revealed that deprotonation of the cyanomethyl moiety is involved in the rate-determining step of the oxidation. Thus the lability of the cyanomethyl hydrogens is an important factor that influences the chemical reactivity of (**1**).

We report here the results of a kinetic study of hydrogen–deuterium exchange of (**1**) in aqueous solution. As far as we know, only two precedents have been reported on the kinetics of the H–D exchange of nitrosamines. Fraser and his co-workers reported the rate of H–D exchange of four non-equivalent α-hydrogens of *N*-nitroso-6,7-dihydro-1,11-dimethyl-5*H*-dibenz-[*c*,*e*]azepine in Bu^{t}OK–Bu^{t}OD.[8] They have found the stereospecificity of nitrosamino carbanion in this conformationally rigid system. Singer and Andrews studied the kinetics of benzylmethylnitrosamines [p-$XC_6H_4CH_2N(N{=}O)CH_3$], where the substituent X did not appreciably affect the acidity of the methyl group.[9] The purpose of this paper is to discuss the electronic effect of the group R on the relative stability of the nitrosamino carbanions from (**1**).



(**1**)

R	
a;	Me
b;	Pr^n
c;	Pr^i
d;	Bu^t
e;	$PhCH_2$
f;	p-$MeOC_6H_4CH_2$
g;	p-$ClC_6H_4CH_2$
h;	Ph
i;	p-$MeOC_6H_4$
j;	p-ClC_6H_4
k;	$NCCH_2$

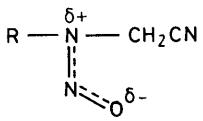
Results and Discussion

The kinetics of the H–D exchange were carried out at 35.1 °C by the use of 60 MHz ^{1}H n.m.r. spectroscopy.

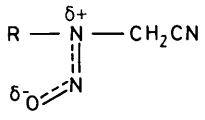
H–D *Exchange of* (**1a**).—When (**1a**) was dissolved in D_2O, H–D exchange of the cyanomethyl protons occurred smoothly without base.† However, the methyl protons did not exchange under the same conditions. It is interesting to note that structurally analogous nitrososarcosine [$MeN(N{=}O)CH_2CO_2H$] did not exchange α-hydrogens (CH_2CO_2H) at all, indicating that the presence of the cyano group in (**1a**) is a crucial factor for the deprotonation.

Restricted rotation around the N–N partial double bond[10] of (**1**) yields two conformers, with either a *syn* or *anti* relationship of the nitroso oxygen to the cyanomethyl moiety. The ratio

† H–D exchange of (**1a**) in neutral D_2O was first reported by S. K. Vohra, G. W. Harrington, and D. Swern, *J. Org. Chem.*, 1978, **43**, 3617.



syn - (**1**)



anti - (**1**)

of the isomers was determined from ^{1}H n.m.r. spectra (Experimental section). The preference for the *syn* isomer can be explained in terms of steric and electronic repulsions between the negatively charged oxygen and the bulky substituent R.[10] The isomers could not be separated by conventional techniques (distillation or column chromatography), so that kinetic measurements were carried out by employing *syn* and *anti* mixtures. As shown in Table 1, pseudo-first-order rate constants for H–D exchange are essentially the same for *syn*- and *anti*-(**1a**) at 35.1 °C.* The n.m.r. spectrum of bis(cyanomethyl)nitrosamine (**1k**) showed two singlet peaks due to *syn*-(δ 4.73) and *anti*-CH_2CN (δ 5.61) which were followed independently to provide the same value of k_{obs}. In the following discussion, therefore, evaluation of the rates refers to the *syn* conformer alone.

As shown in Table 1, an increase of D_2O content resulted in an increase of k_{obs}, suggesting that a solvent of larger dielectric constant increases the stabilizations of the nitrosamino carbanion. It has been shown that the rate constant also increases with an increase of the buffer concentration (0.25—1.0M). However, we cannot determine from the present data whether the buffer species ($D_2PO_4^-$ or DCO_3^-) acts as a general base or as a nucleophile to abstract the cyanomethyl protons in the rate-determining step.

Effect of Substituent R.—Owing to the solubility problem, CD_3COCD_3–D_2O or CD_3CN–deuteriated phosphate buffer was employed as the solvent to evaluate the relative rate of (**1**). The effect of the R group is relatively small but definite as shown in Table 2. Most interesting is the effect of *para* substituents on the phenyl ring. The rate of the H–D exchange are found to be of the order (**1i**) > (**1h**) > (**1j**), correlating inversely with the Hammett σ constant. It is well known that the structure of nitrosamines involves resonance contributions such as $R^1R^2N^+{=}N{-}O^-$, where the lone pair of electrons on the amino nitrogen possibly delocalizes into the π-system of the nitroso group.[12] In this regard, Kupper *et al.* have reported ^{13}C and ^{15}N n.m.r. spectra of a series of substituted *N*-methyl-*N*-nitrosoanilines [$XC_6H_4N(N{=}O)Me$], and discussed the electronic effect of the substituent X on the resonance structure.[13] The chemical shifts observed for the amino nitrogen, the nitroso nitrogen, and the α-methyl carbon when varying the substituent X have led the authors to conclude that an electron-withdrawing substituent in the phenyl ring stabilizes form (**A**), whereas an electron-donating substituent tends to stabilize resonance form (**B**) owing to the presence of the strongly electron-withdrawing nitroso moiety. These findings allow us to say that the electronic effect of the ring substituent differs completely from those observed for a series of *N*-methylanilines.[14] For instance, the positive charge developed on the amino nitrogen in (**1i**) would result in marked stabilization of the adjacent carbanion and thus enhance the rate of H–D exchange. Conversely, the electron-withdrawing substituent in (**1j**) would make the lone pair of electrons on the amino nitrogen less mobile and thus minimize k_{obs}. These results may be helpful in understanding the rate of the H–D exchange of nitrosamines (**1**). Previous studies have revealed that the $n \longrightarrow \pi^*$ transition of the N=O chromophore of dimethylnitrosamine shifts ($\Delta\lambda_{max.}$ 28 nm) to shorter wavelength when the solvent changes from carbon tetrachloride

Table 1. Pseudo-first-order rate constants for H–D exchange of 0.25M-*syn*-(**1a**) at 35.1 °C in various solvent systems

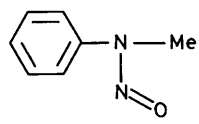
Solvent	pD[a]	10^2k_{obs}/min^{-1} [b]
20% D_2O–80% CD_3COCD_3		0.2 ± 0.0
40% D_2O–60% CD_3COCD_3		3.2 ± 0.2
		3.3 ± 0.3 (*anti*)
50% D_2O–50% CD_3COCD_3		4.6 ± 1.1
D_2O		12 ± 2
		11 ± 2 (*anti*)
30% 0.02M-phosphate buffer–70% CD_3CN	9.12	3.7 ± 0.6
50% 0.02M-phosphate buffer–50% CD_3CN	9.14	5.9 ± 1.0
0.02M-phosphate buffer	8.67	13 ± 2
Phosphate buffer 0.25M[c]	5.72	1.5 ± 0.2
0.50M[c]	5.77	1.8 ± 0.2
0.50M	5.80	1.7 ± 0.3
1.0M[c]	5.87	4.2 ± 0.4
0.25M[c]	6.41	3.0 ± 0.3
0.50M[c]	6.38	3.2 ± 0.4
1.0M[c]	6.40	6.4 ± 0.6
0.50M	7.10	9.1 ± 0.9
0.50M	8.38	21 ± 3
Carbonate buffer 0.25M[c]	8.73	27 ± 2
0.50M[c]	8.79	47 ± 6
0.50M	9.15	53 ± 6

[a] pD = pH_{obs} + 0.40. [b] Average values of two or three runs. [c] Ionic strength was adjusted to 3.0 by NaCl.

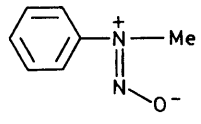
Table 2. Pseudo-first-order rate constants for H–D exchange of 0.25M-*syn*-(**1**) at 35.1 °C in deuteriated acetone–deuterium oxide and deuteriated phosphate buffer–deuterioacetonitrile

	10^2k_{obs}/min^{-1} [a]	
Compound	40% D_2O:60% CD_3COCD_3	30% Phosphate buffer: 70% CD_3CN[b]
(**1a**)	3.2 ± 0.2 (6.4)	3.7 ± 0.6
(**1b**)	3.6 ± 0.4 (7.2)	
(**1c**)	3.7 ± 0.8 (7.4)	
(**1d**)	3.5 ± 0.8 (7.0)	
(**1e**)	0.5 ± 0.1 (k_{rel} 1.0)	0.6 ± 0.2
(**1f**)	0.6 ± 0.1 (1.2)	
(**1g**)	0.6 ± 0.2 (1.2)	
(**1h**)	2.7 ± 0.7 (5.4)	2.3 ± 0.5
(**1i**)	5.8 ± 0.7 (11.6)	
(**1j**)	0.7 ± 0.1 (1.4)	
(**1k**)	7.8 ± 0.9 (15.6)	

[a] Average values of two or three runs. [b] pD 9.12.



(**A**)



(**B**)

* This seems to be in contrast to the complete *syn* selectivity of nitrosamino carbanions reported previously.[8,11] The lack of a *syn* preference in the H–D exchange of (**1a**) could be accounted for, though tentatively, by the rapid interconversion *syn*-(**1a**) ⇌ *anti*-(**1a**) under the experimental conditions. Stefaniak *et al.* reported ΔG^* 86.1 kJ mol^{-1} as the free energy of activation for restricted rotation around the N–N partial double bond of (**1a**) from dynamic ^{1}H n.m.r. analysis (S. Szymanski, L. Stefaniak, M. Witanowski, and A. Ejchat, *Org. Magn. Reson.*, 1977, **9**, 699). This value allows us to calculate *k* 0.96 min^{-1} as the rate constant of interconversion at 35.1 °C, a larger magnitude than the rate constants for H–D exchange obtained in this study.

Table 3. $\lambda_{max.}$ Values of $n \longrightarrow \pi^*$ transition of nitrosamines

$R^1R^2NN{=}O$		$\lambda_{max.}$/nm[a]			$\Delta\lambda_{max.}$/nm
R^1	R^2	CCl_4	MeCN	H_2O	($CCl_4 \longrightarrow H_2O$)
Me	Me	361	353	333	28[b]
Me	CH_2CN (**1a**)	366	359	346	20
Bu^t	CH_2CN (**1d**)	368	362	348	20
$PhCH_2$	CH_2CN (**1e**)	366	360	352	14
$NCCH_2$	CH_2CN (**1k**)	369	365	360	9
F_3CCH_2	CH_2CF_3	372	369	366	6[c]

[a] $\varepsilon_{max.} \leqslant 100$. [b] This study. [c] Ref. 15*a*.

to water. This indicates that the dipolar structure of the nitrosamino moiety is more likely in aqueous solution.[15] However, the shift of the absorption of bis-(2,2,2-trifluoroethyl)nitrosamine is rather small, due to the presence of strongly electron-withdrawal alkyl groups on the amino nitrogen.[15] Thus it is reasonable to suspect, as suggested from the data in Table 3, that the resonance contribution of the dipolar structure of alkylnitrosamines (**1a** and **d**) is pronounced in aqueous solution and advances the rate of H–D exchange. The primary effect on hydrogen acidity of the symmetrical nitrosamine (**1k**) is probably an inductive one by the second CH_2CN group through two σ bonds.[16] The intermediate benzyl group (**1e**) might leave the lone pair of electrons *in situ* to reduce the occurrence of the α-carbanion.

The present study demonstrates that the acidity of the α-hydrogens of nitrosamines is influenced by the electronic effect of the substituent on the amino nitrogen which depends principally on two canonical forms for this class of compound. The results are relevant to the chemical reactivity and oxidative dealkylation of nitrosamines in biological systems.

Experimental

U.v. spectra were recorded on a Shimadzu UV-200 spectrophotometer. ^{1}H N.m.r. spectra were taken by a Hitachi R-24 instrument (60 MHz).

Materials.—Buffer solutions were prepared by D_2O (99.75%) using NaH_2PO_4–Na_2HPO_4 or $NaHCO_3$–Na_2CO_3. The ionic strength was adjusted with NaCl. pH Values were measured on a model FL-5D instrument (Fuji Kagaku Keisoku). The pD was calculated by adding 0.40 to the observed pH.[17] The symmetrical nitrosamine (**1k**) was prepared from the nitrosation of iminodiacetonitrile by $NaNO_2$ in H_2O, m.p. 39—40 °C (lit.,[18] 38—38.5 °C); $\lambda_{max.}^{H_2O}$ 229 nm ($\varepsilon_{max.}$ 5 800). Nitrosamines (**1a**—**j**) were from our previous study.[7] The *syn*:*anti* ratio was determined by ^{1}H n.m.r. spectroscopy in $CDCl_3$: (**1a**) 53:47, (**1b**) 86:14, (**1c**) 89:11, (**1d**) 100:0, (**1e**) 75:25, (**1f**) 80:20, (**1g**) 72:28, (**1h**) 100:0, (**1i**) 100:0, (**1j**) 100:0.

Kinetics of H–D *Exchange.*—An n.m.r. tube containing solvent (200 μl) was placed in the probe at 35.1 °C. After equilibration of the temperature, the calculated amount of (**1**) (5×10^{-5} mol) was added quickly into the tube. The peak areas of the cyanomethyl protons and of the unexchangeable protons of R were integrated from time to time. The latter protons were used as reference. For the kinetics of (**1k**), t-C_4H_9OD (2 μl) was added as reference. The rate constant was calculated from plots of $\ln(A_t/A_0)$ *versus* time, where A = peak area of CH_2CN/peak area of reference protons.

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