

Table 1. Constants for the Cleavage of *m*-Nitrophenyl Acetate in the Presence of Cyclodextrins and Metal Ions^a

Host	M ²⁺ ([host] : [M ²⁺])	K/M ⁻¹	k _o ^{CD} × 10 ² /s ⁻¹	(k _o ^{CD} /k _o ^o) ^b
β-CD	none	100	4.7	86
	none	125 ^c	44.4 ^c	96 ^c
CDen	none	55	11	210
	Zn ²⁺	150	5.1	94
	(1 : 0.25) Cu ²⁺	230	6.5	120
CDdien	none	95	7.1	130
	Zn ²⁺	310	3.2	59
	(1 : 0.75) Cu ²⁺	260	4.8	89

^aAt 25°C, in 0.05 M pH 9.60 borate buffer (I=0.2 M). ^bk_o^o is 5.4 × 10⁻⁴ s⁻¹; ^cLit. values obtained in pH 10.60 carbonate buffer (ref. 9).

reasing order of the binding constant between host and the ester is β-CD ≅ CDdien > CDen, while that of k_o^{CD} is CDen > CDdien > β-CD. Addition of Zn²⁺ or Cu²⁺ to the media containing CDen or CDdien have a contrasting effect on the binding constant and k_o^{CD}: the binding constant increases, whereas k_o^{CD} decreases on the addition of the metal ions.

One possible explanation for the order of the binding constant is that the diethylenetriamine group is sufficiently hydrophilic to stay outside of the cyclodextrin cavity, and the environment and the depth of the cavity are not much different with those of β-CD itself, leading the binding constant of CDdien being essentially same with that of unmodified β-CD. On the other hand, the ethylenediamine group is expected to be included into the cavity, at least in part, causing the shallowness of the cavity and decreased binding of the substrate. This result is in line with our earlier report² of weaker binding of 1-benzyl-1,4-dihydronicotinamide, BNAH, with mono(6-O-tosyl)-β-CD, 6-Ts-CD, compared to that with β-CD itself. The fact that the rate constant k_o^{CD} for the fully complexed substrate is greatest in case of CDen implies that the substrate complexed with CDen is in better position for the nucleophilic interaction between the C-2 oxido group of the CD and the carbonyl carbon atom of the substrate.⁸

The effects of the divalent metal ions on the binding constants and k_o^{CD} for CDen and CDdien can be attributed to the interaction between the metal ion and the amino group.^{4,5,9} The interaction of cyclodextrins functionalized with polyamines with Cu²⁺ or Zn²⁺ results in cyclodextrins flexibly capped by metal ions, which causes increased binding of the substrate.⁴ The difference in the effects of Cu²⁺ and Zn²⁺ seems to arise from different coordination geometry and/or coordination number between Cu²⁺ and Zn²⁺.^{9,10} Contrast to the enhancement of the binding affinity of the substrate with CDen or CDdien by the addition of the metal ions, the k_o^{CD} value gets smaller and becomes not much different with that of β-CD itself. This suggests that the enhanced binding by the flexible capping does not give the proper geometry for the nucleophilic interaction between the C-2 oxido group of

the CD and the carbonyl carbon atom of the substrate.

In conclusion, this work demonstrates that functionalization of β-CD with polyamines increases the reactivity of *m*-nitrophenyl acetate complexed with the host molecules. Addition of divalent metal ions enhances binding affinity of the ester, but decreases the reactivity of the complexed substrate.

Acknowledgement. This work was supported by grant from the Korea Science and Engineering Foundation through Center for Biofunctional Molecules and from Ministry of Education through Basic Science Research Institute Program.

References

- (a) Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer-Verlag: New York, 1978; (b) Breslow, R. *Acc. Chem. Res.* 1981, 13, 170; (c) Tabushi, I. *Acc. Chem. Res.* 1982, 15, 66; (d) D'Souza, V. T.; Bender, M. L. *Acc. Chem. Res.* 1987, 20, 146.
- Park, K. K.; Park, H. S.; Park, J. W. *Bull. Korean Chem. Soc.* 1992, 13, 359.
- Park, K. K.; Yoon, K. Y. *Bull. Korean Chem. Soc.* 1993, 14, 461.
- Tabushi, I.; Shimizu, N.; Sugimoto, T.; Shiozuka, M.; Yamamura, K. *J. Am. Chem. Soc.* 1977, 99, 7100.
- Matsui, Y.; Yokoi, T.; Mochida, K. *Chem. Lett.* 1976, 1037.
- Petter, R. C.; Salek, J. S.; Sikorski, C. T.; Kumaravel, G.; Lin, F. T. *J. Am. Chem. Soc.* 1990, 112, 3860.
- VanEtten, R. L.; Gloves, G. A.; Sebastian, J. F.; Bender, M. L. *J. Am. Chem. Soc.* 1967, 89, 3234, 5253.
- Breslow, R.; Czarniecki, M. F.; Emert, J.; Hamaguchi, H. *J. Am. Chem. Soc.* 1980, 102, 762.
- Kijima, T.; Nakazawa, H.; Kobayashi, M. *Bull. Chem. Soc. Jpn.* 1988, 61, 4277.
- Ogino, K.; Kashihara, N.; Ueda, T.; Isaka, T.; Yoshida, T.; Tagaki, W. *Bull. Chem. Soc. Jpn.* 1992, 65, 373.

Nitrosation Products of *N*-Acyl-*N'*-Substituted Phenylhydrazines

Jack C. Kim* and Sun-Hong Han

Department of Chemistry, Pusan National University,
Pusan 609-735

Received September 27, 1993

A large number of cytostatic 2-chloroethyl-nitrosoureas¹⁻¹² have been synthesized and evaluated for antitumor activity against murine leukemia L1210 implanted either intraperitoneally or intracerebrally. The nitrosoureas decompose to yield an isocyanate and a variety of other reactive species.¹³ The interaction of one or more of these reactive moieties with biologically active macromolecules is thought to be responsible for the anticancer activity and the cytotoxicity of

Table 1. *N*-Acyl-*N'*-Substituted Phenylhydrazines (**1a-k**)

Comp.	G	R	Yield (%)	mp. (°C)	IR (cm ⁻¹)		NMR (δ)			
					C=O	N=H	Ar-NH	CNH	Ar	R
a	H	CH ₃	72	128-129	1650	3310	9.33	7.4	6.2-7.2	1.7
b	3-CH ₃	CH ₃	51	115-117	1640	3300	9.4	7.4	6.1-7.1	1.8
c	4-CH ₃	CH ₃	58	103-105	1640	3250	9.3	7.3	6.2-7.0	1.8
d	3-Cl	CH ₃	60	167-168	1680	3250	9.6	7.9	6.4-7.3	2.1
e	4-Cl	CH ₃	53	145-147	1650	3300	9.7	7.9	6.4-7.5	2.0
f	3-NO ₂	CH ₃	51	125-126	1660	3400	9.3	8.0	6.8-8.2	1.7
g	4-NO ₂	CH ₃	56	208-209	1650	3330	9.6	8.6	6.6-8.2	2.0
h	H	CH ₂ CH ₃	62	151-153	1640	3290	9.3	7.4	6.3-7.2	0.8-2.4
i	H	CH ₂ CH ₂ CH ₃	63	84-86	1660	3350	9.4	7.4	6.3-7.2	0.7-7.3
j	H	CH(CH ₃) ₂	54	137-139	1640	3350	9.5	7.7	6.3-7.4	1.2-2.8
k	H	C(CH ₃) ₃	48	104-106	1650	3280	9.3	7.5	6.3-7.2	1.16

the nitrosoareas.

The nitrosoareas are usually obtained by the conventional route of preparing the urea compound first, then subsequently nitrosating it with a variety of nitrosating agents¹⁴⁻¹⁶, eg, sodium nitrite in acidic medium, nitrous anhydride, nitrosyl chloride, dinitrogen tetroxide or nitrosonium tetrafluoroborate. However, when nitrosoareas are synthesized by the nitrosation of the urea, difficulties in achieving selective nitrosation of the urea at the required position are always encountered, and two regioisomers are produced. Selective nitrosation at the nitrogen bearing the methyl or the 2-chloroethyl group is critical in syntheses of unsymmetrical *N*,

N'-substituted 2-chloroethyl-*N*-nitrosoareas¹⁴ (R-N-C-N-CH₂CH₂Cl).

Furthermore, regioselective nitrosation reaction among three kinds of nitrogen in the antitumor *N*-alkyl-*N'*-anilinoareas

compounds¹⁷ (Φ -N-N-C-N-CH₂CH₂Cl) by the above analogous nitrosation methods will theoretically give three regioisomeric nitrosoareas. The aim of our present work is to investigate how the electronic or steric factor affects the regioselectivity of the *N*-acyl-*N'*-substituted phenylhydrazines (**1a-k**) towards the nitrosation of two kinds of nitrogen atoms. To see the steric informations about the regiochemistry of *N*-nitrosation reactions, the model compounds, *N*-acyl-*N'*-substituted phenylhydrazines (**1a, h-k**) were prepared by the reactions of the phenylhydrazines with the various acids (increasing the bulkiness of alkyl groups; acetic acid, propionic acid, butyric acid, isobutyric acid, and trimethylacetic acid). Of these series of *N*-acyl-*N'*-substituted phenylhydrazines, the phenylhydrazines substituted with either electron-donating or electron-withdrawing groups were also reacted with acetic acid to afford *N*-acetyl-*N'*-substituted phenylhydrazines (**1a-g**) and to examine the role of electron density on the nitrogen atom to the regiochemical change. The regioselectivity in the nitrosation of *N*-acyl-*N'*-substituted phenylhydrazines (**1a, h-k**), and of *N*-acyl-*N'*-substituted phenylhydrazines (**1a-g**) was examined using NaNO₂ and four different acids (diluted HCl, HCOOH, CH₃COOH, CF₃COOH) at -10

°C-0°C. In all cases, the two regioisomeric products, *N*-acyl-*N'*-nitroso-*N'*-substituted phenylhydrazines (**2a-k**) and *N*-acyl-*N*-nitroso-*N'*-substituted phenylhydrazines (**3a-k**) were observed to be formed as major products (Table 2 and 3). However, the *N*-acyl-*N'*-nitroso-*N'*-substituted phenylhydrazines, **2a-k** having on tlc elution mobility higher than that of the *N*-acyl-*N*-nitroso-*N'*-substituted phenylhydrazines, **3a-k** were observed to be formed first, and then rearrange to the **3a-k**. In experiments to examine the occurrence of nitroso group migration in the **2a-k**, we treated the nitrosation reaction of **1a-g** for a prolonged reaction time (3 hours or longer) at 0-5°C, and the **3a-k** were found virtually exclusively as major products with the trace of **2a-k**. Even when the acyl groups were large, eg, isopropyl, and tertiary butyl in **1a-k**, or when there were either electron withdrawing or electron donating groups substituted on the phenyl ring, the **3a-k** were formed exclusively. In each instance, the nitroso group transfer from the **2a-k** to the **3a-k** was unaffected by both the steric and electronic effects^{14,18}. The exclusive formation of the **3a-k** was drastically slowed down under -20°C. Based on these observations, it is clear that products **2a-k** are kinetically controlled products which were formed through the phenyl π -electron assisted nitrosonium ion approach to the phenyl nitrogen, while products **3a-k** are thermodynamically controlled products.

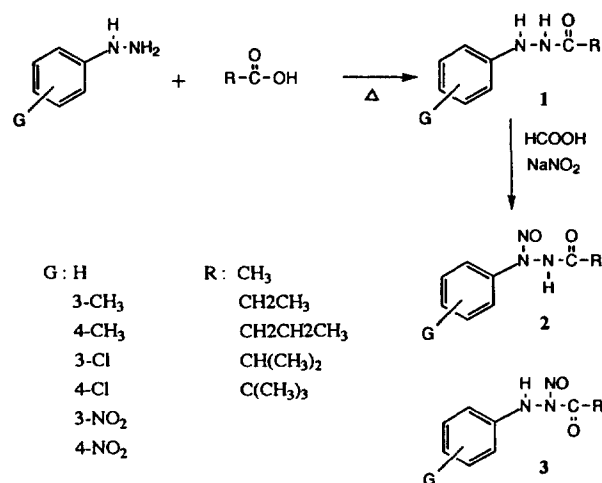


Table 2. *N*-Acyl-*N'*-Nitroso-*N'*-Substituted Phenylhydrazines (**2a-k**)

Comp.	G	R	Yield (%)	mp. (°C)	IR (cm ⁻¹)		NMR (δ)			
					C=O	N=H	N-H	Ar	CNH	R
a	H	CH ₃	32	oil	1710	1495	3300	6.9-7.6	8.73	1.92
b	3-CH ₃	CH ₃	29	oil	1700	1500	3270	6.8-7.4	8.6	2.06
c	4-CH ₃	CH ₃	38	88-89	1690	1490	3250	7.0-7.6	8.0	2.06
d	3-Cl	CH ₃	35	oil	1690	1490	3200	7.0-7.6	8.1	2.1
e	4-Cl	CH ₃	40	84-85	1680	1490	3200	7.1-7.6	10.4	2.1
f	3-NO ₂	CH ₃	39	106-109	1675	1510	3200	7.5-8.5	9.8	2.16
g	4-NO ₂	CH ₃	23	—	—	—	—	—	—	—
h	H	CH ₂ CH ₃	40	oil	1700	1490	3250	7.0-7.6	8.7	0.8-2.4
i	H	CH ₂ CH ₂ CH ₃	37	88-89	1690	1490	3240	6.7-7.5	8.9	0.6-2.25
j	H	CH(CH ₃) ₂	32	72-74	1680	1500	3190	7.0-7.6	8.8	2.76-2.8
k	H	C(CH ₃) ₃	35	108-109	1680	1490	3300	7.13-7.7	8.2	1.3

Table 3. *N*-Acyl-*N*-Nitroso-*N'*-Substituted Phenylhydrazines (**3a-k**)

Comp.	G	R	Yield (%)	mp. (°C)	IR (cm ⁻¹)			NMR (δ)	
					C=O	N=O	N-H	Ar	R
a	H	CH ₃	29	oil	1740	1500	3310	7.2-8.5	2.4
b	3-CH ₃	CH ₃	30	oil	1750	1500	3280	6.9-7.9	2.3
c	4-CH ₃	CH ₃	37	oil	1750	1500	3400	7.2-7.6	2.4
d	3-Cl	CH ₃	22	oil	1715	1480	3250	7.0-8.0	2.4
e	4-Cl	CH ₃	20	oil	1740	1490	3300	7.1-8.1	1.4
f	3-NO ₂	CH ₃	22	oil	1650	1510	3380	7.3-8.6	2.5
g	4-NO ₂	CH ₃	27	oil	1760	1510	3350	7.5-8.5	2.5
h	H	CH ₂ CH ₃	35	oil	1740	1500	3250	7.0-8.3	1.0-1.9
i	H	CH ₂ CH ₂ CH ₃	39	oil	1750	1500	3310	7.0-8.4	0.7-2.9
j	H	CH(CH ₃) ₂	37	oil	1735	1490	3490	7.0-8.4	0.8-3.4
k	H	C(CH ₃) ₃	30	oil	1740	1500	3380	7.2-8.4	1.3

Experimental

Melting points were determined on Electrothermal Capillary Melting Point apparatus and are uncorrected. Tlc was performed on glass plated coated with aluminium oxide (Silica gel 60 F₂₅₄) and compounds were visualized using an uv lamp. ¹H-NMR was obtained with varian EM-360A spectrophotometer (solutions in dimethyl-d₆-sulfoxide with tetramethylsilane as the internal standard). Ultraviolet spectral data were recorded on a Hitachi 124 spectrophotometer, and ir spectra were recorded with Shimadzu 400 spectrophotometer. Pertinent data for synthesized compounds are listed in Table 1, 2, and 3.

General procedure for the preparation of *N*-acyl-*N'*-substituted phenylhydrazines (**1a-k**). Phenylhydrazines or substituted phenylhydrazines (17 mmol) were slowly added to acetic acid (17 mmol) (propionic acid, butyric acid, isobutyric acid, trimethylacetic acid) under ice-cold condition and the reaction mixture was heated at 105-110°C for 30 min. Crystallization from an appropriate solvent afforded an analytically pure solid. Pertinent data for synthesized compounds are listed in Table 1, 2, and 3.

General procedure for the syntheses of *N*-acyl-*N'*-nitroso-

N'-substituted phenylhydrazines (**2a-k**) and *N*-acyl-*N*-nitroso-*N'*-substituted phenylhydrazines (**3a-k**). A solution of *N*-acyl-*N'*-substituted phenylhydrazines (**1a-k**) (14 mmol) in 99% HCOOH (10 ml) was cooled to 0-5°C and treated with NaNO₂ (25 mmol) in small portions. The reaction mixture was stirred for 1 hr at 0-5°C. The reaction mixture was poured into an ice-H₂O mixture (150 ml) and extracted with CHCl₃ (2 × 200 ml). The combined organic layers were washed with H₂O (2 × 150 ml), dried (MgSO₄), filtered and evaporated under reduced pressure to give a solid. Crystallization from an appropriate solvent gave a pure compound. The uncrystallized only residues were applied to a column packed with silica gel and the column was eluted with hexane-ethylacetate (20 : 1, v/v). The fractions containing the **2a-k** were collected first, and concentrated to a sirup which was crystallized from an appropriate solvent. Further elution gave the **3a-k**.

Acknowledgement. This work was supported in part by NON DIRECTED RESEARCH FUND, Korea Research Foundation, 1993 and also supported in part by the Basic Science Research Institute, ministry of Korea, 1993 (BSRI-93-308).

References

1. Johnston, T. P.; McCaleb, G. S.; Opliger, P. S.; Montgomery, I. A. *J. Med. Chem.* **1963**, 6, 669.
2. *ibid*, *J. Med. Chem.* **1966**, 9, 892.
3. *ibid*, *J. Med. Chem.* **1971**, 14, 600.
4. *ibid*, *J. Med. Chem.* **1977**, 20, 279.
5. Kim, J. C.; Cho, I. S. *J. Pharm. Soc.* **1983**, 24, 177.
6. Kim, J. C.; Youn, I. K.; Koh, Y. S.; Yoon, U. C.; Park, M. Y.; Moon, K. H. *J. Pharm. Soc.* **1983**, 27, 309.
7. Kim, J. C.; Kim, M. S.; Lee, H. K. *Arch. Pharm. Res.* **1983**, 6, 7.
8. Kim, J. C.; Park, M. T.; Shin, H. D.; Koh, Y. S.; Yoon, U. C.; Ryu, S. H.; Moon, K. H.; Kim, M. S. *J. Pharm. Soc.* **1984**, 28, 197.
9. Kim, J. C.; Choi, S. K.; Cho, I. S.; Yu, D. S.; Ryu, S. H.; Moon, K. H. *J. Pharm. Soc.* **1985**, 6, 172.
10. Kim, J. C.; Choi, S. K.; Moon, S. H. *Arch. Pharm. Res.* **1986**, 9, 215.
11. Kim, J. C.; Lee, J. C.; Nha, C. S. *Korean. J. Med. Chem.* **1992**, 3, 86.
12. Kim, J. C.; Park, J. I.; Hur, T. H. *Bull. Korean Chem. Soc.* **1993**, 14, 176.
13. Montgomery, J. A. *Cancer Treat. Rep.* **1976**, 60, 651.
14. Kim, J. C.; Cho, I. S.; Choi, S. K. *J. Korean Chem. Soc.* **1991**, 35, 240.
15. Won, M. S.; Kim, J. C.; Shim, Y. B. *J. Korean Chem. Soc.* **1991**, 35, 707.
16. Won, M. S.; Kim, J. C.; Shim, Y. B. *Bull. Korean Chem. Soc.* **1992**, 13, 214.
17. Kim, J. C.; Lim, Y. G.; Min, B. T. *J. Med. Chem.* in press.
18. Singer, S. S.; Cole, B. B. *J. Org. Chem.* **1981**, 46, 3461.