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}	$k_\psi^{CD}\times10^2/s^{-1}$	$(k_\psi^{CD}/k_\psi^{\circ})^b$
β-CD	none	100	4.7	86
	none	125[c]	44.4[c]	96[c]
CDen	none	55	11	210
	Zn^{2+} (1 : 0.25)	150	5.1	94
	Cu^{2+} (1 : 0.50)	230	6.5	120
CDdien	none	95	7.1	130
	Zn^{2+} (1 : 0.75)	310	3.2	59
	Cu^{2+} (1 : 0.75)	260	4.8	89

[a] At 25℃, in 0.05 M pH 9.60 borate buffer (I=0.2 M). [b] k_ψ° is $5.4\times10^{-4}\,s^{-1}$; [c] Lit. 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_ψ^{CD} is CDen >CDdien>β-CD. Addition of Zn^{2+} or Cu^{2+} to the media containing CDen or CDdien have a contrasting effect on the binding constant and k_ψ^{CD}: the binding constant increases, whereas k_ψ^{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 report[2] 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_ψ^{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_ψ^{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 Cu^{2+} or Zn^{2+} results in cyclodextrins flexibly capped by metal ions, which causes increased binding of the substrate.[4] The difference in the effects of Cu^{2+} and Zn^{2+} seems to arise from different coordination geometry and/or coordination number between Cu^{2+} and Zn^{2+}.[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_ψ^{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

1. (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.
2. Park, K. K.; Park, H. S.; Park, J. W. *Bull. Korean Chem. Soc.* **1992**, *13*, 359.
3. Park, K. K.; Yoon, K. Y. *Bull. Korean Chem. Soc.* **1993**, *14*, 461.
4. Tabushi, I.; Shimizu, N.; Sugimoto, T.; Shiozuka, M.; Yamamura, K. *J. Am. Chem. Soc.* **1977**, *99*, 7100.
5. Matsui, Y.; Yokoi, T.; Mochida, K. *Chem. Lett.* **1976**, 1037.
6. Petter, R. C.; Salek, J. S.; Sikorski, C. T.; Kumaravel, G.; Lin, F. T. *J. Am. Chem. Soc.* **1990**, *112*, 3860.
7. VanEtten, R. L.; Gloves, G. A.; Sebastian, J. F.; Bender, M. L. *J. Am. Chem. Soc.* **1967**, *89*, 3234, 5253.
8. Breslow, R.; Czarniecki, M. F.; Emert, J.; Hamaguchi, H. *J. Am. Chem. Soc.* **1980**, *102*, 762.
9. Kijima, T.; Nakazawa, H.; Kobayashi, M. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 4277.
10. 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-nitrosoureas[1-12] 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. (℃)	IR (cm^{-1}) C=O	IR (cm^{-1}) N=H	NMR (δ) Ar-NH	NMR (δ) CNH	NMR (δ) Ar	NMR (δ) 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_2CH_3	62	151-153	1640	3290	9.3	7.4	6.3-7.2	0.8-2.4
i	H	$CH_2CH_2CH_3$	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 nitrosoureas.

The nitrosoureas are usually obtained by the conventional route of preparing the urea compound first, then subsequently nitrosating it with a variety of nitrosating agents[14-16], eg, sodium nitrite in acidic medium, nitrous anhydride, nitrosyl chloride, dinitrogen tetraoxide or nitrosonium tetrafluoroborate. However, when nitrosoureas 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-nitrosoureas[14] (R-N-C-N-CH_2CH_2Cl).

Furthermore, regioselective nitrosation reaction among three kinds of nitrogen in the antitumor *N*-alkyl-N′-anilinourea compounds[17] (Φ-N-N-C-N-CH_2CH_2Cl) by the above analogous nitrosation methods will theoretically give three regioisomeric nitrosoureas. 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′-unsubstituted-phenylhydrazines (**1a, h-k**), and of *N*-acetyl-N′-substituted phenylhydrazines (**1a-g**) was examined using $NaNO_2$ and four different acids (diluted HCl, HCOOH, CH_3COOH, CF_3COOH) at −10 ℃-0℃. 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℃, 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℃. 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.

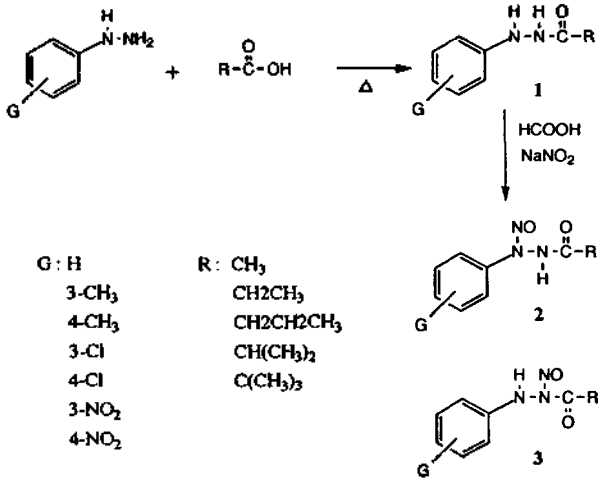

Table 2. *N*-Acyl-N'-Nitroso-N'-Substituted Phenylhydrazines (**2a-k**)

Comp.	G	R	Yield (%)	mp. (℃)	IR (cm^{-1}) C=O	IR (cm^{-1}) N=H	NMR (δ) N-H	NMR (δ) Ar	NMR (δ) CNH	NMR (δ) 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_2CH_3	40	oil	1700	1490	3250	7.0-7.6	8.7	0.8-2.4
i	H	$CH_2CH_2CH_3$	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. (℃)	IR (cm^{-1}) C=O	IR (cm^{-1}) N=O	IR (cm^{-1}) N-H	NMR (δ) Ar	NMR (δ) 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_2CH_3	35	oil	1740	1500	3250	7.0-8.3	1.0-1.9
i	H	$CH_2CH_2CH_3$	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 (Silca gel 60 F_{254}) and compounds were visualized using an uv lmap. ^{1}H-NMR was obtained with varian EM-360A spectrophotometer (solutions in dimethyl-d_6-sulfoxide with tetromethylsilane as the internal standard). Ultraviolet spactiral 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℃ 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 m*l*) was cooled to 0-5℃ and treated with $NaNO_2$ (25 mmol) in small portions. The reaction mixture was stirred for 1 hr at 0-5℃. The reaction mixture was poured into an ice-H_2O mixture (150 m*l*) and extracted with $CHCl_3$ (2× 200 m*l*). The combined organic layers were washed with H_2O (2×150 m*l*), dried ($MgSO_4$), 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.; Pack, 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 Pham. 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.