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Nitrosation of 1-Phenyl-3-(pyridylmethyl)ureas and the Reactivity of Two Nitrosated Isomers

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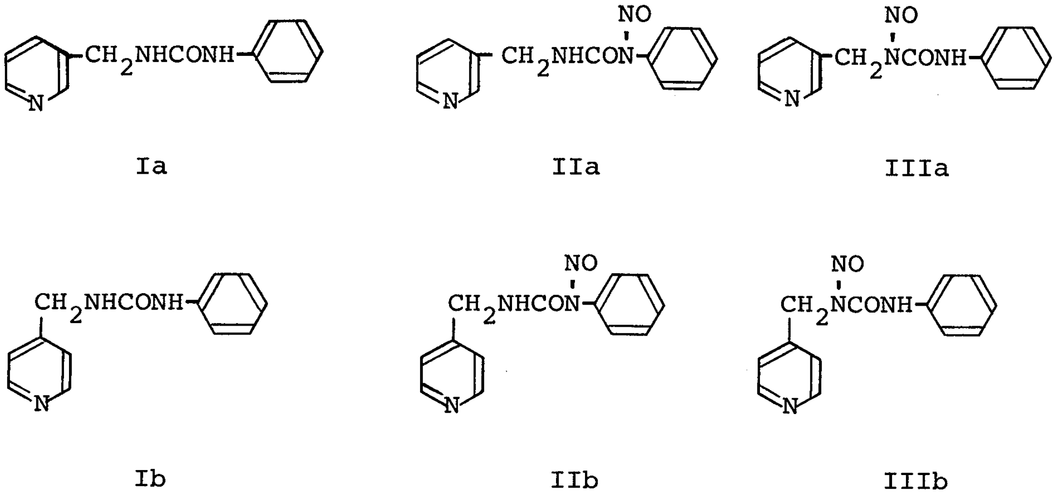
1-Phenyl-3-(pyridylmethyl)ureas (I) were nitrosated with sodium nitrite under acidic conditions to give two positional isomers, 1-nitroso-1-phenyl-3-(pyridylmethyl)urea (II) and 3-nitroso-1-phenyl-3-(pyridylmethyl)urea (III). The nitrosation was carried out using several nitrosating reagents under various conditions, and each isomer was determined by means of high-performance liquid chromatography. The regioselectivity depended on the medium and reagents used. With gaseous nitrosating reagents in organic solvents at 0 °C, I generated mainly III. The combination of sodium nitrite and acid (1 M perchloric acid, 1 M hydrochloric acid or 99% formic acid) gave II predominantly. However, with N_2O_3, the predominant product was dependent on the pH of the medium. The differences of chemical and antitumor activity of the nitroso isomers were also examined.

Keywords——1-phenyl-3-(pyridylmethyl)urea; nitrosation; sodium nitrite; dinitrogen trioxide; dinitrogen tetroxide; nitrosyl chloride; nitrosonium tetrafluoroborate; regioselectivity

N-Nitrosoureas have been known to have antitumor activity since the 1960's,[1] and have been used in clinical treatment. When 1,3-disubstituted ureas are nitrosated, two positional isomers of *N*-nitrosated ureas are generally produced. Since the two isomers are expected to give different reactive intermediates and to have different antitumor activities,[2] regioselective nitrosation is desirable to obtain the more active isomer. In our preceding paper, it was reported that when diarylureas were nitrosated with gaseous reagents (nitrosyl chloride (NOCl), dinitrogen trioxide (N_2O_3) and dinitrogen tetroxide (N_2O_4)), the formation ratio of the two positional isomers of diarylnitrosoureas was affected by the electron density around the ureido nitrogen atoms and by the reagent used.[3] Kamiya, and Kamiya and Sueyoshi



Ia IIa IIIa

Ib IIb IIIb

Chart 1

reported preparative isolation of 1-nitroso-1-phenyl-3-(pyridylmethyl)ureas and 1-(2-chloroethyl)-3-(3-pyridylmethyl)nitrosoureas.[4] In this paper, we report a study on the regioselectivity of nitrosation of 1-phenyl-3-(3-pyridylmethyl)urea (Ia) and 1-phenyl-3-(4-pyridylmethyl)urea (Ib) by means of high-performance liquid chromatography (HPLC), and we describe the antitumor activities of the isomers.

By the treatment of 1-phenyl-3-(3-pyridylmethyl)urea (Ia) with sodium nitrite and aqueous hydrochloric acid, each nitrosated isomer was successfully prepared. Addition of an aqueous solution of sodium nitrite to a hydrochloric acid solution of Ia, followed by neutralization, gave 1-nitroso-1-phenyl-3-(3-pyridylmethyl)urea (IIa)[4] in 80% yield. The other isomer, 3-nitroso-1-phenyl-3-(3-pyridylmethyl)urea (IIIa), was obtained in 95% yield by addition of powdered sodium nitrite to a hydrochloric acid solution of Ia followed neutralization. Treatment of 1-phenyl-3-(4-pyridylmethyl)urea (Ib) as described for IIIa afforded an isomeric mixture of 1-nitrosated (IIb) and 3-nitrosated (IIIb) isomers, and the mixture was chromatographed on a silica gel column (eluent: chloroform and methanol) to isolate 3-nitroso-1-phenyl-3-(4-pyridylmethyl)urea (IIIb). The structures of these isomers (IIa, b and IIIa, b) were confirmed by elemental analysis, and proton nuclear magnetic resonance (^{1}H-NMR), ^{13}C-NMR and infrared (IR) spectral analysis.

We examined the regioselectivity in the nitrosation of Ia using various nitrosating agents under several conditions, by means of HPLC. The results are summarized in Table I. Nitrosation by NOCl, N_2O_3 or N_2O_4 occurred rapidly in chloroform at 0 °C, and within 30 min the reaction reached equilibrium. The urea (Ia) gave mainly IIIa with NOCl, N_2O_3, or N_2O_4 in chloroform.

On the other hand, in 1 M perchloric acid, Ia afforded only the 1-nitrosated isomer (IIa) with all the three nitrosating reagents. In the case of nitrosation with sodium nitrite under acidic conditions (1 M hydrochloric acid, or formic acid) at 0 °C, the nitroso group was similarly introduced at the N-1 position of the urea.

Next, Ia was nitrosated with a 0.1 M chloroform solution of dinitrogen trioxide at various pH values (6.86, 4.5, 3.2, 1.5) at 0 °C. In weakly acidic solution (over pH 4.5) IIIa was the

TABLE I. Effects of the Reagent and Medium on the Isomer Ratio (II : III) (%)

Reagent	Medium	Isomer ratio		Yield
		II	III	(%)
1-Phenyl-3-(3-pyridylmethyl)urea (Ia)				
NOCl	$CHCl_3$	9	91	9
	1 M $HClO_4$	100	0	14
N_2O_3	$CHCl_3$	5	95	30
	1 M $HClO_4$	100	0	20
N_2O_4	$CHCl_3$	3	97	16
	1 M $HClO_4$	100	0	22
$NaNO_2$	1 M $HClO_4$	96	4	13
	1 M HCl	99	1	27
	99% HCOOH	100	0	12
N_2O_3	pH 6.86 phosphate buffer	17	83	6
	pH 4.5 HCl	40	60	10
	pH 3.2 HCl	82	18	12
	pH 1.5 HCl	87	13	31
$NOBF_4$-$(C_2H_5)_3$	$CHCl_3$	0	100	Trace
1-Phenyl-3-(4-pyridylmethyl)urea (Ib)				
N_2O_3	$CHCl_3$	0	100	40
	pH 6.86 phosphate buffer	28	72	9
	pH 2.0 HCl	100	0	6

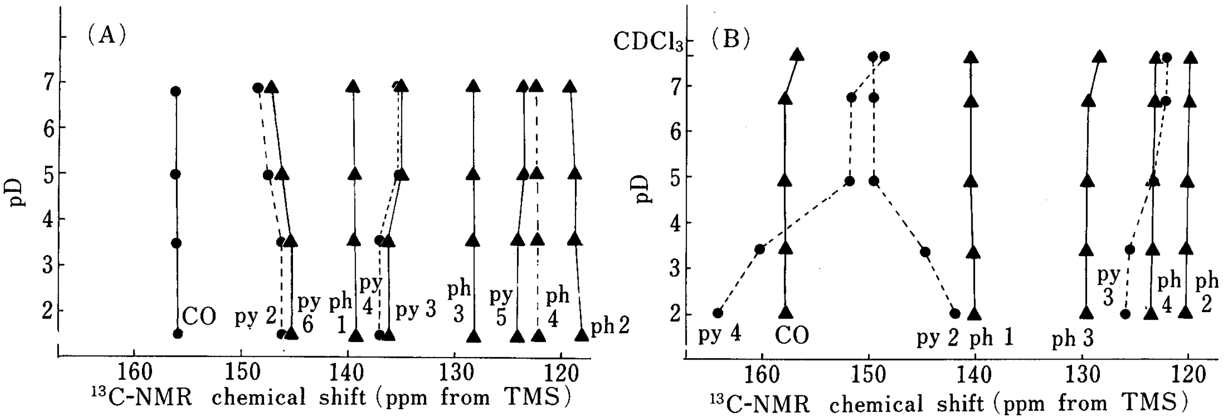


Fig. 1. ^{13}C-NMR Chemical Shifts of 1-Phenyl-3-(3-pyridylmethyl)urea (Ia) and 1-Phenyl-3-(4-pyridylmethyl)urea (Ib) in Various pH Values

(A) 1-phenyl-3-(3-pyridylmethyl)urea (Ia); (B) 1-phenyl-3-(4-pyridylmethyl)urea (Ib). py, pyridyl ring; ph, phenyl ring; CO, carbonyl carbon.

main product, but in more acidic solution (below pH 3.2), IIa was predominant.

Nitrosation of Ia with nitrosonium tetrafluoroborate in the presence of equimolar triethylamine in chloroform afforded only a trace of IIIa.

Compound Ib was nitrosated under typical conditions (N_2O_3 in chloroform, with pH 6.86 buffer solution and pH 2.0 hydrochloric solution). The results were similar to those with Ia.

^{13}C-NMR spectra of Ia and Ib were measured at various values of pD. As shown in Fig. 1, the chemical shifts at all positions of the pyridine ring depended on the pD of the solution. From the results, both Ia and Ib were concluded to be protonated below pD 4.5.

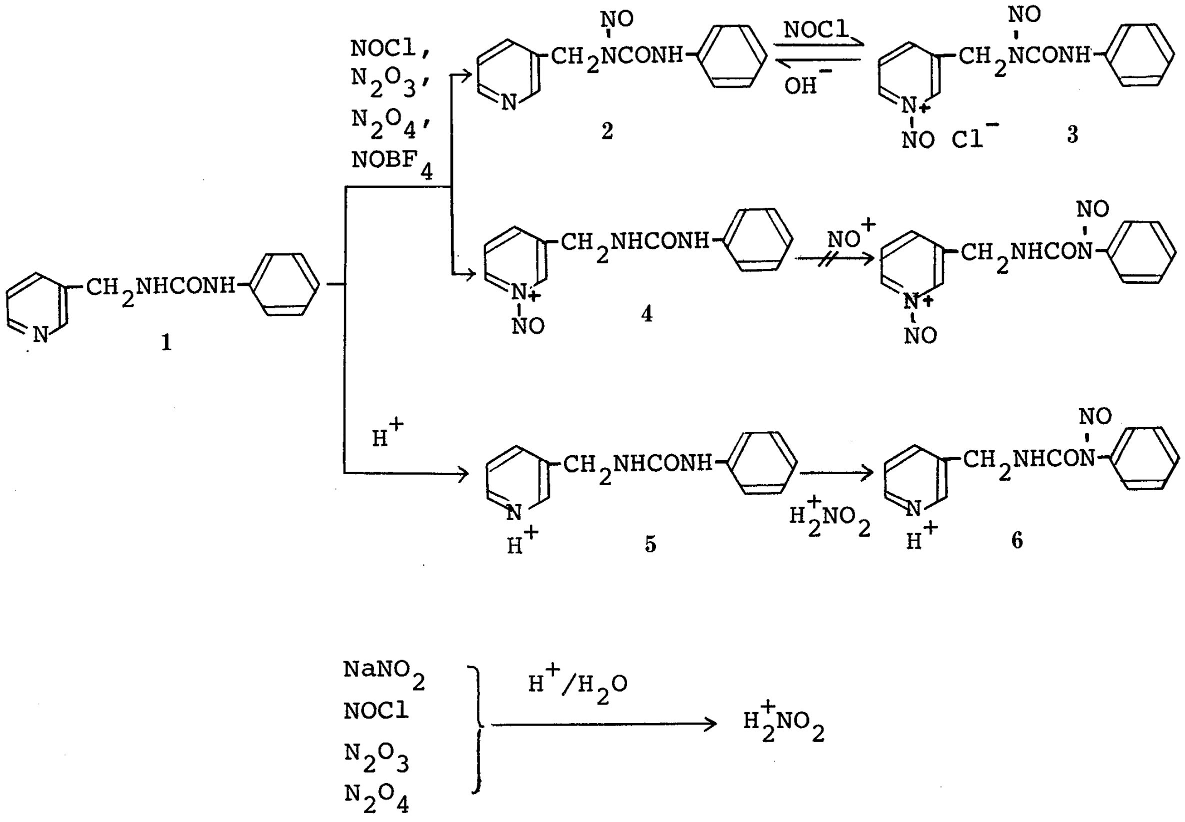


Chart 2

Monitoring of the time courses of IIa and IIIa in diluted acid and organic solvents by means of HPLC indicated that the nitroso groups of IIa and IIIa did not migrate.

Since IIa and IIIa were not interconvertible in diluted acid or organic solvents, the following mechanisms of the regioselective nitrosation of Ia may be considered. Compound **1** (Ia) gives **2** (IIIa) mainly because of the sterical and electronic factors favoring N^3 as the site of nitrosation. Nitrosation at N^3 is also favored by the fact that both substrate and reagent have no charge under these conditions. At low acidities (above pH 2), the main nitrosating reagents formed from sodium nitrite in diluted acid is dinitrogen trioxide, having no charge.[5] Then **2** is nitrosated to give the 1-phenyl-3-nitroso-3-(*N*-nitroso-3-pyridiniomethyl)urea salt (**3**). The N_{arom}-nitrosated ureas (**4**) can not give the *N*-nitrosated ureido derivative, because N_{arom}-nitrosation reduces the reactivity of ureido N^3 atom or N^1 atom.

Under acidic conditions, the protonated pyridinium ion (**5**) is in equilibrium with **1**. The amount of **5** is inversely proportional to pH above the pK_a of the N_{arom}. Compared with the N^3 atom of **5**, N^1 is relatively favorable for nitrosation to give mainly **6** (IIa), because of the strong electron-withdrawing effect of pyridinium ion, the stabilizing effect on the 1-nitrosated isomer, and the repulsion between the protonated pyridinium ion of the urea and the protonated nitrosating reagents (gaseous nitrosating reagents are known to decompose to give nitrous acidium ion ($H_2^+NO_2$)).[6] These nitrosation mechanisms are also conceivable for Ib.

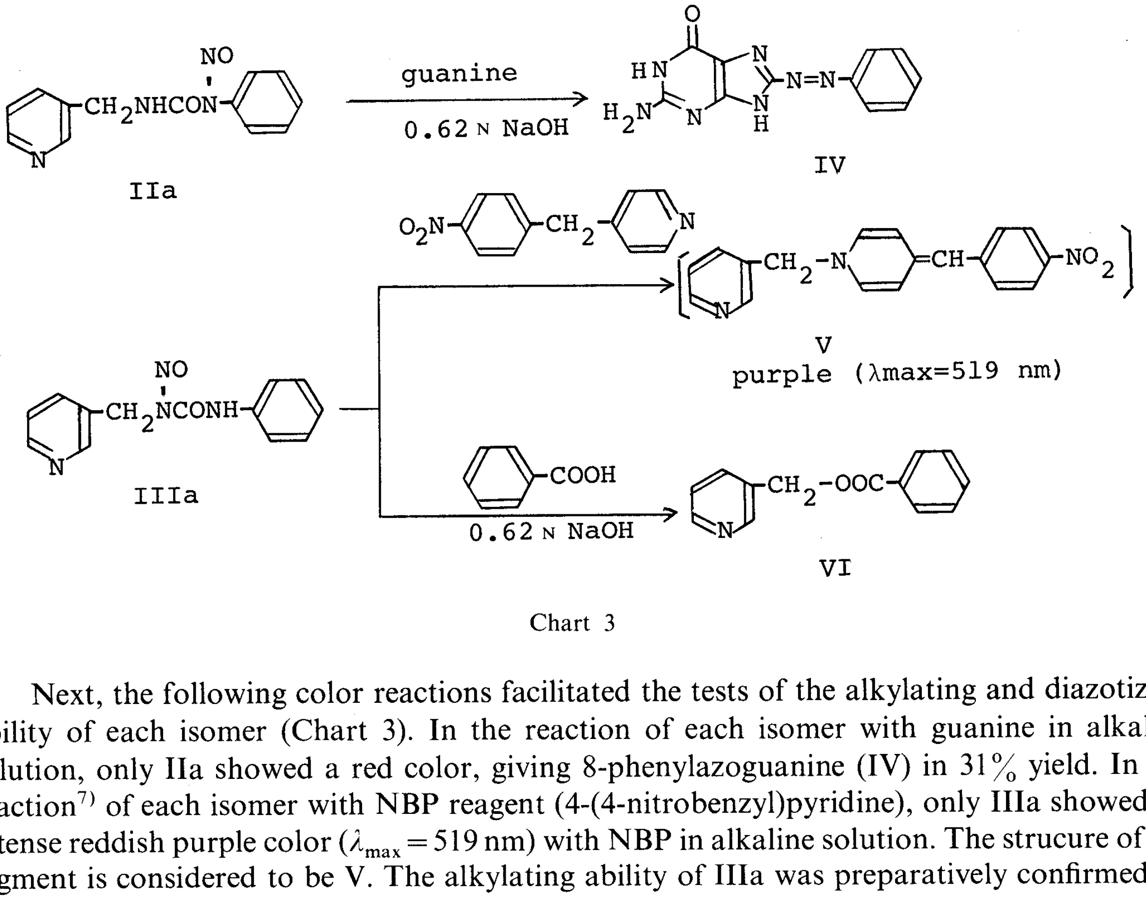


Chart 3

Next, the following color reactions facilitated the tests of the alkylating and diazotizing ability of each isomer (Chart 3). In the reaction of each isomer with guanine in alkaline solution, only IIa showed a red color, giving 8-phenylazoguanine (IV) in 31% yield. In the reaction[7] of each isomer with NBP reagent (4-(4-nitrobenzyl)pyridine), only IIIa showed an intense reddish purple color ($\lambda_{\max} = 519$ nm) with NBP in alkaline solution. The strucure of the pigment is considered to be V. The alkylating ability of IIIa was preparatively confirmed by the formation of the benzoate VI[4a] in 6% yield. These results showed considerable differences of reactivity between 1-nitrosated and 3-nitrosated isomers.

Each isomer (IIa, IIIa) was examined for antitumor activity against rat ascites hepatoma AH13 and mouse lymphoid leukemia L1210[8] (Table II). Each isomer was comparatively effective against AH13 but ineffective against L1210. Nevertheless, there were difference in the reactivity and toxicity of these isomers, and so it is important to synthesize selectively various nitrosated isomers and to evaluate each of them separately.

TABLE II. Antitumor Effects of 1-Phenyl-3-(3-pyridylmethyl)-1-nitrosourea (IIa) and 1-Phenyl-3-(3-pyridylmethyl)-3-nitrosourea (IIIa)

Compound	AH13 (*i.p.*–*i.p.* system)				L1210 (*i.p.*–*i.p.* system)[d]	
	Day 3 MTD[a] (mg/kg)	Days 3—7[b] dose (mg/kg)	$T/C\%$[c]	60 d survivors	Days 2, 6 dose (mg/kg)	Result[e]
IIa	100	5	141	0/6	6.25—50.0	Negative
		10	>332	2/6		
		20	>317	2/6		
IIIa	250	12.5	94	0/6	12.6—100.0	Negative
		25	141	0/6		
		50	>337	2/6		

a) Maximum tolerated dose (MTD) in single intraperitoneal administration, 3 d after intraperitoneal inoculation of 10^6 AH13 cells/rat. *b*) All compounds were administered intraperitoneally once a day on days 3—7 after intraperitoneal inoculation of 10^6 AH13 cells/rat. *c*) $T/C\%$ (T, survival time of treated animals; C, survival time of control animals). *d*) All compounds were administered intraperitoneally on days 2 and 6 after intraperitoneal inoculation of 10^5 L1210 cells/mouse. *e*) $T/C\% < 120$ in the L1210 system was evaluated as negative.

Experimental

General Notes——All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Visible spectra were measured on a Shimadzu UV-240 spectrophotometer. IR were recorded on a JASCO IR A-102 spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were measured with a JEOL FX-200 (operating at a frequency of 50 MHz for ^{13}C-NMR or 199.5 MHz for ^{1}H-NMR), using tetramethylsilane as an internal standard in deuterochloroform ($CDCl_3$). Mass spectra were measured with a JEOL JMX-DX300 mass spectrometer. The analytical silica gel HPLC column (ODS C_{18} column) was prepared with Partisil-5 ODS-3. A JASCO "TWINCLE" high-pressure liquid chromatograph pump was used, equipped with a syringe-loading sample injector (injection volume 2—8 μl) and a Toyo Soda UV-8 spectrophotometer. HPLC was carried out with eluent containing aqueous acetonitrile (water : acetonitrile = 1 : 1), 5 mM potassium perchlorate and 4 mM sodium hexanesulfonate at a flow rate of 2 ml. The eluate was monitored at 254 nm.

Preparation of *N*-Nitroso Ureido Isomers——1-Nitroso-1-phenyl-3-(3-pyridylmethyl)urea (IIa) and 1-nitroso-1-phenyl-3-(4-pyridylmethyl)urea (IIb) were prepared by the reported method.[4]

3-Nitroso-1-Phenyl-3-(3-pyridylmethyl)urea (IIIa): Solid sodium nitrite (5.5 g, 0.08 mol) was added to a solution of 9 g (0.04 mol) of Ia in 18% hydrochloric acid (50 ml), and the mixture was allowed to stand at 0—5 °C for 90 min. A part of the N_{arom}-nitrosated salt (3) precipitated. If necessary, the precipitate was filtered off. The reaction mixture was neutralized with an aqueous saturated solution of sodium bicarbonate. The precipitate formed was filtered off and the filtrate was extracted with chloroform (20 ml, three times). The combined extracts were dried over anhydrous sodium sulfate and the solvent was distilled off under reduced pressure. The residue and precipitate were IIIa. Yellow crystalline powder, mp 47—48 °C (dec.). Yield, 10 g (95%). IR ν_{max}^{Nujol} cm^{-1}: 3280 (NH), 1705 (CO). ^{1}H-NMR ($CDCl_3$) δ: 5.10 (s, $PyCH_2$, 2H), 7.22 (d, PyH-4, 1H), 7.24 (t, PyH-5, 1H), 7.40 (t, PyH-3, PhH-5, 2H), 7.59 (d, PhH-2, PhH-6, 2H), 7.64 (t, PhH-4, 1H), 8.51 (d, PyH-6, 1H), 8.61 (s, PyH-2, 1H), 8.87 (s, NH, 1H). ^{13}C-NMR ($CDCl_3$) δ: 40.22 ($-CH_2-$), 120.24 (PhC-2, PhC-6), 123.41 (PhC-4), 125.06 (PyC-5), 129.15 (PhC-3, PhC-5), 130.12 (PyC-3), 135.77 (PhC-1), 136.45 (PyC-4), 149.15 (PyC-6), 150.17 (PyC-2), 150.22 (CO).

3-Nitroso-3-(*N*-nitroso-3-pyridiniomethyl)-1-phenylurea Chloride (3): mp 136 °C. *Anal.* Calcd for $C_{13}H_{12}ClN_5O_3$: C, 48.53; H, 3.76; N, 21.77. Found: C, 48.37; H, 4.04; N, 21.74.

3-Nitroso-1-phenyl-3-(4-pyridylmethyl)urea (IIIb): Solid sodium nitrite (69 mg, 1 mmol) was added to a solution of 227 mg (1 mmol) of Ib in 18% hydrochloric acid (0.5 ml), and the mixture was allowed to stand at 0—5 °C for 60 min, then neutralized with an aqueous saturated solution of sodium bicarbonate. The precipitate formed was filtered off and the filtrate was extracted with chloroform (20 ml, three times). The combined extracts were dried over anhydrous sodium sulfate and the solvent was distilled off under reduced pressure. The residue and precipitate were dissolved in chloroform and chromatographed on a silica gel column using a mixture of chloroform and methanol (49 : 1). Yellow crystalline powder, mp 88 °C (dec.). Yield, 31 mg (12%). IR ν_{max}^{Nujol} cm^{-1}: 3160 (NH), 1700 (CO). ^{1}H-NMR ($CDCl_3$) δ: 5.10 (s, $PyCH_2$, 2H), 7.17 (t, PhH-3, PhH-5, 2H), 7.19 (d, PhH-4, 1H), 7.27 (t, PyH-3, PyH-5, 2H), 7.48 (t, PhH-4, 1H), 7.60 (d, PhH-2, PhH-6, 2H), 8.64 (d, PyH-6, PyH-2, 2H), 9.02 (s, NH, 1H). ^{13}C-NMR ($CDCl_3$) δ: 41.63 ($-CH_2-$), 120.15 (PhC-2, PhC-6), 123.40 (PhC-4), 122.87 (PyC-3, PyC-5), 129.20 (PhC-3, PhC-5), 136.33 (PhC-1), 142.77 (PyC-4), 150.12 (PyC-6, PyC-2), 150.65 (CO). *Anal.* Calcd for $C_{13}H_{12}N_4O_2$: C, 60.93; H, 4.72; N, 21.87. Found: C, 60.73; H, 4.74; N, 21.64.

Procedure for Nitrosation with Gaseous Reagents in Organic Solvent——Nitrosating reagents (0.1 M; 10 ml) in organic solvent were placed in a flask equipped with a drying tube (calcium chloride) and cooled to the desired purpose temperature. Then, 0.001 M urea (1 ml) in organic solvent was added with stirring.

Procedure for Nitrosation with Sodium Nitrite and Acid——A mixture of an acid (10 ml) and 0.0001 M urea in aqueous solution (1 ml) was cooled, then a 0.1 M aqueous solution of sodium nitrite (1 ml) was added with stirring.

Procedure for Nitrosation with Gaseous Reagents in Aqueous Media——Stock solution of 0.1 M nitrosating reagent in chloroform was added with vigorous stirring at 0 °C to a mixture of 0.0001 M aqueous solution of urea (1 ml) and phosphate buffer (10 ml) at desired pH. The organic and aqueous layers were analyzed.

Nitrosation with Nitrosonium Tetrafluoroborate——A mixture of nitrosonium tetrafluoroborate (12 mg) in chloroform (10 ml) and 0.001 M urea (1 ml) was cooled at 0 °C with stirring, then 0.1 M triethylamine (2 ml) in chloroform was added.

The Reaction of NBP Reagent with Nitrosoureido Derivatives (IIa and IIIa)——Each nitrosated compound (0.03 mmol) was dissolved in ethylene glycol monomethyl ether (1.4 ml), and the solution was mixed with 5% NBP solution (1.4 ml) in ethylene glycol monomethyl ether. The mixture was allowed to stand for 15 min at 37 °C, then piperidine (0.2 ml) was added (final concentration: 0.014 mmol/3.0 ml). After 5 min, the absorbance was determined at the maximum wavelength.

The Reaction of IIa and Guanine——IIa (1.28 g, 50 mmol) and guanine (0.75 g, 50 mmol) were dissolved in 0.62 N sodium hydroxide solution and the mixture was stirred for 40 min at 0 °C. Precipitated denitrosated urea was filtered off. The filtrate was neutralized with 1 N hydrochloric acid. Precipitated red crystals were collected and recrystallized from ethanol. Yield, 369 mg (31%). This compound was identical with an authentic sample which has already been reported.⁹⁾

Reaction of IIIa and Benzoic Acid——IIIa (512 mg, 2 mmol) dissolved in methanol (5 ml) was added to a solution of benzoic acid (244 mg, 2 mmol) in 0.62 N NaOH (20 ml) at 0 °C, and the mixture was stirred for 75 min, then extracted with ether. The extracts were dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was chromatographed on silica gel with a mixture of chloroform and methanol (2 : 1). Oil, 27 mg (6%). ^{1}H-NMR (DMSO-d_6) δ: 4.68 (s, 2H, CH_2), 7.41—7.1 (m, 4H, PhH-2, PhH-3), 7.41 (t, 1H, PhH-4), 7.70 (d, 1H, PyH-4), 8.42 (d, 1H, PyH-6), 8.52 (s, 1H, PyH-2). This compound was identical with an authentic sample prepared from pyridylmethanol and benzoyl chloride.⁴⁾

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