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Reactions of 1-Nitroso-1-phenyl-3-(4-pyridylmethyl)ureas[1]

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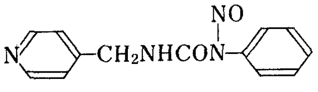
Treatment of 1-nitroso-1-phenyl- and 1-nitroso-1-(4-tolyl)-3-(4-pyridylmethyl)ureas (Ia, b) with methanol and ethanol at room temperature gave methyl and ethyl *N*-(4-pyridylmethyl)-carbamates (IIIa, b) in almost quantitative yields, together with benzene (IVa) and toluene (IVb), respectively. Treatment of Ia, b with benzene at 70 °C gave 4-pyridylmethylamine (V) with biphenyl (VIa) and its 4-methyl derivative (VIb), respectively. Treatment of Ia, b with sodium azide in aqueous acetone at −5 °C gave 1,3-bis(4-pyridylmethyl)urea (VII) and phenylazides (VIIIa, b) in more than 90% yields.

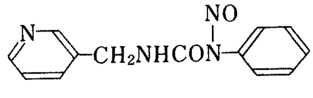
Compound Ia showed antitumor activity against rat ascites hepatoma AH13, but not against mouse lymphoid leukemia L1210. The mechanisms of these reactions and the mechanism of antitumor action of Ia are discussed.

Keywords——decomposition; nitrosourea; 1-nitroso-1-phenyl-3-(4-pyridylmethyl)urea; *N*-(4-pyridylmethyl)carbamate; phenylazide; antitumor activity; rat ascites hepatoma AH13; mouse lymphoid leukemia L1210

Many *N*-nitrosourea derivatives have been prepared in the hope of finding more potent and more stable *N*-nitrosourea antitumor agents in this laboratory.[2-4] Among these derivatives, 1-nitroso-1-phenyl-3-(4-pyridylmethyl)urea (Ia) was highly reactive with various types of reagents as compared with the 3-pyridyl derivative (IIa). For instance, in warm ethanol, the 4-derivative Ia immediately decomposed to produce a new compound, whereas the 3-derivative IIa was stable enough to be recrystallized from ethanol.



Ia



IIa

This paper describes the reactions of 1-nitroso-1-phenyl- and 1-nitroso-1-(4-tolyl)-3-(4-pyridylmethyl)ureas (Ia, b) to produce various kinds of compounds. The antitumor activities of the compounds against rat ascites hepatoma AH13 and mouse lymphoid leukemia L1210 were also examined in connection with their chemical reactivities.

When solutions of 1-nitroso-1-phenyl-3-(4-pyridylmethyl)urea (Ia) and its 1-(4-tolyl) derivative (Ib) in methanol were allowed to stand at room temperature for 10 min, methyl *N*-(4-pyridylmethyl)carbamate (IIIa) was produced almost quantitatively, with evolution of nitrogen gas. During this reaction the smell of benzene and toluene, respectively, was perceptible and these compounds were identified by means of reverse-phase high performance liquid chromatography (HPLC) on an ODS-18 column using a mixture of acetonitrile and water (7:3). Similar treatment of Ia in ethanol at 50 °C also gave ethyl *N*-(4-pyridylmethyl)carbamate (IIIb) quantitatively. These alcoholyses began at room temperature.

The time-course of ultraviolet (UV) absorption of Ia in methanol was recorded over a period of 1 h at room temperature. As shown in Fig. 1, the intensity of the 255 nm band

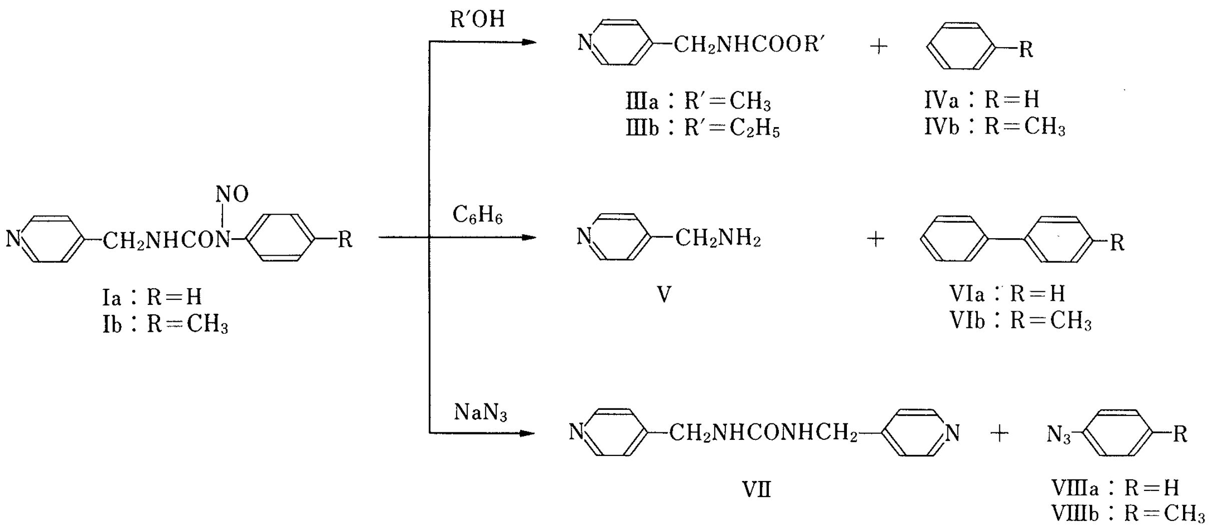


Chart 1

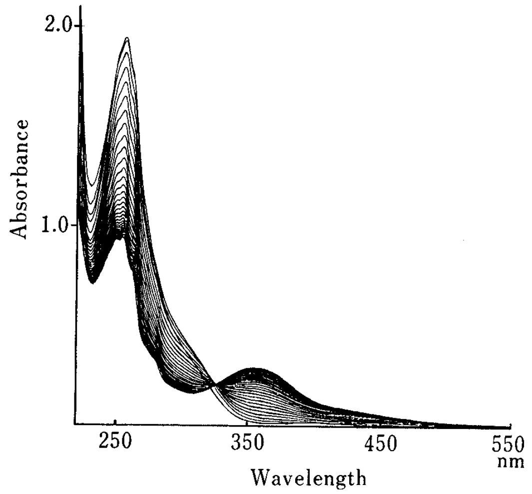


Fig. 1. Time-Course of UV Absorption of 1-Nitroso-1-phenyl-3-(4-pyridylmethyl)urea (Ia) in Methanol at Room Temperature

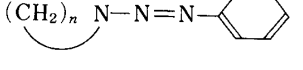
($\varepsilon = 1.9 \times 10^4$) decreased with time and that of the new band at 360 nm (finally, $\varepsilon = 2.8 \times 10^3$) increased gradually. A distinct isosbestic point at 276 nm was seen on the absorption curves.

When Ia was heated in benzene at 70 °C for 10 min, the products were 4-pyridylmethylamine (V) in 82% yield and biphenyl (VIa) in 34% yield. Similar treatment of Ib in benzene also gave V and 4-methylbiphenyl (VIb) in 37% yield.

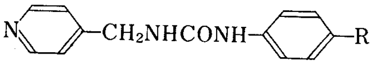
When solutions of Ia and Ib in chloroform were treated with a solution of sodium azide in aqueous acetone at −5 °C, phenylazide (VIIIa) and 4-tolylazide (VIIIb) were obtained in 94% and 91% yields, respectively. A bisurea, 1,3-bis(4-pyridylmethyl)urea (VII) was produced in both reactions, in 41—44% yields.

Formation of phenylazide and 4-tolylazide from the *N*-nitrosoureas Ia, b suggested that phenylpentazenes[5] [N_5–C_6H_4–R(*p*)] were the first products, and they decomposed to give phenylazides with evolution of nitrogen gas.

Bisurea VII was separately synthesized by the reaction of 4-pyridylmethylamine (V) and 1-methyl-1-nitroso-3-(4-pyridylmethyl)urea [4-py$CH_2NHCON(NO)CH_3$] in 62% yield.



IXa: *n*=4
IXb: *n*=5



Xa: R=H
Xb: R=CH_3

Treatment of Ia with pyrrolidine in chloroform at room temperature gave a triazene, 1-phenyl-3,3-tetramethylenetriazene (IXa), in 57% yield, and similar treatment of Ia with piperidine gave 1-phenyl-3,3-pentamethylenetriazene (IXb) in 45% yield. These triazenes were separately prepared by the reaction of benzenediazonium hexafluorophosphate with the corresponding amines.

In all reactions of Ia, b mentioned above, denitrosated ureas, 1-phenyl-3-(4-pyridylmethyl)ureas (Xa, b) were always isolated in 1—5% yields.

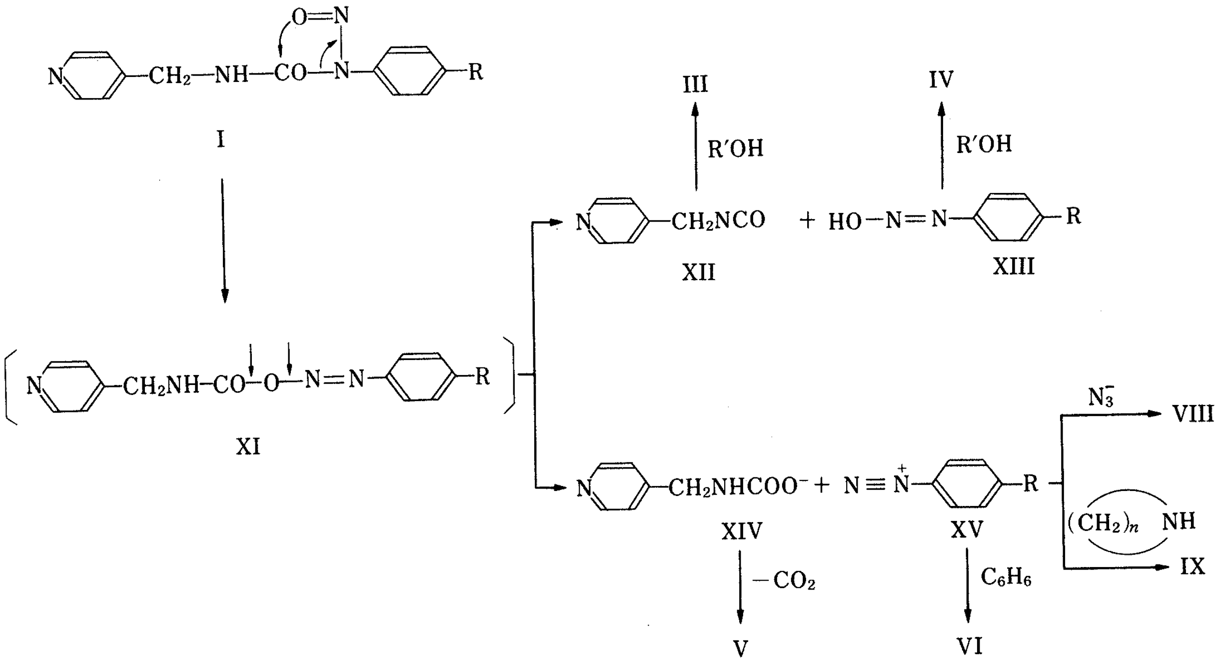


Chart 2

Possible reaction mechanisms in the reactions of Ia, b are presented in Chart 2. In solution, due to the strong electron-withdrawing effect of the 4-pyridyl nucleus, I readily rearranges to a diazo carbamate,[6] phenyldiazo *N*-(4-pyridylmethyl)carbamate (XI), which (according to the arrows) decomposes through two routes, although we cannot rule out a concerted mechanism in which the reagents directly attack the indicated bonds of the active intermediate XI. The first route leads to the formation of 4-pyridylmethyl isocyanate (XII) and phenyldiazo hydroxide (XIII), and the second route leads to the 4-pyridylmethylcarbamate anion (XIV) and phenyldiazonium ion (XV). The isocyanate XII reacts with alcohols to give the carbamate III, and the diazo hydroxide XIII is reduced by alcohols to give benzene derivatives. The carbamate anion (XIV) releases carbon dioxide to give 4-pyridylmethylamine (V), and the diazonium ion XV reacts with benzene itself, azide ion and secondary amines to give the products VI, VIII and IX. Thus, compounds such as I appear to be good phenyldiazonium sources.

The antitumor activity of the 4-derivative Ia was examined in our first screening system consisting of AH13 and L1210. As noted in the experimental section, the 4-derivative Ia was effective against AH13 cells (60 d survivors: 5/6 at a dose of 25 mg/kg), but was ineffective against L1210 cells. The 3-derivative, 1-nitroso-1-phenyl-3-(3-pyridylmethyl)urea (IIa), was less effective against AH13 cells (60 d survivors: 2/6 at a dose of 50 mg/kg), and was also ineffective against L1210 cells.

As far as ascites hepatoma AH13 is concerned, the chemical reactivities of the 4-derivative Ia and the 3-derivative IIa correspond to their antitumor activities. Presumably the active chemical species, phenyldiazonium ion XV and phenyldiazo hydroxide XIII, are more readily produced in cells from the 4-derivative Ia than from the 3-derivative IIa.

Experimental

All melting points are uncorrected. Infrared (IR) spectra were measured on a JASCO A-102 spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were measured on a Varian EM 360A spectrometer with tetramethylsilane as an internal standard. The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; py, pyridyl group.

1-Nitroso-1-phenyl-3-(4-pyridylmethyl)ureas (Ia, b)——1-Nitroso-1-phenyl-3-(4-pyridylmethyl)urea (Ia): Pale yellow granules (from ether), mp 114 °C (dec.).[3] 1-Nitroso-3-(4-pyridylmethyl)-1-(4-tolyl)urea (Ib) was similarly prepared. Pale yellow granules (from ether), mp 121—122 °C (dec.). Yield, 92%. ^{1}H-NMR(CDCl$_3$) δ: 4.75 (d, J=4 Hz, CH$_2$NH). *Anal.* Calcd for $C_{13}H_{12}N_4O_2$: C, 60.93; H, 4.72; N, 21.87. Found: C, 60.92; H, 4.55; N, 21.98.

Decomposition of 1-Nitroso-1-phenyl-3-(4-pyridylmethyl)ureas (Ia, b) in Alcohols——Methyl *N*-(4-Pyridylmethyl)carbamate (IIIa): When a solution of 0.51 g (0.002 mol) of Ia in 20 ml of methanol was allowed to stand at room temperature, nitrogen gas evolved gradually, and soon the mixture became clear. The methanol was distilled off, and during the distillation the odor of benzene was perceptible. The IR spectrum of the oily residue, bp 138—141 °C (6 mmHg), was identical with that of the authentic carbamate IIIa. Yield, 0.33 g (quantitative).

Ethyl *N*-(4-Pyridylmethyl)carbamate (IIIb): A mixture of Ia and ethanol was warmed in a water bath at 50 °C for 10 min, and the reaction mixture was similarly treated. Colorless liquid, bp 146—151 °C (5 mmHg). The IR spectrum of this product was identical with that of the authentic carbamate IIIb. Yield, quantitative. Oxalate: Colorless plates (from ethanol), mp 157—158 °C (dec.). *Anal.* Calcd for $C_9H_{12}N_2O_2 \cdot 0.5\ (COOH)_2$: C, 53.33; H, 5.78; N, 12.67. Found: C, 53.21; H, 6.03; N, 12.46.

The authentic carbamates IIIa, b were prepared by the reaction of 4-pyridylmethylamine and methyl or ethyl chloroformate in the presence of an equimolar amount of sodium hydroxide. IIIa: Colorless oil, bp 138—141 °C (6 mmHg). IR (liq.) cm^{-1}: 3290, 1710, 1260 ($-NHCOOCH_3$). IIIb: Yellowish oil, bp 146—151 °C (5 mmHg). IR (liq.) cm^{-1}: 3290, 1710, 1260 ($-NHCOOC_2H_5$).

Compound Ib decomposed analogously in methanol and ethanol to give the carbamates, IIIa and IIIb, respectively; the toluene produced was gas chromatographically identified.

Decomposition of 1-Nitroso-1-phenyl-3-(4-pyridylmethyl)ureas (Ia, b) in Benzene ——A typical experiment is described with Ia. A mixture of 2.56 g (0.001 mol) of Ia and 25 ml of benzene was heated in a water bath at 70 °C for 10 min. The solid product was a denitrosated urea, 1-phenyl-3-(4-pyridylmethyl)urea (Xa), mp 138—139 °C (from acetone). Yield, 0.12 g (5%). The benzene layer was extracted with 10% hydrochloric acid three times, dried over anhydrous sodium sulfate, and poured onto an aluminum oxide column. The column was washed with benzene, the pale yellow band was eluted with further benzene, and the eluant was evaporated to dryness under reduced pressure. The IR spectrum of the product, mp 71—72 °C, was identical with that of authentic biphenyl. Yield, 0.27 g (34%). From the acidic extract, 0.87 g (82%) of 4-pyridylmethylamine (V) was obtained.

Similar treatment of Ib in benzene gave V in 89% yield and 4-methylbiphenyl (VIb), mp 47—48 °C, in 37% yield.

Phenylazides (VIIIa, b) and 1,3-Bis(4-pyridylmethyl)urea (VII) from 1-Nitroso-1-phenyl-3-(4-pyridylmethyl)ureas (Ia, b)——A solution of 2.56 g (0.01 mol) of Ia in 15 ml of chloroform was added dropwise to a solution of 1.30 g (0.02 mol) of sodium azide in aqueous acetone (1 : 1) at -5 °C with stirring, during which time vigorous evolution of nitrogen gas was observed. The reaction mixture was further stirred for 10 min. The chloroform layer was washed with 5% hydrochloric acid, and dried over anhyd. sodium sulfate, then the chloroform was evaporated off under reduced pressure. The IR spectrum of the oily residue, bp 20 °C (5 mmHg), was identical with that of authentic phenylazide. Yield, 1.01 g (94%). The aqueous layer of the reaction mixture was concentrated under reduced pressure, and the solution was allowed to stand overnight. The separated 1,3-bis(4-pyridylmethyl)urea (VII) was filtered. Pale brown plates (from hot water), mp 181—182 °C. Yield, 0.53 g (44%). ^{1}H-NMR (DMSO-d_6) δ: 4.20 (d, J=4 Hz, CH$_2$), 7.76 (d, J=3.5 Hz, H^{3,5}), 8.46 (d, J=3.5 Hz, H^{5,6}). *Anal.* Calcd for $C_{13}H_{14}N_4O$: C, 64.44; H, 5.82; N, 23.13. Found: C, 64.11; H, 5.73; N, 22.83.

4-Tolylazide (VIIIb): VIIIb was analogously obtained by the reaction of Ib and sodium azide in 91% yield. Yellow oil, bp 30—32 °C (4 mmHg). ^{1}H-NMR (CDCl$_3$) δ: 2.02 (s, CH$_3$), 6.60, 6.87 (a pair of doublets). Bisurea VII, 41% yield.

1,3-Bis(4-pyridylmethyl)urea (VII) was separately synthesized. 1) 1-Methyl-1-nitroso-3-(4-pyridylmethyl)urea: Pale yellow needles (from ether), mp 68 °C (dec.). *Anal.* Calcd for $C_8H_{10}N_4O_2$: C, 49.48; H, 5.19; N, 28.85. Found: C, 49.26; H, 5.21; N, 29.07. Oxalate; Yellow pillars (from methanol), mp 140—141 °C (dec.). *Anal.* Calcd for $C_8H_{10}N_4O_2 \cdot (COOH)_2$: C, 42.25; H, 4.26; N, 19.71. Found: C, 42.17; H, 4.39; N, 19.60. 2) A solution of 1.08 g (0.011 mol) of 4-pyridylmethylamine in 3 ml of water was added dropwise to a solution of 1.94 g (0.01 mol) of finely powdered 1-methyl-1-nitroso-3-(4-pyridylmethyl)urea in aqueous ethanol (1 : 1) under ice-cooling with stirring. The reaction mixture was further stirred for 30 min. The separated crystals were filtered, washed with ice-water, and recrystallized from hot water. Colorless needles, mp 181—182 °C. Yield, 1.5 g (62%).

TABLE I

AH13 *i.p.–i.p.* system				L1210 *i.p.–i.p.* system		
Days 3	Days 3—7			Days 2, 6		
MTD (mg/kg)	Dose (mg/kg)	T/C%	60 d survivors	Dose (mg/kg)	T/C%	30 d survivors
50	5	210	0/6	12.5	106	0/3
	10	190	0/6	25	110	0/3
	20	>410	1/6	50	110	0/3
	25	>629	5/6	100	Toxic	0/3
	50	Toxic	0/6			

1-Phenyl-3,3-tetramethylenetriazene (IXa) and 1-Phenyl-3,3-pentamethylenetriazene (IXb) from 1-Nitroso-1-phenyl-3-(4-pyridylmethyl)urea (Ia)——1-Phenyl-3,3-tetramethylenetriazene (IXa): A solution of 0.20 g (0.0008 mol) of Ia in 10 ml of chloroform was mixed with a solution of 0.14 g (0.0016 mol) of pyrrolidine in 10 ml of chloroform, and the mixture was stirred for 2 h at room temperature. The reaction mixture was poured onto a silica gel column, and the column was washed with chloroform. The chloroform eluate was evaporated off under reduced pressure, and the residue was recrystallized from ether. Brownish plates, mp 48—49 °C. Yield, 71 mg (57%). An authentic sample was prepared by the reaction of benzenediazonium hexafluorophosphate and pyrrolidine in ether at room temperature. Brown plates, mp 48—49 °C.[7] Yield, 27%.

Similarly the reaction of Ia and piperidine in chloroform gave IXb in 45% yield. Brownish plates (from ether), mp 41—42 °C. An authentic sample was synthesized by the reaction of benzenediazonium hexafluorophosphate and piperidine in ether at room temperature. Brown plates, mp 43—44 °C. Yield, 30%.

Antitumor Activity——The compounds were tested according to the screening method reported in our previous paper.[8] The results of Ia are shown in the Table I.

References and Notes

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