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Pyridine Analogs of 1-Methyl-3-nitro-1-nitrosoguanidine and Related Compounds*,[1]

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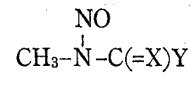
3-Pyridylmethyl analogs of 1-methyl-3-nitro-1-nitrosoguanidine (MNNG), 1-methyl-1-nitrosourea (MNU) and N-methyl-N-nitrosourethan (MNUT) were synthesized by nitrosation of the corresponding N-acyl-N-(3-pyridyl)methylamines. 3-Alkyl(aryl)-3-nitroso-1-(3-pyridyl)methylureas were also synthesized by nitrosation of 3-alkyl(aryl)-1-(3-pyridyl)methylureas.

The reaction of some of these N-nitroso compounds with alkali, was examined.

Among these N-nitroso compounds the mutagenic activity of 3-nitro-1-nitroso-1-(3-pyridyl)methylguanidine was as potent as the ethyl analog of MNNG in the mutation assay using streptomycin-dependent *E. coli* Sd-4.

Many N-acyl-N-nitrosamines, RN(NO)C(=X)Y, have potent carcinogenic activity for experimental animals. For instance, 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) induces adenocarcinoma in the glandular stomach of rats,[3] and 1-methyl-1-nitrosourea[4] (MNU) does tumors of the peripheral nurvous system and forestomach in high frequency, both with continuous oral administration. N-Methyl-N-nitrosourethan (MNUT) induces tumors of the stomach, esophagus, small intestine and lung, with one or a few doses.[4,5]



MNNG: X=NH, Y=$NHNO_2$
MNU: X=O, Y=NH_2
MNUT: X=O, Y=$COOC_2H_5$

On the other hand, these carcinogenic N-acyl-N-nitrosamines are potent mutagens for bacteria. Among them, MNNG is the most potent mutagen yet discovered.[6]

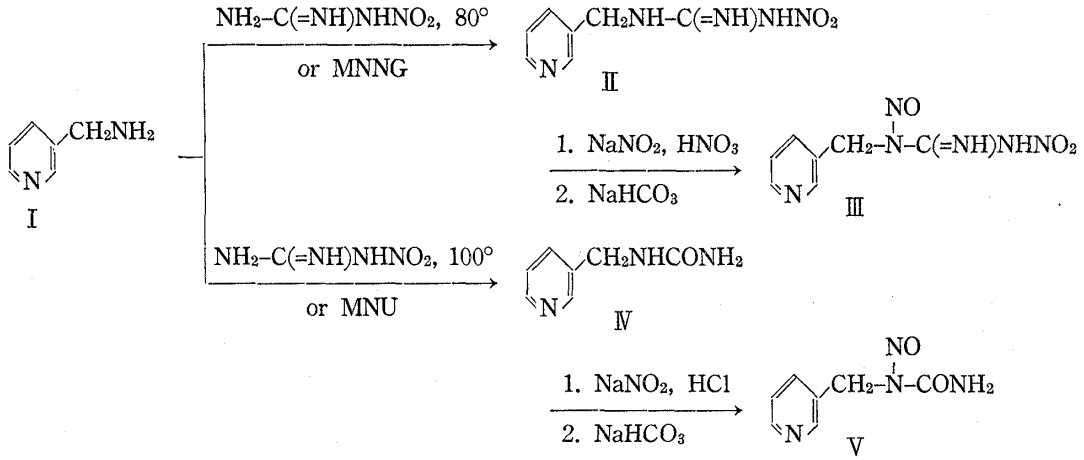


Chart 1

* Dedicated to the memory of Prof. Eiji Ochiai.

1) S. Kamiya, *Chem. Pharm. Bull.* (Tokyo), **20**, 2497 (1972).
2) Location: *Kamiyoga 1-18-1, Setagaya, Tokyo.*
3) T. Sugimura, S. Fujimura and T. Baba, *Cancer Res.*, **30**, 455 (1970).
4) S. Odashima, *J. Food Hyg. Soc. Japan.*, **15**, 419 (1974).
5) R. Schoenthal, *Nature*, **188**, 420 (1960); *ibid.*, **199**, 190 (1963).
6) S. Iwahara, K. Yanagimachi, S. Kamiya, M. Nakadate and I. Suzuki, *Chem. Pharm. Bull.* (Tokyo), **19**, 1914 (1971).

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This paper describes the synthesis of pyridine analogs of MNNG, MNU and MNUT, which directs to the preparation of new types of chemical carcinogens and mutagens.

When a mixture of 3-pyridylmethylamine (I) and nitroguanidine in water was heated at 80° for 20 minutes, 3-nitro-1-(3-pyridyl)methylguanidine (II) was obtained in 66% yield. While, this mixture was heated at the boiling temperature for 3 hours, the product was 1-(3-pyridyl)methylurea (IV) in 60% yield.

Nitrosation of II with sodium nitrite and nitric acid, followed by neutralization with sodium bicarbonate, yielded 3-nitro-1-nitroso-1-(3-pyridyl)methylguanidine (III) as yellow needles in 51% yield. Since the nuclear magnetic resonance (NMR) spectrum of this nitroso compound in DMSO-d_6 showed a 2H singlet at 4.90 τ, the nitrosated nitrogen was determined to be the nitrogen adjacent to the pyridylmethyl group. Nitrosation of IV with sodium nitrite and hydrochloric acid, followed by neutralization with sodium bicarbonate, gave 1-nitroso-1-(3-pyridyl)methylurea (V).

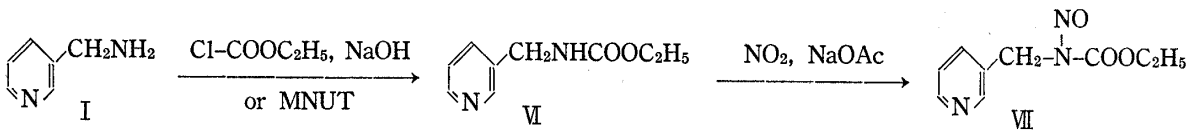

Chart 2

As shown in Chart 2, N-nitroso-N-(3-pyridyl)methylurethan (VII) was synthesized by nitrosation of the corresponding urethan (VI) with nitrogen tetroxide in the presence of anhyd. sodium acetate, as a red oil in 77% yield.

The reaction of I with MNNG, MNU, and MNUT at 0—5°, also gave II, IV, and VI, respectively, in 60—90% yields, with vigorous evolution of nitrogen (Chart 1, 2).

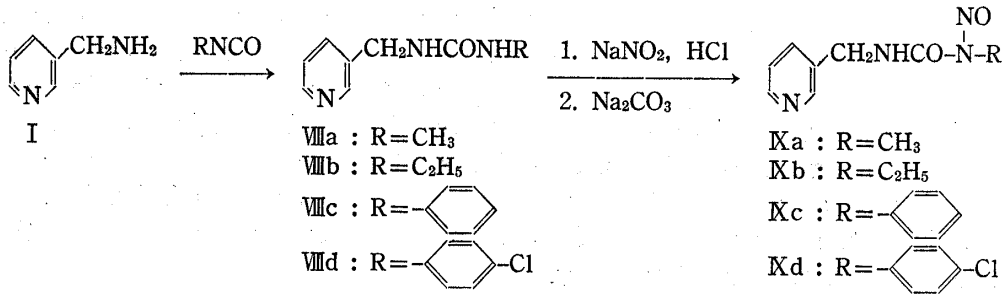

Chart 3

Another kind of pyridine analogs of MNU were then synthesized according to the route shown in Chart 3. Treatment of I with alkyl isocyanates gave 3-alkyl-1-(3-pyridyl)methylureas (VIIIa,b). 3-Aryl-1-(3-pyridyl)methylureas (VIIIc, d) were also prepared by the reaction of I with aryl isocyanates.

Nitrosation of VIIIa and VIIIb with sodium nitrite and 10% hydrochloric acid, followed by neutralization with sodium carbonate, gave 3-alkyl-3-nitroso-1-(3-pyridyl)methylureas, IXa and IXb, in over 90% yields. The same nitrosation of VIIIc and VIIId unexpectedly yielded the 3-nitrosoureas, IXc and IXd, in over 90% yields. In both cases the corresponding 1-nitroso compounds could not be isolated. The NMR spectra of IXa-d in DMSO-d_6 or deudeuterochloroform showed a 2H doublet due to $\underline{CH_2}NH$, which changed to a singlet on deuteration with deuterium oxide, and a 1H triplet due to $CH_2\underline{NH}$.

The methylenes in the groups, $-CH_2NH-C(=X)Y$ and $-CH_2N(NO)-C(=X)Y$, were well characterized by NMR spectroscopy. In the group, $-CH_2NH-C(=X)Y$, the methylene signal generally appeared at 5.5—5.8 τ as a doublet in DMSO-d_6, and on nitrosation, they shifted to 4.9—5.2 τ as a singlet. Also, in the IR spectra, a characteristic shift up to 50 cm^{-1} for the urethan CO and 80—100 cm^{-1} for the urea CO was observed on nitrosation.

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Some of these N-nitroso derivatives are practically insoluble in water, but their hydrochlorides and organic acid salts are well soluble in water.

Then, the reaction of some of these N-nitroso compounds with alkali was examined (Chart 4).

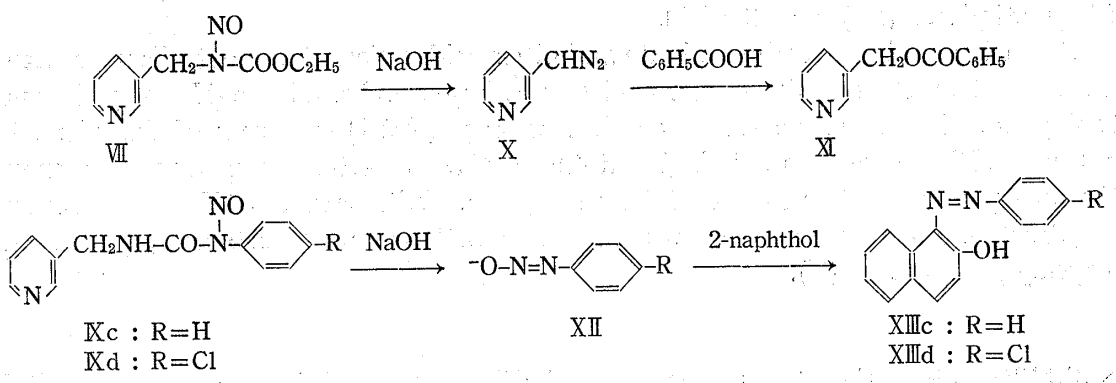


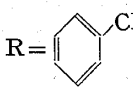
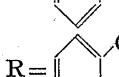
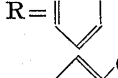
Chart 4

When VII covered with ether was treated with a 20% sodium hydroxide solution, 3-pyridyldiazomethane (X) was obtained as a yellow liquid, of which IR spectrum showed the presence of a diazo group at 2080 cm^{-1}. As a matter of fact, X reacted with benzoic acid to produce 3-benzoyloxymethylpyridine (XI), with evolution of nitrogen.

Treatment of IXc and IXd with a 10% sodium hydroxide solution in the presence of 2-naphthol, afforded the corresponding 1-arylazo-2-naphthol, XIIIc and XIIId. This reaction is the additional proof for the structure of IXc and IXd.

Though carcinogenicity of the N-nitroso derivatives synthesized here has been not examined yet, their mutagenicity was tested by the mutation assay using streptomycin-dependent *E. coli* Sd-4. The method for the detection of induced mutation (from the streptomycin-dependent strain to streptomycin-nondependent) has been reported in our previous paper.[6,7] As the result, 3-nitro-1-nitroso-1-(3-pyridyl)methylguanidine (III) was the most potent mutagen among the N-acyl-N-nitrosamines containing a pyridine nucleus, and its comparative activity was as potent as the ethyl analog of MNNG, as shown in Table I. However, IXa-d, of which nitroso groups attached to the nitrogen far from the pyridylmethyl group, showed no activity by this mutation assay.

Table I. Mutagenic Activity of MNNG and its Analogs (Mutation from Streptomycin-dependent to Streptomycin-nondependent in *E. coli* Sd-4)

R-N(NO)-C(=NH)NHNO$_2$	Concentration (μg/ml)	Mutants (per 10^8)
R=CH_3	5	236
MNNG	20	960
R=C_2H_5	10	120
	50	416
R=C_3H_7	10	145
	50	376
R=C_4H_9	10	89
	50	294
R=C_5H_{11}	10	8
	50	34
R= 	10	8
	50	22
R= 	10	38
	50	60
R=  III	10	196
	50	465

7) K. Koshinuma, S. Iwahara, S. Kamiya, M. Nakadate and I. Suzuki, *Eisei Shikenjo Hokoku*, **88**, 118 (1970).

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Experimental[8]

3-Nitro-1-(3-pyridyl)methylguanidine (II)——1) By the reaction of 3-pyridylmethylamine[9] (I) with nitroguanidine: A mixture of 10.8 g (0.10 mole) of I, 12.5 g (0.12 mole) of nitroguanidine and 100 ml of water was heated at 70—80° for 30 minutes, and the clear solution was allowed to stand overnight. The separated crystals were filtered, washed with water, and recrystallized from ethanol. Colorless needles, mp 200—201° (decomp.). NMR (τ, DMSO-d_6): 5.53 (d, 4 Hz, CH_2). λ_{max}^{EtOH} nm (log ε): 269 (4.98). Yield, 12.8 g (66%). *Anal.* Calcd. for $C_7H_9O_2N_5$: C, 43.07; H, 4.65; N, 35.89. Found: C, 42.92; H, 4.96; N, 36.20. 2) By the reaction of 3-pyridylmethylamine (I) with MNNG: To an ice-cooled mixture of 1.47 g (0.01 mole) of MNNG[10] and 10 ml of water was added dropwise 1.62 g (0.015 mole) of I, with stirring. After stirring for further 1 hour at room temperature, the separated crystals were filtered, washed with water, and recrystallized from ethanol, mp 200—201° (decomp.). Yield, 1.41 g (72%).

3-Nitro-1-nitroso-1-(3-pyridyl)methylguanidine (III)——A solution of 1.38 g (0.02 mole) of sodium nitrite in 3 ml of water was added dropwise to an ice-cooled solution of 1.56 g (0.008 mole) of II in 6 ml of conc. nitric acid with stirring. The reaction mixture was stirred for 30 minutes, and neutralized with sodium bicarbonate under ice-cooling. The crystals separated were filtered, washed with ice-water, and dried in a desiccator. Recrystallization from ethanol gave yellow needles, mp 162—163° (decomp.). λ_{max}^{EtOH} nm (logε): 270 (4.19). Yield, 0.80 g (51%). *Anal.* Calcd. for $C_7H_8O_3N_6$: C, 37.50; H, 3.60; N, 37.49. Found: C, 37.57; H, 3.81; N, 37.80.

1-(3-Pyridyl)methylurea (IV)——1) By the reaction of 3-pyridylmethylamine (I) with nitroguanidine: A mixture of 2.16 g (0.02 mole) of I, 2.60 g (0.025 mole) of nitroguanidine and 40 ml of water was heated at the boiling temperature for 3 hours. The reaction mixture was concentrated under reduced pressure, the crystals separated were filtered, and washed with ice-water. Colorless leaflets (from ethanol), mp 180—181°. IR (cm^{-1}, nujol): 1650 (CO). Yield, 1.80 g (46%). *Anal.* Calcd. for $C_7H_9ON_3$: C, 55.61; H, 6.00; N, 27.80. Found: C, 55.59; H, 6.14; N, 27.80. 2) By the reaction of 3-pyridylmethylamine (I) and MNU: A solution of 11.9 g (0.11 mole) of I in 15 ml of water was added dropwise to an ice-cooled mixture of 10.3 g (0.10 mole) of MNU[11] and 50 ml of water, with stirring. The reaction mixture was further stirred till the evolution of nitrogen ceased. The reaction mixture was concentrated under reduced pressure, and the residue was treated with ethanol. The crystals separated were filtered, and recrystallized from ethanol to give colorless leaflets, mp 182°. Yield, 9.21 g (61%).

1-Nitroso-1-(3-pyridyl)methylurea (V)——A solution of 1.72 g (0.025 mole) of sodium nitrite in 4 ml of water was added dropwise to a solution of 3.02 g (0.02 mole) of IV in 20 ml of 10% hydrochloric acid, with stirring, at −5——10°. The reaction mixture was neutralized to pH 7.0 with sodium bicarbonate, and the solution was extracted with chloroform several times. Without washing with water the chloroform layer was dried over anhyd. sodium sulfate, and the chloroform was evaporated to dryness under reduced pressure. The yellow residue was recrystallized by dissolving it into methanol, followed by evaporating the solution under reduced pressure. Yellow leaflets, exploded at about 80°. IR (cm^{-1}, nujol): 3260—2900, 1740 ($N(NO)CONH_2$). NMR (τ, DMSO-d_6): 5.12 (s, CH_2NNO), 1.92 (broad, $NCONH_2$). Yield, 0.38 g (11%). *Anal.* Calcd. for $C_7H_8O_2N_4$: C, 46.66; H, 4.48; N, 31.10. Found: C, 46.40; H, 4.28; N, 31.41. Oxalate: Pale yellow granules (from ethanol), mp 114° (decomp.). *Anal.* Calcd. for $C_7H_8O_2N_4 \cdot C_2H_2O_4$: C, 40.00; H, 3.73; N, 20.74. Found: C, 39.82; H, 3.60; N, 20.58.

N-(3-Pyridyl)methylurethane (VII)——1) By the Reaction of 3-Pyridylmethylamine (I) with Ethyl Chloroformate: To a solution of 5.40 g (0.005 mole) of I in 50 ml of ether was added dropwise 5.43 g (0.005 mole) of ethyl chloroformate under ice-cooling. When half of the ethyl chloroformate was added, a solution of 2.00 g (0.005 mole) of sodium hydroxide in 10 ml of water was added dropwise, together with the remaining ethyl chloroformate. After stirring for further 30 minutes, the ether layer was separated, and the water layer was extracted with ether. The combined ether layer was dried over anhyd. potassium carbonate, the ether was evaporated, and the residue was distilled under reduced pressure. Colorless liquid, bp 142—144°/5 mmHg. IR (cm^{-1}, liquid): 3280, 1710 ($NHCOOC_2H_5$). NMR (τ, $CDCl_3$): 8.80 (t, $CH_2\underline{CH_3}$), 5.90 (q, $\underline{CH_2}CH_3$), 5.72 (d, $\underline{CH_2}NH$). Yield, 5.78 g (70%). Oxalate: Colorless pillars (from ethanol), mp 128—129°. *Anal.* Calcd. for $C_9H_{12}O_2N_2 \cdot C_2H_2O_4$: C, 48.89; H, 5.22; N, 10.37. Found: C, 48.93; H, 5.44; N, 10.20. 2) By the Reaction of 3-Pyridylmethylamine (I) and MNUT: To 13.2 g (0.10 mole) of MNUT[10] was added dropwise 10.8 g (0.10 mole) of I, with vigorous stirring under ice-cooling. After the evolution of

8) All melting points are uncorrected. Infrared spectra were measured on a JASCO Model-S spectrophotometer. NMR spectra were measured on a Japan Electron Optics JNMC-60 H spectrometer, and tetramethylsilane was used as an internal standard for hexadeuterodimethyl sulfoxide (DMSO-d_6) and deuterochloroform ($CDCl_3$).

9) Purchased from Aldrich Chemical Co., Inc., Milwaukee, U.S.A.

10) Purchased from Tokyo Kasei Kogyo Co., Ltd., Tokyo.

11) F. Arndt, "Organic Syntheses," Coll. Vol. II, ed. by A.H Blatt, John Wiley and Sons, Inc., New York, 1943, p. 461.

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nitrogen ceased, the reaction mixture was heated on a water bath for 30 minutes, and distilled under reduced pressure. Colorless liquid, bp 142—145°/5 mmHg. Yield, 15.0 g (91%).

N-Nitroso-N-(3-pyridyl)methylurethan (VII)——A cooled solution of 5.52 g (0.06 mole) of nitrogen tetroxide in 5 ml of carbon tetrachloride was added to a cooled mixture of 4.92 g (0.06 mole) of anhyd. sodium acetate and 50 ml of carbon tetrachloride, at −5——10°, with stirring. To this mixture was added dropwise a solution of 3.30 g (0.02 mole) of VI in 5 ml of carbon tetrachloride, and stirring was continued for 1 hour. The solvent was evaporated under reduced pressure, the residue was extracted with chloroform, and the chloroform layer was washed with water. After drying over anhyd. sodium sulfate, the chloroform was evaporated under reduced pressure. Since this compound could not be distilled because of decomposition, it was purified by passing its ether solution through a silica gel column. IR (cm^{-1}, liquid): 1750 (CO), 1389, 905 (NNO). NMR (τ, $CDCl_3$): 5.14 (s, $CH_2N(NO)$), 8.57 (t, $COOCH_2\underline{CH_3}$), 5.49 (q, $COO\underline{CH_2}CH_3$). Yield, 3.22 g (77%). Oxalate: Pale yellow granules (from a mixture of methanol and diisopropyl ether), mp 74—75° (decomp.). *Anal.* Calcd. for $C_9H_{11}O_3N_3 \cdot C_2H_2O_4$: C, 44.15; H, 4.38; N, 14.04. Found: C, 44.20; H, 4.36; N, 13.67.

3-Alkyl(aryl)-1-(3-pyridyl)methylureas (VIIIa, b, c, d)——A typical synthesis for VIIIa—d is described with 3-methyl-1-(3-pyridyl)methylurea (VIIIa). To a solution of 1.08 g (0.01 mole) of 3-pyridylmethylamine in 20 ml of ethanol was added dropwise 0.68 g (0.012 mole) of methyl isocyanate with stirring, and the mixture was concentrated under reduced pressure, and the residue was treated with a hydrochloric acid—ethanol mixture. The separated crystals were filtered, and recrystallized from a mixture of methanol and ethanol to give colorless needles, mp 138—141° (decomp.). IR (cm^{-1}, nujol): 1600, 1630 (CO). Yield, 1.78 g (88%). *Anal.* Calcd. for $C_8H_{11}ON_3 \cdot HCl$: C. 47,61; H, 6.01; N, 20.83. Found: C, 47.91; H, 5.86; N, 21.28. 3-Ethyl-1-(3-pyridyl)methylurea (VIIIb): Yield, 87%. Hydrochloride: Colorless needles (from a mixture of ethanol and diisopropyl ether), mp 145—147° (decomp.). *Anal.* Calcd. for $C_9H_{13}ON_3 \cdot HCl$: C, 50.11; H, 6.54; N, 19.48. Found: C, 49.97; H, 6.32; N, 19.18. 3-Phenyl-1-(3-pyridyl)methylurea (VIIIc): Colorless plates (from a mixture of ethanol and diisopropyl ether), mp 103—105°. IR (cm^{-1}, nujol): 3320, 1650 (NHCONH). Yield, 91%. *Anal.* Calcd. for $C_{13}H_{13}ON_3$: C, 68.70; H, 5.77; N, 18.49. Found: C, 68.81; H, 5.79; N, 18.73. 3-(4-Chlorophenyl)-1-(3-pyridyl)methylurea (VIIId): Colorless needles (from acetone), mp 169°. Yield, 82%.

3-Alkyl(aryl)-3-nitroso-1-(3-pyridyl)methylureas (IXa, b, c, d)——A typical synthesis for IXa, b, c, d is described with IXa. A solution of 1.04 g (0.015 mole) of sodium nitrite in 3 ml of water was added dropwise to a solution of 2.02 g (0.01 mole) of VIIIa hydrochloride in 10 ml of 10% hydrochloric acid with stirring under ice-cooling. The reaction mixture was neutralized with a 20% sodium carbonate solution, the crystals separated were filtered, and washed with ice-water. The crystals were recrystallized from a mixture of methanol and water by evaporating the solution under reduced pressure. Pale yellow needles, mp 74° (decomp.). IR (cm^{-1}, nujol): 1715 (CO). NMR (τ, CD_3COCD_3): 6.82 (s, CH_3), 5.32 (d, 6 Hz, CH_2) changed to a singlet on deuteration with deuterium oxide. Yield, 1.62 g (83%). *Anal.* Calcd. for $C_8H_{10}O_2N_4$: C, 49.48; H, 5.19; N, 28.85. Found: C, 49.47; H, 5.19; N, 29.36. 3-Ethyl-3-nitroso-1-(3-pyridyl)methylurea (IXb): Yellow oil. IR (cm^{-1}, liquid): 3400, 1720 (NHCON(NO)). Yield, 85%. Oxalate: Pale yellow leaflets (from ethanol), mp 129—130° (decomp.). *Anal.* Calcd. for $C_9H_{12}O_2N_4 \cdot C_2H_2O_4$: C, 44.30; H, 4.73; N, 18.79. Found: C, 44.01; H, 4.56; N, 18.45. 3-Nitroso-3-phenyl-1-(3-pyridyl)methylurea (IXc): Yellow pillars (from acetone), mp 83—84° (decomp.). IR (cm^{-1}, nujol): 3160, 1710 (NHCON(NO)). NMR (τ, $CDCl_3$): 5.35 (d, 4 Hz, $\underline{CH_2}NH$). Yield, 93%. *Anal.* Calcd. for $C_{13}H_{12}O_2N_4$: C, 60.93; H, 4.72; N, 21.87. Found: C, 61.18; H, 4.66; N, 22.23. 3-(4-Chlorophenyl)-3-nitroso-1-(3-pyridyl)methylurea (IXd): Pale yellow granules (from acetone), mp 123° (decomp.). NMR (τ, DMSO-d_6): 5.53 (d, 4 Hz, $\underline{CH_2}NH$), changed to a singlet on deuteration with deuterium oxide. *Anal.* Calcd. for $C_{13}H_{11}O_2N_4Cl$: C, 53.71; H, 3.81; N, 19.29. Found: C, 53.27; H, 3.58; N, 19.08.

The Reaction of Some of the N-Nitroso Compounds with Sodium Hydroxide——1) N-Nitroso-N-(3-pyridyl)methylurethan (VII): To 1.94 g (0.01 mole) of VII covered with 40 ml of ether, was added dropwise 10 ml of a 20% sodium hydroxide solution, with vigorous stirring, under ice-cooling. The ether layer was washed with a 2% sodium hydroxide solution, then with water twice. After the ether layer was dried over anhyd. sodium sulfate, the ether was distilled off. 3-Pyridyldiazomethane (X), yellow liquid. IR (cm^{-1}, liquid): 2080 (CHN_2). Yield, 0.42 g (35%). This compound reacted with benzoic acid to give 3-benzoyloxymethylpyridine (XI), bp 192—196°/15 mmHg. NMR (τ, $CDCl_3$): 4.70 (s, CH_2). IR (cm^{-1}, liquid): 1720 (COO), 1265 (C–O–C).

2) 3-Aryl-3-nitroso-1-(3-pyridyl)methylureas (IXc, d): To an ice-cooled solution of 0.26 g (0.001 mole) of IXc, were added 10 ml of a 10% sodium hydroxide solution and a solution of 0.15 g of 2-naphthol in 10 ml of methanol. After the reaction mixture was stirred for 10 minutes, 25 ml of water was added to the mixture. The separated crystals were filtered, and washed with water. Dark red leaflets (from acetic acid), mp 132—133°. This product coincided with the authentic 1-phenylazo-2-naphthol by mixed melting point determination. Yield, 62 mg (25%). The same reaction of IXd gave 55 mg (22%) of 1-(4-chlorophenylazo)-2-naphthol (XIIId), dark red needles (from acetic acid), mp 161—162°.

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