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Synthesis of Antitumor Pyridine and Pyridazine N-Oxides having (2-Chloroethyl)nitrosoureidomethyl and Bis(2-chloroethyl)-aminomethyl Groups[1)]

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Treatment of 1-(2-chloroethyl)-1-nitroso-3-(3-pyridylmethyl)urea (Ia) and the 3-nitroso isomer (Ib) with *m*-chloroperbenzoic acid, gave the corresponding N-oxides, IIa and IIb.

The 3-nitrosoureas (Ib, IIb) isomerized to the 1-nitroso isomers (Ia, IIa) by dissolving them in formic acid.

The reaction of 6-bis(2-chloroethyl)aminomethyl-3-hydroxypyridazine 1-oxide (III) with bromine gave the 4-bromo derivative (IV).

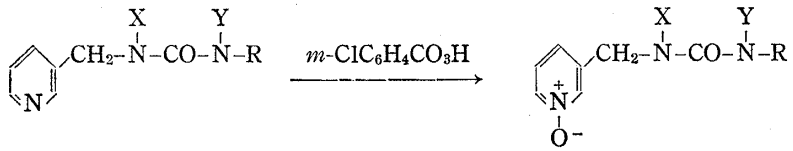
1-(2-Chloroethyl)-1-nitroso-3-(3-pyridylmethyl)urea N-oxide (IIa) showed the most marked activity against rat ascites hepatom AH-13 and mouse lymphoid leukemia L-1210.

Keywords——antitumor activity; antitumor pyridine N-oxide; antitumor pyridazine N-oxide; 2-chloroethylnitrosoureidomethyl group; bis(2-chloroethyl)aminomethyl group; 1-(2-chloroethyl)-1-nitroso-3-(3-pyridylmethyl)urea N-oxide; 1-(2-chloroethyl)-3-nitroso-3-(3-pyridylmethyl)urea N-oxide; isomerization of nitrosourea; 6-bis(2-chloroethyl)aminomethyl-3-hydroxypyridazine 1-oxide

In the preceding paper[3)] we reported the antitumor activity of 4-nitropyridazine 1-oxides and 4-nitrocinnoline 1-oxides against rat ascites hepatoma AH-13 and mouse lymphoid leukemia L-1210. Antitumor heterocyclic compounds which have one or two of the antitumor functional groups in a molecule are being synthesizing in this laboratory.

This paper describes the synthesis of antitumor pyridine and pyridazine N-oxides having (2-chloroethyl)nitrosoureidomethyl and bis(2-chloroethyl)aminomethyl groups. The experimental tumors used for our primary screening system are rat ascites hepatoma AH-13 and mouse lymphoid leukemia L-1210.

Treatment of 1-(2-chloroethyl)-1-nitroso-3-(3-pyridymethyl)urea[4)] (Ia) and the 3-nitrosourea[4)] (Ib) with *m*-chloroperbenzoic acid in chloroform at room temperature gave 1-(2-chloroethyl)-1-nitroso-3-(3-pyridylmethyl)urea N-oxide (IIa) in 96% yield and the 3-nitrosourea



Ia: X=H, Y=NO, R=CH_2CH_2Cl
Ib: X=NO, Y=H, R=CH_2CH_2Cl
Ic: X=H, Y=NO, R=CH_3

IIa: X=H, Y=NO, R=CH_2CH_2Cl
IIb: X=NO, Y=H, R=CH_2CH_2Cl
IIc: X=H, Y=NO, R=CH_3

Chart 1

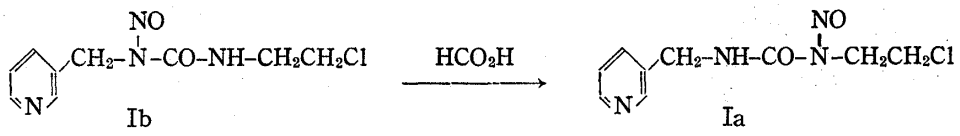
1) This work was presented at the 98th Annual Meeting of the Pharmaceutical Society of Japan, Okayama April, 1978.
2) Location: *1-18-1, Setagaya, Tokyo 158 Japan.*
3) S. Kamiya, M. Anzai, T. Nakashima, S. Sueyoshi, M. Tanno, I. Suzuki, M. Ishidate, Jr. and S. Odashima, *Chem. Pharm. Bull.* (Tokyo), **25**, 504 (1977); *Eisei Shikensho Hokoku*, **94**, 148 (1976).
4) S. Kamiya, *Chem. Pharm. Bull.* (Tokyo), **25**, 1121 (1977).

N-oxide (IIb) in 62% yield, respectively. Since the existence of the N-nitroso group in IIa and IIb was confirmed by their diazo-coupling reaction using sulfanilic acid and N-(1-naphthyl)-ethylenediamine, they were not N-nitroureas but N-nitrosourea N-oxides.

Nitrosation of 1-(2-chloroethyl)-3-(3-pyridylmethyl)urea N-oxide with sodium nitrite and hydrochloric acid gave an approximately 6:1 mixture of the isomers, IIa and IIb, from which IIa was isolated in 65% yield by recrystallization.

An N-methyl-N-nitrosoureido structure [NH-CO-N(NO)CH_3] is also an antitumor functional group which is present in an antitumor antibiotic, streptozotocin isolated from *Streptomyces achromogenes* fermentation broth. The same N-oxidation of 1-methyl-1-nitroso-3-(3-pyridylmethyl)urea (Ic) gave the corresponding N-oxide (IIc) in 82% yield.

The inherent lipid solubility of nitrosourea antitumor agents results in less or no solubility in water, and they are comparatively unstable. These two problems make thier injection troublesome. Attention has recently been focussed upon the stable nitrosourea antitumor agents which have water-soluble property and reduced bone marrow toxicity. These nitrosourea N-oxides were soluble in water as expected.



Ib Ia

Chart 2

During this study we observed an interesting isomerization from the 3-nitrosourea to the 1-nitroso isomer. When a solution of the 3-nitrosourea (Ib) in formic acid was allowed to stand at room temperature for five days, 13% yield of the 1-nitroso isomer (Ia), together with 32% recovery of the 3-nitrosourea (Ib), was isolated. This type of isomerization was also observed in the 3-nitrosourea N-oxide (IIb). The 1-nitrosoureas (Ia, IIa), in which the nitroso group is located at the ureido nitrogen for the 2-chloroethyl group, seem to be thermodynamically more stable than the 3-nitrosoureas (Ib, IIb), in which the nitroso group is located at the ureido nitrogen for the 3-pyridylmethyl group. Further investigation on the isomerization of nitrosoureas of this type is in progress. The details will be reported in a separate paper.

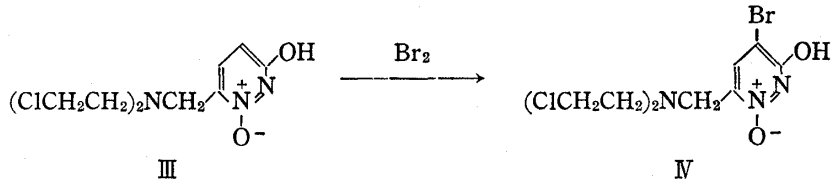


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Chart 3

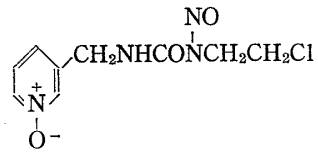
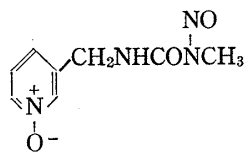
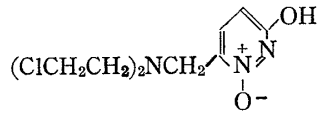
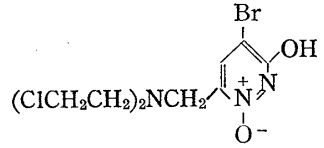
Bromination of 6-bis(2-chloroethyl)aminomethyl-3-hydroxypyridazine 1-oxide[5] (III) with bromines gave the 4-bromo derivative (IV) in 42% yield.

As shown in Table I, compound IIa showed marked activity against both tumors. Especially for L-1210 compound IIa was effective even at a lower dose of 2.5 mg/kg with a positive value of 190. Compound IIc, a pyridine N-oxide analog of carcinogenic and also carcinostatic 1-methyl-1-nitrosourea, was not effective against AH-13, and it was, however, effective to some extent against L-1210.

6-Bis(2-chloroethyl)aminomethylpyridazine derivatives, III and IV, were effective against AH-13, but they were not effective against L-1210.

5) G. Okusa, S. Kamiya and T. Itai, *Chem. Pharm. Bull.* (Tokyo), **15**, 1172 (1967).

TABLE I. Effect of the Pyridine and Pyridazine N-Oxides against Rat Ascites Hepatoma AH-13 and Mouse Lymphoid Leukemia L-1210

No.	Compound	Antitumor activity against AH-13 (*i.p.*) MTD[a] (mg/kg)	MED[b] (mg/kg)	Dose (mg/kg)	*T/C*[c] (%)	No. of survivors 30 days	No. of survivors 60 days	Antitumor activity against L-1210 (*i.p.*) Dose (mg/kg)	*T/C* (%)	No. of survivors 30 days
IIa		25	1	1.25	>450	4/6	3/6	2.5	190	0/3
				2.5	>400	2/6	2/6	5.0	213	0/3
				5.0	>505	4/6	3/6	10	>365	2/3
								20	>417	3/3
IIc		>500	250	25	104	0/6	—	100	133	0/3
				50	110	0/6	—	200	147	0/3
				100	134	0/6	—	400	164	0/3
III		50	0.5	1	218	1/6	0/6	5	109	0/3
				2	218	0/6	—	10	116	0/3
				5	>343	3/6	2/6	20	113	0/3
IV		100	5	1	223	0/6	—	25	122	0/3
				2	215	0/6	—	50	130	0/3
				5	>354	1/6	1/6	100	99	0/3

a) Maximum tolerated dose: single dose on Donryu rats bearing AH-13 cells.
b) Minimum effective dose: single dose on Donryu rats bearing AH-13 cells.
c) Mean survival time of controled groups/mean survival time of treated group×100. *T/C*% was calculated at 60 days after inoculation.

Experimental[6]

1-(2-Chloroethyl)-1-nitroso-3-(3-pyridylmethyl)urea (Ia) and 1-(2-Chloroethyl)-3-(3-pyridylmethyl)urea (Ib)——A mixture of Ia and Ib, which was prepared according to our previous report,[4] was chromatographed on a silica gel column using $CHCl_3$ as an eluent. The first fraction contained the 3-nitroso isomer (Ib), the second one was a mixture of Ia and Ib, and the third one contained the 1-nitroso isomer (Ia). Their yields were 59% for Ia and 29% for Ib. Ia: mp 88—91° (dec.). Ib: mp 86—87° (dec.).

1-(2-Chloroethyl)-1-nitroso-3-(3-pyridylmethyl)urea N-Oxide (IIa)——To a solution of 2.43 g (0.01 mol) of Ia in 50 ml of $CHCl_3$ was added 2.50 g (0.01 mol) of *m*-chloroperbenzoic acid [*m*-CPBA] (purity: 70%) under ice-cooling. The reaction mixture was allowed to stand at room temperature overnight. The $CHCl_3$ solution was washed with two 20 ml portions of a saturated aqueous solution of $NaHCO_3$. The water layer was then saturated with NaCl, and the solution was extracted with $CHCl_3$ twice. The $CHCl_3$ layer and the extracts were combined, the mixture was dried over anhyd. Na_2SO_4, and the $CHCl_3$ was evaported to dryness under reduced pressure. The residue was recrystallized from $CHCl_3$ to give yellow needles, mp 140—141° (dec.). Yield, 2.40 g (96%). IR (cm^{-1}, nujol): 3120, 1710 (NHCONNO), 1260 (N-O). NMR (τ, $CDCl_3$): 5.32 (d, 4 Hz, CH_2NH), 5.78, 6.47 (a pair of triplet, $(N(NO)CH_2CH_2Cl)$. *Anal.* Calcd. for $C_9H_{11}ClN_4O_3$: C, 41.78; H, 4.29; N, 21.66. Found: C, 41.77; H, 4.20; N, 21.92.

1-(2-Chloroethyl)-3-nitroso-3-(3-pyridylmethyl)urea N-Oxide (IIb)——This compound was similarly synthesized from Ib, as in the case of IIa. Pale yellow needles (from a mixture of $CHCl_3$ and ether), mp 114° (dec.). Yield, 62%. IR (cm^{-1}, nujol): 3180, 1720 (N(NO)CONH), 1260 (N-O). NMR (τ, $CDCl_3$): 5.06 (s, $CH_2N(NO)$), 6.25 (m, $NHCH_2CH_2Cl$). *Anal.* Calcd. for $C_9H_{11}ClN_4O_3$: C, 41.78; H, 4.29; N, 21.66. Found: C, 41.75; H, 4.29; N, 21.92.

1-(2-Chloroethyl)-3-(3-pyridylmethyl)urea N-Oxide and Its Nitrosation——1-(2-Chloroethyl)-3-(3-pyridylmethyl)urea N-Oxide: To a solution of 4.27 g (0.02 mol) of 1-(2-chloroethyl)-3-(3-pyridylmethyl)urea in 100 ml of $CHCl_3$ was added 5.00 g of *m*-CPBA (purity: 70%) under ice-cooling, and the reaction mixture was allowed to stand for 6 hr. The $CHCl_3$ was evaporated under reduced pressure, the residue was treated with 10 ml of 10% HCl, and the mixture was extracted with ether. The water layer was then neutralized with Na_2CO_3, extracted with $CHCl_3$, and the $CHCl_3$ layer was dried over anhyd. Na_2SO_4. The $CHCl_3$ was evaporated to give a syrupy product. Yield, 2.85 g (62%). Picrate: Yellow pillars (from ethanol), mp 149° (dec.). *Anal.* Calcd. for $C_{15}H_{15}ClN_6O_9$: C, 39.27; H, 3.30; N, 18.32. Found: C, 39.76; H, 3.38; N, 19.09.

Nitrosation: A solution of 2.07 g (0.03 mol) of $NaNO_2$ in 5 ml of water was added dropwise into an ice-cooled solution of 4.59 g (0.02 mol) of the N-oxide in 20 ml of 10% HCl with stirring, during which time the temperature did not exceed 0°. The reaction mixture was neutralized with $NaHCO_3$, extracted with $CHCl_3$ repeatedly, and the $CHCl_3$ layer was dried over anhyd. Na_2SO_4. The $CHCl_3$ was evaporated to dryness under reduced pressure to give a mixture which consisted of IIa (86%) and IIb (14%). Recrystallization of the mixture from ethanol gave 2.70 g (65%) of pure IIa, mp 140° (dec.). This synthesis chemically proved the structure of IIa.

1-Methyl-1-nitroso-3-(3-pyridylmethyl)urea N-Oxide (IIc)——1-Methyl-3-(3-pyridylmethyl)urea: A solution of 5.5 g (0.1 mol) of methyl isocyanate in 50 ml of ether was added dropwise into a solution of 10.8 g (0.1 mol) of 3-pyridylmethylamine in 200 ml of ether under ice-cooling with stirring, and the reaction mixture was further stirred for 30 min. The separated crystals were filtered and recrystallized from a mixture of $CHCl_3$ and ether. Colorless, fine needles, mp 97—98°. Yield, 13.2 g (79%). *Anal.* Calcd. for $C_8H_{11}N_3O$: C, 58.16; H, 6.71; N, 25.44. Found: C, 58.01; H, 6.50; N, 25.38.

1-Methyl-1-nitroso-3-(3-pyridylmethyl)urea (Ic): 1-Methyl-3-(3-pyridylmethyl)urea was nitrosated with $NaNO_2$ and 10% HCl in the same manner mentioned above. The NMR spectrum of the product showed that the reaction produced only the 1-nitroso isomer. Yield, 70%. Pale yellow needles (from a mixture of $CHCl_3$ and ether), mp 74—75° (dec.). *Anal.* Calcd. for $C_8H_{10}N_4O_2$: C, 49.48; H, 5.19; N, 28.85. Found: C, 49.08; H, 5.12; N, 28.28.

1-Methyl-1-nitroso-3-(3-pyridylmethyl)urea N-Oxide (IIc): To a solution of 1.94 g (0.01 mol) of Ic in 50 ml of $CHCl_3$ was added 2.50 g (0.01 mol) of *m*-CPBA (purity: 70%), and the reaction mixture was allowed to stand at room temperature overnight. The reaction mixture was similarly treated as noted in the synthesis of IIa and IIb. Pale yellow, fine needles (from a mixture of $CHCl_3$ and ether), mp 108° (dec.). IR (cm^{-1}, nujol): 1715 (NCON(NO)), 1260 (N-O). Yield, 1.72 g (82%). *Anal.* Calcd. for $C_8H_{10}N_4O_3$: C, 45.71; H, 4.80; N, 26.60. Found: C, 45.50; H, 4.58; N, 26.32.

Isomerization of 1-(2-Chloroethyl)-3-nitroso-3-(3-pyridylmethyl)urea (Ib) to the 1-Nitroso Isomer (Ia)——A solution of 0.60 g of Ib in 2 ml of formic acid, was allowed to stand at room temperature for five days. The reaction mixture was neutralized with $NaHCO_3$, extracted with $CHCl_3$, and the $CHCl_3$ layer was dried over

6) 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-60H spectrometer, and tetramethylsilane was used as an internal standard for deuterochloroform.

anhyd. Na_2SO_4. The $CHCl_3$ solution was chromatographed on a silica gel column by using $CHCl_3$ as an eluent. The first fraction gave 190 mg (32%) of Ib, the second one gave 20 mg of a mixture of Ia and Ib, and the third one gave 81 mg (13%) of Ia.

4-Bromo-6-bis(2-chloroethyl)aminomethyl-3-hydroxypyridazine 1-Oxide Hydrobromide (IV)——In a hood, 2.45 g (0.01 mol) of bromine was added into a solution of 3.03 g (0.01 mol) of 6-bis(2-chloroethyl)aminomethyl-3-hydroxypyridazine 1-oxide hydrochloride[5] (III) in 20 ml of water, with vigorous stirring, and the reaction mixture was then stirred for 30 min. The reaction mixture was evaporated to dryness under reduced pressure, and the residue was recrystallized from ethanol to give the hydrobromide, colorless leaflets, mp 185—186° (dec.). NMR (τ, DMSO-d_6): 6.68, 6.12 (a pair of doublet, $((ClCH_2CH_2)_2N)$, 5.78 (s, CH_2). Yield, 1.54 g (42%). *Anal.* Calcd. for $C_9H_{12}BrCl_2N_3O_2 \cdot HBr$: C, 25.38; H, 3.06; N, 9.86. Found: C, 25.84; H, 3.17; N, 10.07.

Screening Method——Complete details of the screening methods have been described in our previous report.[3]

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Electrophilic Substitution of 1,2-Benzisothiazol-3-acetic Acid

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The bromination, the nitration, the chlorosulfonation, the formylation and the Mannich reaction of 1,2-benzisothiaizol-3-acetic acid (1) were investigated and it was shown that the 3α-methylene group is also activated to the electrophilic substitutions as in 1,2-benzisoxazol-3-acetic acid.

Keywords——1,2-benzisothiazole; electrophilic substitution; bromination; nitration; chlorosulfonation; formylation; Mannich reaction

In the previous paper,[2] it has been demonstrated that 3α-methylene group of 1,2-benzisoxazol-3-acetic acid is strongly activated to the electrophilic substitution. As an extension of this study, several electrophilic substitution reactions of 1,2-benzisothiazol-3-acetic acid-(**1**)[3] were investigated.

As the result, electrophilic substitution reactions occurred to 3α-methylene group of **1** and it was shown that 3α-methylene group of **1** is also activated to the electrophilic substitu tion as in the 1,2-benzisoxazole derivative.

When brominated with 2 molar equivalents of bromine in acetic acid, **1** gave 3α-bromo-1,2-benzisothiazol-3-acetic acid (**2**) expectedly. However, the reaction of **1** with 4 molar equivalents of N-bromosuccinimide (NBS) did not afford the expected compound, 3-tribromomethyl-1,2-benzisothiazole (**3**) but gave 3-dibromomethyl-1,2-benzisothiazole (**4**), the analogue of which, 3-dibromomethyl-1,2-benzisoxazole, was not obtained on the bromination of 1,2-benzisoxazole derivative under the same condition. Compound **3** was obtained by the reaction of **1** with 4 molar equivalent of bromine in acetic acid at 80°. The decarboxylation of **2** afforded 3-bromomethyl-1,2-benzisothiazole (**5**), which was converted to monoiodomethyl derivative (**6**) by the reaction with potassium iodide in acetone.

The nitration of **1** with fuming nitric acid on an ice-bath for 1 hour and then at room temperature for 2 hours afforded a mixture of three products, 5-nitro-3-trinitromethyl-1,2-

1) Location: *33-94, Enoki-cho, Suita, Osaka.*
2) H. Uno and M. Kurokawa, *Chem. Pharm. Bull.* (Tokyo), **26**, 3498 (1978).
3) M. Giannella, F. Gualtieri and C. Melchiorri, *Phytochemistry*, **10**, 539 (1971).