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Reactions of 1-(2-Chloroethyl)-1-nitroso-3-(3-pyridylmethyl)urea N_{arom}-Oxide with Several Biological Model Compounds

MICHIKO MIYAHARA,* SHOKO SUEYOSHI and SHOZO KAMIYA

National Institute of Hygienic Sciences, 1–18–1 Kamiyoga, Setagaya-ku, Tokyo 158, Japan

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The mechanism of antitumor action of 1-(2-chloroethyl)-1-nitroso-3-(3-pyridylmethyl)urea N_{arom}-oxide (I) was chemically studied using several biological model compounds. Compound I reacted with piperidine (a model compound for nucleic acid bases) in acetone to form 1-(2-chloroethyl)piperidine, 1,2-bispiperidinoethane and 1,1-pentamethylene-3-(3-pyridylmethyl)urea N_{arom}-oxide (III). Moreover, 1-(2-chloroethyl)piperidine reacted with piperidine in acetone to form 1,2-bispiperidinoethane. Compound I reacted with N_{α}-acetyl-L-lysine in weakly alkaline conditions and with *N*-acetyl-DL-penicillamine in acidic methanol (both are model compounds for enzyme proteins having NH_2 and SH groups) to form 1-(5-acetylamino-5-carboxy-1-pentamethylene)-3-(3-pyridylmethyl)urea N_{arom}-oxide and *N*-acetyl-*S*-nitroso-DL-penicillamine, respectively. Based on these chemical data, a possible antitumor action mechanism of compound I is discussed.

Keywords——1-(2-chloroethyl)-1-nitroso-3-(3-pyridylmethyl)urea N_{arom}-oxide; piperidine; alkylation; 1-(2-chloroethyl)piperidine; 1,2-bispiperidinoethane; 2-chloroethyl-substituted DNA; cross-linked DNA; *N*-carbamoylation; *S*-nitrosation; DNA repair enzyme

N-Nitrosoureas of various types have been synthesized and tested for antitumor activities against rat ascites hepatoma AH13 and mouse lymphoid leukemia L1210 in this laboratory. Among these *N*-nitrosoureas, 1-(2-chloroethyl)-1-nitroso-3-(3-pyridylmethyl)urea N_{arom}-oxide (I) was the most effective against both experimental tumors. On intraperitoneal (*i.p.*) administration of I, animals inoculated with AH13 or L1210 cells intraperitoneally recovered completely[1] (survivors: 3/5 at the 60th day or 3/3 at the 30th day after inoculation). Most *N*-nitrosourea antitumor agents are insoluble in water and are unstable. However, since I is comparatively soluble in water and is stable, it should be advantageous as regards both preparation and application.

This paper deals with the chemical reactions of I with piperidine as a model compound for nucleic acid bases, and with N_{α}-acetyl-L-lysine and *N*-acetyl-DL-penicillamine as model compounds for enzyme proteins having NH_2 and SH groups. The antitumor mechanism of I is discussed in the light of the results.

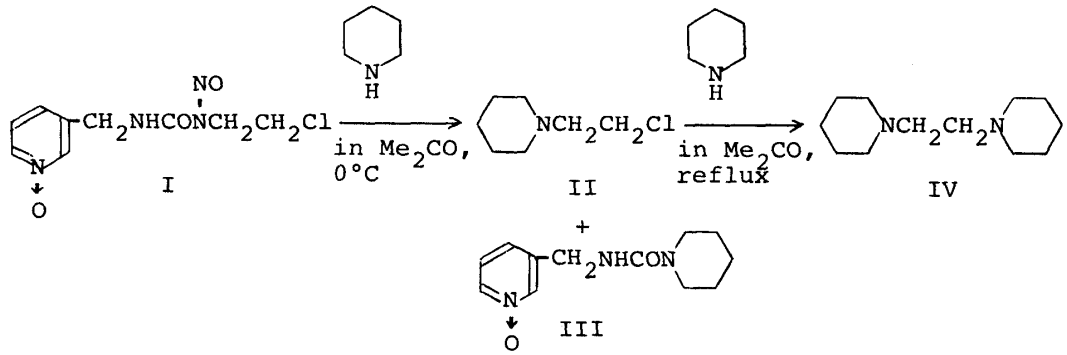

Chart 1

Piperidine was chosen as a model compound because it was soluble in the experimental system and the alkylated products were easily separable and detectable. Although the reaction of I with adenine and guanine was tried several times, the separation of the products was very difficult. The reaction of I with piperidine in acetone at 0 °C gave 1-(2-chloroethyl)piperidine (II) in 20% yield and 1,1-pentamethylene-3-(3-pyridylmethyl)urea N_{arom}-oxide (III) in 94% yield, while the reaction at reflux gave a mixture of II, III and 1,2-bispiperidinoethane (IV) (Chart 1). Compounds II and IV were identified by means of gas chromatography (GC) and nuclear magnetic resonance spectroscopy (NMR). Authentic samples of II and IV were separately prepared by the reaction of piperidine with 1-bromo-2-chloroethane and 1,2-dibromoethane, respectively. Carbamoylated compound III was determined by means of NMR and mass spectroscopy. Moreover, the reaction of 1-(2-chloroethyl)piperidine (II) and piperidine in acetone at reflux gave 1,2-bispiperidinoethane (IV).

The alkylating activity of I was also indicated by the formation of an intense purple color (λ_{max} 559 nm) in the reaction of I and NBP reagent [4-(4-nitrobenzyl)pyridine].[2)]

These chemical data suggest that such alkylation, which produces II and IV, occurs in tumor cells to form *N*-(2-chloroethyl)-substituted deoxyribonucleic acid (DNA) [>N–CH_2CH_2Cl], which, by loss of its chloro group, further reacts with other DNA to form cross-linked DNA [>N–CH_2CH_2–N<]; such carbamoylation to produce III may occur in proteins.

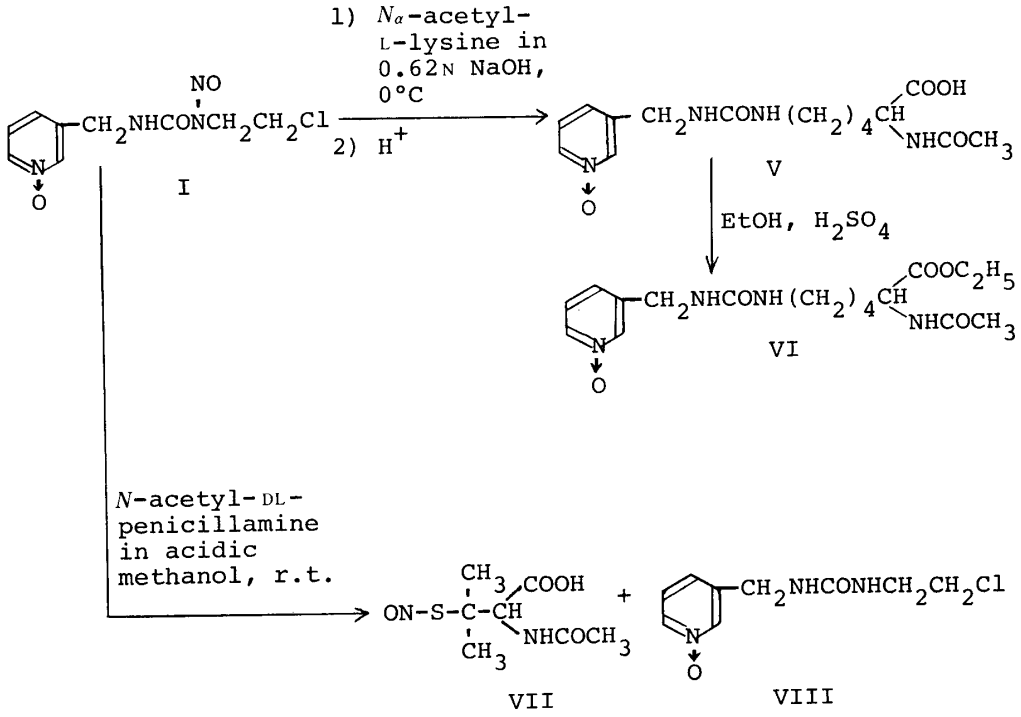

Chart 2

The reactions of I with N_α-acetyl-L-lysine and *N*-acetyl-DL-penicillamine were then examined in order to cast light on the reactivities of I with enzyme proteins having active NH_2 and SH groups. Treatment of I with N_α-acetyl-L-lysine under weakly alkaline conditions, followed by acidification, gave 1-(5-acetylamino-5-carboxy-1-pentamethylene)-3-(3-pyridylmethyl)urea N_{arom}-oxide (V) in 18% yield. The identity of V was also confirmed by deriving it to the corresponding ester, 1-(5-acetylamino-5-ethoxycarbonyl-1-pentamethylene)-3-(3-pyridylmethyl)urea N_{arom}-oxide (VI). The reaction of I with *N*-acetyl-DL-penicillamine in acidic methanol gave *N*-acetyl-*S*-nitrosopenicillamine (VII) in 42% yield, together with a denitrosated urea (VIII) in 73% yield (Chart 2).

From the carbamoylation of the terminal amino group and the nitrosation of the SH group in each model compound, it is expected that the reaction of enzymes with I will reduce their activities. In fact, the carbamoylation of DNA polymerase II inhibits its DNA replication activity.[3]

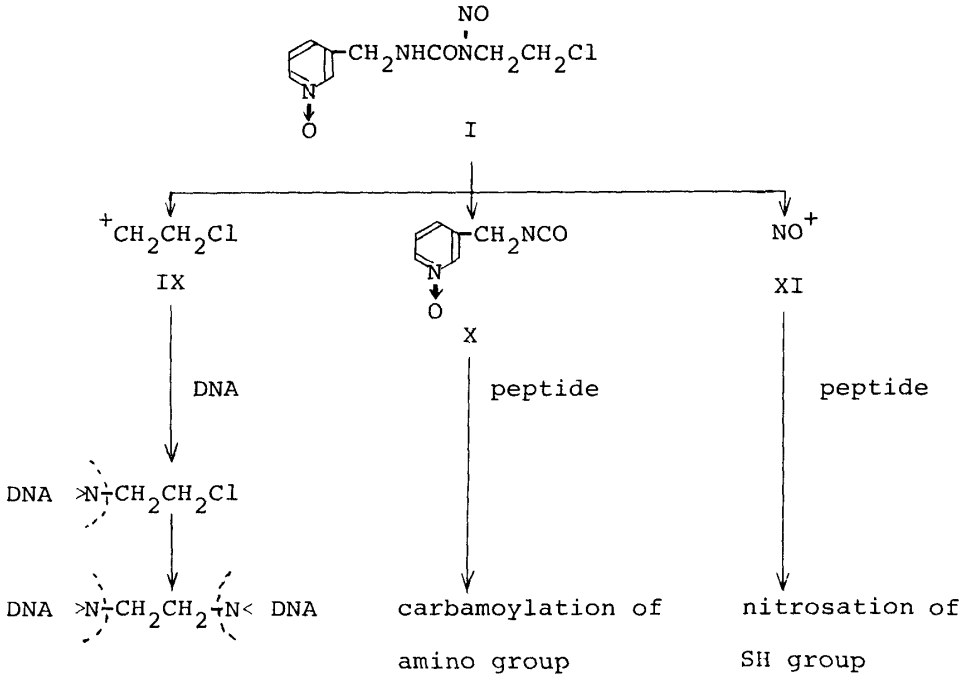

Chart 3

Consequently, on the basis of these chemical model reactions, the antitumor mechanism of I is proposed to be as follows. Compound I is degradated to produce 2-chloroethyl cation (IX), an isocyanate (X) and nitrosonium ion (XI) in tumor cells. The 2-chloroethyl cation forms 2-chloroethyl-substituted DNA and this yields cross-linked DNA. This alkylation of DNA will result in DNA scission, DNA repair or cell death. In addition, the *N*-carbamoylation and *S*-nitrosation of DNA repair enzymes will cause inhibition of DNA repair. Due to these actions on the tumor cells, the life-span of treated, tumor-bearing animals is prolonged.

Experimental

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. ^{1}H-NMR spectra were measured with a Varian EM 360A spectrometer, and ^{13}C-NMR spectra were measured with a JEOL FX-200 spectrometer, in deuterochloroform ($CDCl_3$). Tetramethylsilane and $CDCl_3$ were used as internal standards. Mass spectra (MS) were measured with a JEOL LMS-01 SG-2 spectrometer. GC-MS were measured with a JEOL JMX-DX 300 mass spectrometer. GC was done on a Shimadzu GC-7A instrument using a hydrogen flame ionization detector (OV-1, 2 m, injection temp. 170 °C, column temp. 150 °C). The abbreviations used are as follows: pip, piperidine; py, pyridine.

1-(2-Chloroethyl)-1-nitroso-3-(3-pyridylmethyl)urea N_{arom}-Oxide (I)——This compound was prepared according to our previous report.[4]

Reaction of 1-(2-Chloroethyl)-1-nitroso-3-(3-pyridylmethyl)urea N_{arom}-Oxide (I) with Piperidine——A solution of 341 mg (4 mmol) of piperidine in 20 ml of acetone was mixed with 214 mg (1 mmol) of I, and the mixture was allowed to stand at 0 °C for 3 h. The precipitate which formed was filtered off, and the filtrate was evaporated under reduced pressure. The oily residue was partially extracted with ether, and the ether extract was subjected to GC and NMR spectroscopy. The ether extract gave 1-chloro-2-piperidinoethane (II) which was identified by comparison with an authentic sample prepared by the method described below. Yield, 26 mg (20%). The residue was then extracted with chloroform. The extract mainly contained a carbamoylated piperidine, 1,1-pentamethylene-3-(3-pyridylmethyl)urea

N_{arom}-oxide (III), which was identified by means of NMR and mass spectroscopy. Yellow oil. Yield, 220 mg (94%). ^{13}C-NMR: 24.64 (4-pip-C), 26.10 (3,5-pip-C), 41.81 (py$\underline{C}H_2$–), 45.78 (2,6-pip-C), 127.65, 131.16, 138.11, 138.28 (2,4,5,6-py-C), 141.57 (3-py-C), 159.47 ($\underline{C}$O). High MS: molecular ion *m/e* at 235.1189. Calcd 235.1317.

A similar experiment was done under reflux. From the ether extract, alkylated piperidines (II and IV) were obtained, and from the chloroform extract, a carbamoylated piperidine (III) was obtained.

Reaction of 1-Chloro-2-piperidinoethane (II) with Piperidine——A solution of 852 mg (10 mmol) of piperidine in 20 ml of acetone was mixed with 210 mg (1.4 mmol) of II, and the whole was refluxed for 5 h. After filtration of the precipitate, which was identified as piperidine hydrochloride (colorless needles, mp 246—247 °C, yield, 136 mg, 79%), the filtrate was evaporated under reduced pressure to give 1,2-bispiperidinoethane (IV). Yield, 96 mg (35%).

1-Chloro-2-piperidinoethane (II)—A solution of 17 g (0.2 mol) of piperidine in 50 ml of acetone was mixed with 14.3 g (0.1 mol) of 1-bromo-2-chloroethane, and the whole was allowed to stand at 0 °C for 3 h. The precipitate was filtered off, the filtrate was concentrated to dryness under reduced pressure, and the oily residue was extracted by ether. The ether extract was evaporated to dryness, and the oily residue was identified as 1-chloro-2-piperidinoethane.[5] ^{1}H-NMR: 1.46 (br, 3,4,5-pip-H, 6H), 2.37 (t, J=5.2 Hz, 2,6-pip-H, 4H), 2.59 (t, J=8.0 Hz, CH_2Cl, 2H), 3.55 (t, J=8.0 Hz, $C\underline{H}_2CH_2Cl$, 2H). ^{13}C-NMR: 23.80 (4-pip-C), 25.50 (3,5-pip-C), 40.63 (CH_2Cl), 54.18 (2,6-pip-C), 60.29 ($NC\underline{H}_2CH_2Cl$). Picrate: Yellow needles (from acetone), mp 120 °C (lit.,[5] 116—117 °C). ^{13}C-NMR: 21.40 (4-pip-C), 22.59 (3,5-pip-C), 37.77 (CH_2Cl), 52.82 (2,6-pip-C), 56.79 ($NC\underline{H}_2CH_2Cl$), 125.34 (3,5-phenyl-C). *Anal.* Calcd for $C_{13}H_{17}ClN_4O_7$: C, 41.44; H, 4.55; N, 14.87. Found: C, 41.38; H, 4.44; N, 14.91.

1,2-Bispiperidinoethane (IV)——A solution of 680 mg (8 mmol) of piperidine in 40 ml of acetone was mixed with 376 mg (2 mmol) of 1,2,-dibromoethane, and the whole was refluxed for 4 h. The precipitate was filtered off, and the filtrate was evaporated to dryness under reduced pressure. The residue was identified as 1,2-bispiperidinoethane.[6] ^{13}C-NMR: 23.00 (4-pip-C), 24.51 (3,5-pip-C), 53.58 (2,6-pip-C), 55.38 (NCH_2). GC-MS, *m/e*: 196 (M^+), 112 ($C_7H_{14}N$), 99 ($C_6H_{13}N$), 83 (C_5H_9N), 69 (C_4H_7N), 55 (C_3H_5N). Hydrobromide[6]: Colorless fine leaflets (from aqueous ethanol), mp >280 °C. ^{13}C-NMR (D_2O): 20.69 (4-pip-C), 22.69 (3,5-pip-C), 49.95 (NCH_2), 54.04 (2,6-pip-C). *Anal.* Calcd for $C_{12}H_{26}Br_2N_2$: C, 40.24; H, 7.32; N, 7.82. Found: C, 39.94; H, 7.60; N, 7.63. Picrate: Yellow needles (from a mixture of ethanol and acetone), mp 225—227 °C (dec.) [lit.,[6b] 225 °C (dec.)].

Reaction of 1-(2-Chloroethyl)-1-nitroso-3-(3-pyridylmethyl)urea N_{arom}-Oxide (I) with NBP Reagent——This test was done according to our previous report.[7] The colored reaction mixture showed λ_{max} at 559 nm.

Reaction of 1-(2-Chloroethyl)-1-nitroso-3-(3-pyridylmethyl)urea N_{arom}-Oxide (I) with N_α-Acetyl-L-lysine——Powdered I (427 mg, 2 mmol) was added to a solution of 376 mg (2 mmol) of N_α-acetyl-L-lysine in 12 ml of 1.2% sodium hydroxide solution at 0 °C. The mixture was left for 2 h, and then evaporated to dryness under reduced pressure. The residue was mixed with methanol, and the mixture was filtered. The filtrate was evaporated to dryness under reduced pressure, and the residue was identified as 1-(5-acetylamino-5-carboxy-1-pentamethylene)-3-(3-pyridylmethyl)urea N_{arom}-oxide sodium salt. Yellow solid. Yield, 132 mg (18%). ^{13}C-NMR: 22.89, 29.80, 32.23, 40.55 (lysine-CH_2), 23.47 ($CO\underline{C}H_3$), 41.23 (py$\underline{C}H_2$–), 56.11 (lysine-CH), 127.68, 131.18, 138.11, 138.16 (2,4,5,6-py-CH), 141.45 (3-py-C), 160.81 (NHCONH), 173.94 ($NH\underline{C}OCH_3$), 179.88 (COONa). After neutralization of the salt with dil. hydrochloric acid, the solution was evaporated to dryness under reduced pressure. The residue was mixed with conc. sulfuric acid and ethanol, and the mixture was allowed to stand for 3 h at 0 °C. The reaction mixture was neutralized with saturated sodium carbonate solution. The neutralized mixture was evaporated to dryness under reduced pressure, and the residue was extracted with chloroform. The chloroform extract was concentrated under reduced pressure to give a yellow oil which mainly consisted of 1-(5-acetylamino-5-ethoxycarbonyl-1-pentamethylene)-3-(3-pyridylmethyl)urea N_{arom}-oxide (IV). ^{13}C-NMR: 13.97 ($CH_2\underline{C}H_3$), 22.39, 29.51, 31.44, 39.34 (lysine-CH_2), 22.66 ($CO\underline{C}H_3$), 40.73 (py$\underline{C}H_2$–), 52.23 (lysine-CH), 61.11 ($O\underline{C}H_2CH_3$), 125.70, 126.40, 137.11, 137.54 (2,4,5,6-py-CH), 140.88 (3-py-C), 158.66 (NHCONH), 170.43 ($\underline{C}OCH_3$), 172.38 ($\underline{C}OOC_2H_5$). High MS: molecular ion *m/e* at 366.1889. Calcd 366.1897.

Reaction of 1-(2-Chloroethyl)-1-nitroso-3-(3-pyridylmethyl)urea N_{arom}-Oxide (I) with *N*-Acetyl-DL-penicillamine——*N*-Acetyl-DL-penicillamine (191 mg, 1 mmol) was dissolved in 15.5 ml of a mixture of methanol, 0.5 N hydrochloric acid and conc. sulfuric acid (5 : 10 : 0.5). Then I (213 mg, 1 mmol) was added, and the reaction mixture was kept at 25 °C for 8 h with vigorous stirring, and extracted with ether. The aqueous layer was extracted with a mixture of methanol and ether (1 : 1). The combined extracts gave *N*-acetyl-*S*-nitroso-DL-penicillamine. Deep green crystals with red reflections, mp 150 °C (dec.) [lit.,[8] 150—152 °C (dec.)]. Yield, 93 mg (42%). The aqueous layer was neutralized with saturated sodium carbonate solution, and extracted repeatedly with a mixture of chloroform and methanol (1 : 1). The combined extracts were evaporated to dryness under reduced pressure to give 1-(2-chloroethyl)-3-(3-pyridylmethyl)urea N_{arom}-oxide,[4] which was identical with an authentic sample. Colorless solid, mp 135—137 °C. Yield, 107 mg (73%).

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