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Studies on Antitumor-active 2,3-Dioxopiperazine Derivatives. I. Degradation Products of 1-(2-Chloroethyl)-3-(4-substituted-2,3-dioxo-1-piperazinyl)-alkyl-1-nitrosourea in Aqueous Solution

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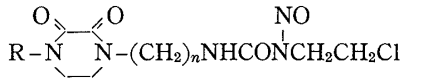
Degradation products of 1-(2-chloroethyl)-3-(4-substituted-2,3-dioxo-1-piperazinyl)-alkyl-1-nitrosourea in aqueous solution were investigated. Among them, a symmetrical urea derivative, 1,3-bis[4-(4-ethyl-2,3-dioxo-1-piperazinyl)butyl]urea (**3c**), was found to have antitumor activity. A synthetic unsymmetrical urea derivative, 1-[2-(4-ethyl-2,3-dioxo-1-piperazinyl)ethyl]-3-methylurea (**4a**), also showed antitumor activity against Ehrlich carcinoma (solid form).

Keywords——degradation products of nitrosourea derivatives; antitumor activity; symmetrical urea derivatives; 2,3-dioxopiperazine derivatives; unsymmetrical urea derivatives; antitumor agent of new type

In our previous paper[1] on the physico-chemical properties of the antitumor agent 1-(2-chloroethyl)-3-(4-substituted-2,3-dioxo-1-piperazinyl)alkyl-1-nitrosourea (**1**), it was shown that the stability of **1** in aqueous solution was correlated with its methylene number n (Chart 1).



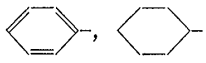
1: R=H, alkyl (C_1—C_8),  , n=1—6

Chart 1. Chemical Structure of 1-(2-Chloroethyl)-3-(4-substituted-2,3-dioxo-1-piperazinyl)alkyl-1-nitrosourea

Montgomery *et al.*[2] reported that the degradation products of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea(CCNU) in aqueous solution are cyclohexylamine and N,N′-biscyclohexylurea. However, the antitumor activity of these degradation products of nitrosourea derivatives in aqueous solution has not yet been reported.

Thus, we decided to elucidate the structures of the degradation products of a series of compounds of type **1** and to evaluate their antitumor activity. This paper describes the results, which might lead to the development of a new type of antitumor agent.

First, the time courses of the degradation of **1a** (R=C_2H_5, n=2) in aqueous solution at pH 4, 7, and 9 were examined qualitatively by thin-layer chromatography (TLC). At pH 4, a spot of a new product (**2a**) was observed on the thin-layer chromatogram and at pH 7 and 9, **3a** which had a larger *Rf* value than **2a**, was also observed (Fig. 1). It was found that **1** decomposed in aqueous solution in the same way as CCNU, and that **2a** was an amino compound (1-aminoethyl-4-ethyl-2,3-dioxopiperazine) while **3a** was a urea derivative, (N,N′-bis[2-(4-ethyl-2,3-dioxopiperazin-1-yl)ethyl]urea). The *Rf* value of **2a** on TLC was identical with that of the amino compound[1] synthesized as the starting material for **1a**, and the *Rf* value of **3a** on TLC was identical with that of the urea derivative synthesized independently by the route shown in Chart 2. Further, these results were confirmed by the fact that one of the degradation products of **1b** (R=Ph, n=2) in aqueous solution was isolated and indentified as the expected symmetrical urea derivative (**3d**) (R=Ph, n=2) by elemental analysis. The stability of the compounds **1** of this series in aqueous solution was found to be correlated with the methylene number, n, in the previous report,[1] but in the present investigation it was found that the degradation pattern of **1** was the same regardless of the methylene number n. Chart 3 sum-

marizes these results.

Next, the antitumor effects of **2** and **3** against Ehrlich carcinoma (solid form) were studied. It is of interest that a symmetrical urea derivative (**3c**) (R=C_2H_5, n=4) was found to have antitumor activity (Table I).

Our investigations were next extended from symmetrical to unsymmetrical urea derivatives. Unsymmetrical urea derivatives (**4**) were synthesized by the method shown in Chart 2

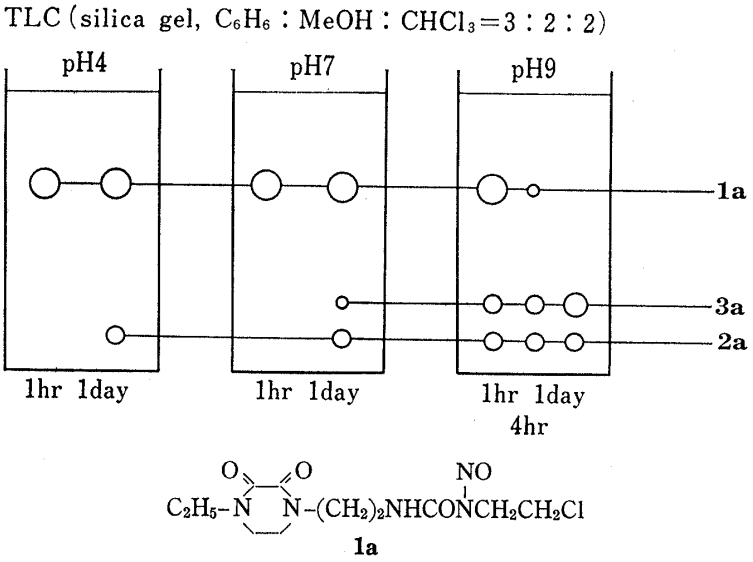


Fig. 1. Thin-layer Chromatograms of Aqueous Solutions of **1a** at pH 4,7, and 9 at 37°

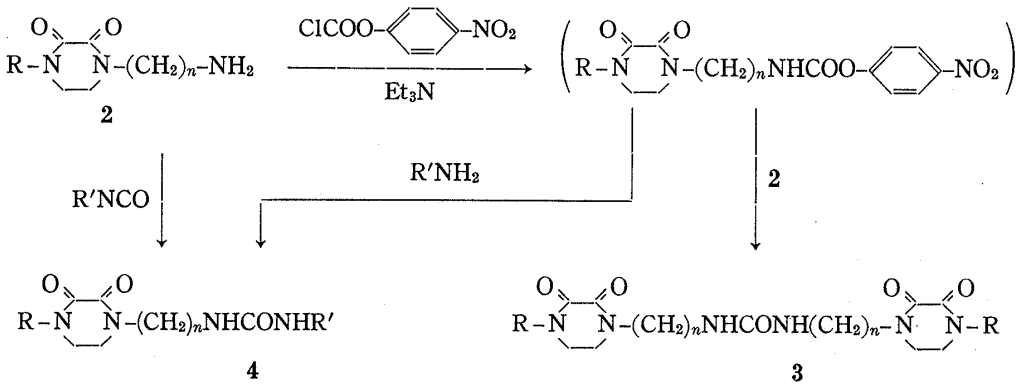


Chart 2

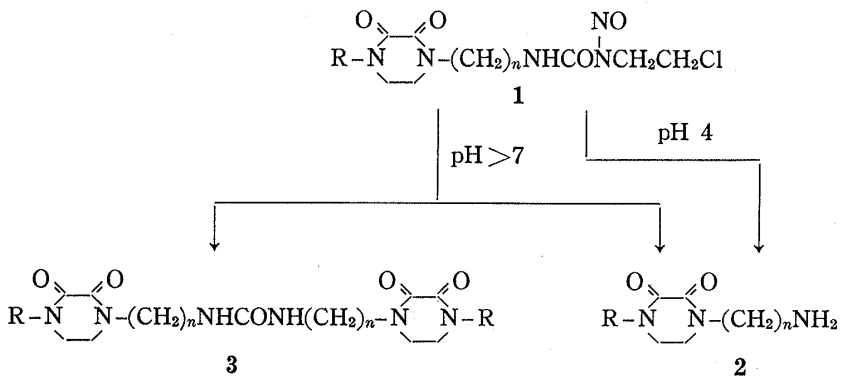


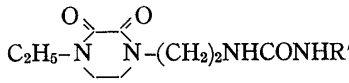
Chart 3

Table I. Antitumor Activities of Symmetrical Urea Compounds

Compd. No.	n	EAC(*s.c.–i.p.*)[a] Dose (mg/kg)	T/C (%)
3a	2	500×5	72
3b	1	500×5	67
3c	4	500×5	58

a) Animal: SLC-ICR (♀) mice, 5 mice/group. Inoculum size: EAC 1×10^6 cells/mouse, *s.c.* Treatment: Days 1—5, *i.p.* Determined on day 14.
$T/C(\%)=\frac{\text{mean tumor weight of treated}}{\text{mean tumor weight of control}}\times100$

Table II. Antitumor Activities of Unsymmetrical Urea Compounds



Compd. No.	R′	EAC(*s.c.–i.p.*)[a] Dose (mg/kg)	T/C (%)
4a	CH_3	500×5	56
4b[1]	CH_2CH_2Cl	500×5	96
4c		500×5	61

a) See legend to Table I.

and their antitumor activities were investigated. It was found that **4a** ($R=C_2H_5$, $R'=CH_3$, $n=2$) had antitumor activity against Ehrlich carcinoma (solid form) (Table II).

It seems noteworthy that 2,3-dioxopiperazine derivatives without antitumor active groups, such as N-(2-chloroethyl)-N-nitrosourea, had antitumor activity. This finding might lead to the development of a new type of antitumor agent having a 2,3-dioxopiperazine ring.

Experimental[3]

Time Course of Degradation in Aqueous Solution——Solutions of the test compounds were prepared at a concentration of 3000 μg/ml in 0.1 M pH 7.0 phosphate buffer solution, 0.1 M pH 4.0 citric acid–sodium phosphate buffer solution or 0.2 M pH 9.0 boric acid–NaOH buffer solution. The solutions were stored at 37°, and aliquots were withdrawn at intervals; a 4 μl portion was used for thin-layer chromatography (silica gel).

Degradation of 1-(2-Chloroethyl)-3-[2-(4-phenyl-2,3-dioxo-1-piperazinyl)ethyl]-1-nitrosourea (1b) in Aqueous Solution at pH 9.0——**1b** (300 mg) was dissolved in a mixture of pH 9.0 buffer solution (100 ml) and acetone (30 ml) and allowed to stand for 3 hr at 37°. It was then concentrated to one-half of the original volume, and 1,3-bis[2-(4-phenyl-2,3-dioxo-1-piperazinyl)ethyl]urea (**3d**) precipitated as colorless crystals. Yield 60 mg. mp 262—264° (dec.). IR ν_{max}^{KBr} cm^{-1}: 3340, 3270 (NH), 1665 (C=O). *Anal.* Calcd for $C_{25}H_{28}N_6O_5\cdot H_2O$: C, 58.81; H, 5.92; N, 16.46. Found: C, 58.73; H, 5.76; N, 16.26.

1,3-Bis[4-(4-ethyl-2,3-dioxo-1-piperazinyl)butyl]urea (3c)——A solution of a mixture of 4-(4-ethyl-2,3-dioxo-1-piperazinyl)butylamine (3.9 g) and triethylamine (2.56 ml) in CH_2Cl_2 (50 ml) was added dropwise to a solution of *p*-nitrophenyl chloroformate (3.7 g) in CH_2Cl_2 (50 ml) over a period of 30 min at −40°. The reaction mixture was stirred for 1 hr at −40 to −30°, then H_2O (30 ml) was added. The CH_2Cl_2 layer was separated, washed with H_2O and dried over $MgSO_4$. Next, 4-(4-ethyl-2,3-dioxo-1-piperazinyl)butylamine (3.9 g) in CH_2Cl_2 (30 ml) was added to the above CH_2Cl_2 solution at room temperature over a period of 10 min, and the whole was stirred for 1 hr at room temperature. Evaporation to dryness gave a residue, which was washed with IPE and recrystallized from IPA–AcOEt to afford colorless crystals of mp 162°. Yield 3.7 g (45%). IR ν_{max}^{KBr} cm^{-1}: 3340 (NH), 1650 (C=O). NMR ($CDCl_3$) δ: 1.19 (6H, t, $J=7$ Hz, $2\times CH_3$),

1.20—1.90 (8H, m, $4\times CH_2$), 2.80—3.80 (20H, m, $10\times CH_2$), 6.16 (2H, m, $2\times NH$). *Anal.* Calcd for $C_{21}H_{36}N_6O_5$: C, 55.74; H, 8.02; N, 18.57. Found: C, 55.70; H, 8.10; N, 18.48. The *Rf* value of this material was identical with that of one of the degradation products of **1c** ($R=C_2H_5$, $n=4$) in aqueous solution at pH 7.

The following compounds were obtained similarly. **3a** ($R=C_2H_5$, $n=2$); mp 234—235° (EtOH). **3b** ($R=C_2H_5$, $n=1$); mp 287—289° (MeOH).

1-[2-(4-Ethyl-2,3-dioxo-1-piperazinyl)ethyl]-3-methylurea (4a)——Methylisocyanate (2.0 g) was added dropwise to a suspension of 2-(4-ethyl-2,3-dioxo-1-piperazinyl)ethylamine (5.5 g) in abs. tetrahydrofuran (50 ml) at room temperature. The mixture was stirred for 1 hr, then $CHCl_3$ (10 ml) was added. **4a** precipitated as a white solid. Yield 5.4 g (75%). Recrystallization from iso-PrOH afforded colorless needles of mp 168°. IR ν_{max}^{KBr} cm^{-1}: 3370, 3305 (NH), 1650 (C=O). NMR ($CDCl_3$) δ: 1.18 (3H, t, $J=6.6$ Hz, CH_3), 2.73 (3H, d, $J=4.2$ Hz, CH_3), 3.31—3.61 (10H, m, $3\times CH_2$, piperazine ring 5 and 6 CH_2), 5.96 (1H, q, $J=4.2$ Hz, NH), 6.39 (1H, m, NH). *Anal.* Calcd for $C_{10}H_{18}N_4O_3$: C, 49.57; H, 7.49; N, 23.13. Found: C, 49.74; H, 7.53; N, 23.02.

Compound (**4c**) ($R=C_2H_5$, $R'=Ph$, $n=2$) was obtained similarly. mp 236° (MeOH).

Antitumor Activity against EAC (*s.c.-i.p.*)——A group of mice consisting of 5,6-week-old female SLC-ICR mice, weighing 22 ± 1 g, was inoculated subcutaneously with Ehrlich ascites carcinoma cells (1×10^6/cells/head/0.2 ml physiological saline). The test compounds dissolved in H_2O or suspended in 0.3% CMC–physiological saline were intraperitoneally administered daily for 5 successive days. On day 14 the solid tumors were isolated and their weights were measured. The average tumor weight of the above group was compared with that of the untreated control group and T/C(%) was calculated.

$$T/C(\%) = \frac{\text{mean tumor weight in treated group}}{\text{mean tumor weight in control group}} \times 100$$

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References and Notes

1) T. Hori, K. Momonoi, Y. Kiba, C. Yoshida, H. Sakai, R. Takeno, T. Ohashi, S. Kishimoto, and I. Saikawa, *Yakugaku Zasshi*, **99**, 730 (1979).
2) J.A. Montgomery, R. James, G.S. McCaleb, and T.P. Johnston, *J. Med. Chem.*, **10**, 668 (1967); D.J. Reed, H.E. May, R.B. Boose, K.M. Gregory, and M.A. Beilstein, *Cancer Res.*, **35**, 568 (1975).
3) All melting points are uncorrected. Infrared absorption (IR) spectra were recorded on a Hitachi 215 spectrometer. NMR spectra were measured with a Hitachi R24 (60 MHz) spectrometer. Chemical shift values are expressed in ppm relative to internal tetramethylsilane. Abbreviations are as follows: s, singlet; d, doublet; t, triplet; m, multiplet. pH values were measured with a Toa Denpa HM-5A pH meter.