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Studies on Antitumor-active 2,3-Dioxopiperazine Derivatives. II.[1] Synthesis and Structure-Antitumor Activity Relationship of 1-Benzyl-2,3-dioxopiperazine Derivatives

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2,3-Dioxopiperazine derivatives, which are antitumor agents of a new type, were synthesized and the structure–activity relationship was investigated from the viewpoint of lipophilic–hydrophilic balance. It was found that 1-(4-diethylaminobenzyl)-4-*n*-hexyl-2,3-dioxopiperazine (**12n**) possessed significant *in vitro* cytotoxicity and *in vivo* antitumor activity against transplanted tumor, but this *in vivo* antitumor activity did not reflect the *in vitro* cytotoxicity. The metabolism of **12n** in rats and mice was then studied. It was found that the Et_2N-group of **12n** was easily metabolized to an AcNH-group *in vivo*.

Keywords——new type of antitumor agent; 2,3-dioxopiperazine derivatives; structure-activity relationship; cytotoxicity; antitumor activity; Ehrlich ascites carcinoma; HeLa S3 cell; metabolism

In the preceding paper,[1] we reported that antitumor agents of a new type could be developed from 2,3-dioxopiperazine derivatives. It has already been reported that highly lipophilic polyene compounds such as vitamin A, its analogs and coenzyme Q, possess tumor preventive effect or antitumor effect.[2] On the other hand, the 2,3-dioxopiperazinyl group is known to be a hydrophilic group.[3]

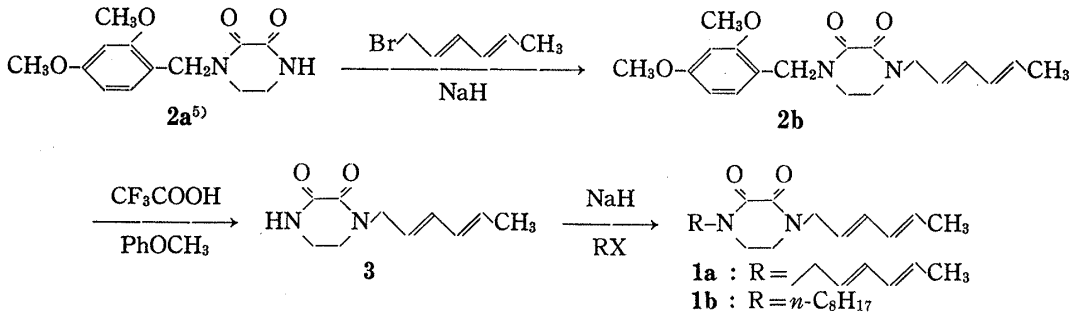
This work was undertaken from the viewpoint of lipophilic–hydrophilic balance in an attempt to develop a new antitumor agent having a 2,3-dioxopiperazine moiety. In the present paper we wish to report the antitumor activity of 1-(4-diethylaminobenzyl)-4-*n*-hexyl-2,3-dioxopiperazine (**12n**) and some problems regarding its *in vivo* activity.

First, in order to find antitumor-active 2,3-dioxopiperazine derivatives having polyene groups, 1-(2,4-hexadien-1-yl)-2,3-dioxopiperazine derivatives (**1a**, **b**) were synthesized by two methods, as shown in Chart 1: method A *via* the intermediate (**2b**) and method B through the cyclization of (**5a**, **b**) to form the 2,3-dioxopiperazine ring, followed by introduction of a 2,4-hexadienyl group. In method A, the yield in the removal of the 2,4-dimethoxybenzyl protecting group[4,5] of the amide nitrogen of the 2,3-dioxopiperazine ring was poor, and synthetically method B was superior to method A.

The minimum inhibitory concentrations (MIC values) against HeLa S3 cells and Ehrlich ascites carcinoma (EAC) cells of **1a**, **b**, **2b**, and **3** were tested. Among them, only **2b** showed high cytotoxicity (MIC: 3.13 μg/ml against HeLa S3 and EAC cells). This result suggests that the 2,4-dimethoxybenzyl group contributes to the cytotoxicity and that the polyene group does not participate (Table I). Next, the relation between the nature of 4-substituents of 1-(2,4-dimethoxybenzyl)-2,3-dioxopiperazine derivatives (**2**) and cytotoxicity was investigated. Compounds (**2c**—**n**) were synthesized by the same method as **2b**. The cytotoxicity of **2** was greatest when the carbon number of the 4-substituent was 6. Introduction of polar groups such as OH, NH_2, and COOH, into the 4-substituent decreased the cytotoxicity. **2b** and **2c** showed the highest cytotoxicity (Table II).

In order to find more effective compounds than **2b**, **c**, chemical modification of the A,

method A



method B

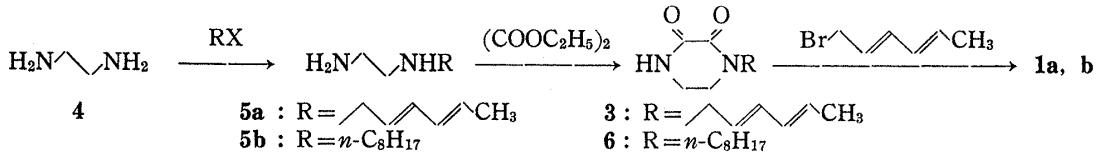
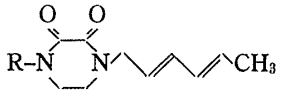


Chart 1

TABLE I. Structure–Cytotoxicity Relationship of 1-(2,4-Hexadien-1-yl)-2,3-dioxopiperazine Derivatives



Compd. No.	R	MIC (μg/ml)[a] HeLaS3	EAC
3	H	>100	>100
1a	CH_3	25	25
1b	*n*-C_8H_{17}-	50	50
2b	CH_3O- (2,5-dimethoxybenzyl)-	3.13	3.13

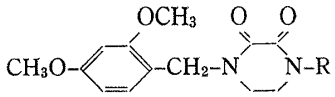
a) Microplate method.
Incubation medium: Eagle's MEM supplemented with 20% calf serum.
Inoculum size: 2×10^4 cells/ml.
Incubation period: 4 days.
Determination: Giemsa staining.

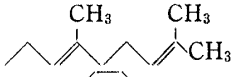
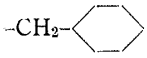
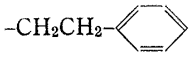
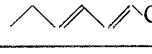
B, and C moieties of **2e** (Fig. 1) was carried out and the structure–activity relationship was investigated. In the A moiety, the substituent effect on the benzene ring was studied. Compounds (**12a—q**) were synthesized by method A from substituted benzaldehydes (**7**) or by method B from substituted benzyl halides **13** (Chart 2). Table III shows the *in vitro* cytotoxicities of **12a—q** and **2e**. Among them, the 2,5-dimethoxy compound (**12d**), 2,3,4-trimethoxy compound (**12h**) and 4-diethylamino compound (**12n**) showed high cytotoxicities, as did the 2,4-dimethoxy compound (**2e**).

These four compounds showed cytotoxic effects against HeLa and Ehrlich cells *in vitro*, but only **12n** showed antitumor activity against transplanted EAC; the other three compounds showed no therapeutic effect (Table IV). It may be concluded from the results of this investigation that the 4-diethylamino group is the preferred substituent on the benzene ring. The 4-diethylaminophenyl group was therefore chosen as the A moiety.

Next, the B moiety in Fig. 1 was investigated. Compounds (**14a—d**) were synthesized by method shown in Chart 3. In particular, it is of interest that **14a** was easily obtained by

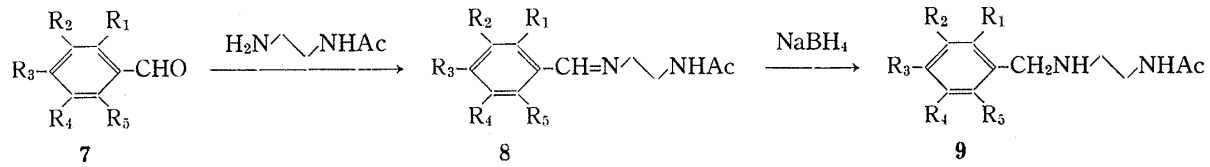
Table II. Structure–Cytotoxicity Relationship of 1-(2,4-Dimethoxybenzyl)-2,3-dioxopiperazine Derivatives



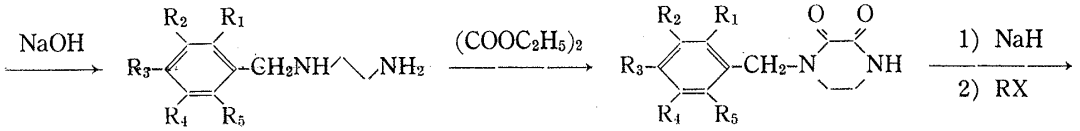
Compd. No.	R	MIC(μg/ml)[a] HeLaS3	EAC
2a	H	100	100
2c	$-CH_2CH=CH_2$	100	100
2d	n-C_4H_9	12.5	12.5
2e	n-C_6H_{13}	3.13	3.13
2f	n-C_7H_{15}	6.25	6.25
2g	n-C_8H_{17}	25	25
2h		25	25
2i		6.25	6.25
2j		12.5	12.5
2k	$-(CH_2)_6Br$	12.5	6.25
2l	$-(CH_2)_6OH$	50	100
2m	$-(CH_2)_6NH_2$	25	12.5
2n	$-CH_2-CH=CH-CH=C(CH_3)COOH$	100	100
2b		3.13	3.13

a) See the legend to Table I.

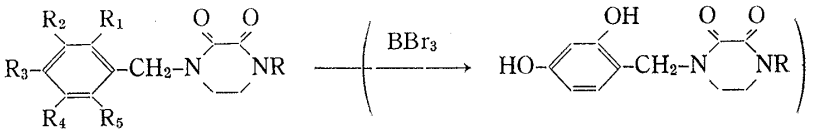
method A



7 **8** **9**

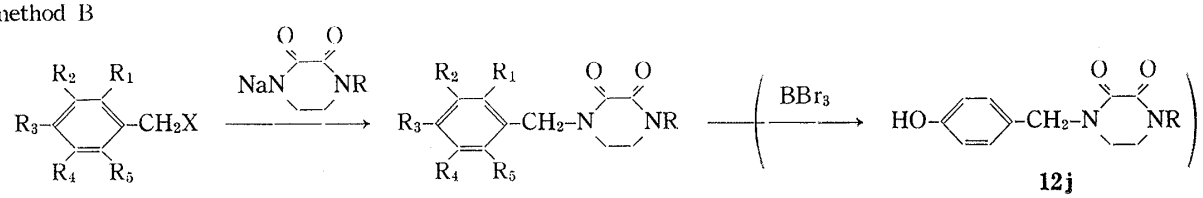


10 **11**



12g, n, o, 18a—k **12k**

method B



13 (X=halogen) **12a—f, h, i, l, m, p, q** **12j**

Chart 2

the reaction of 1-*n*-hexyl-2,3-dioxopiperazine with an aromatic iodine compound in the presence of K_2CO_3 and Cu.[4] Table V shows the cytotoxicities of **14a—d** and **12n** against HeLa S3 and EAC cells. **12n**, in which the B moiety is $-CH_2-$, showed the highest cytotoxicity. Thus, the 4-diethylaminobenzyl group was chosen as the AB moiety.

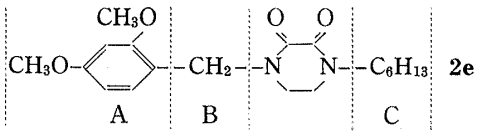
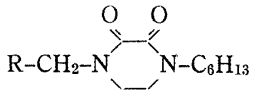
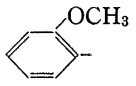
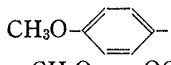
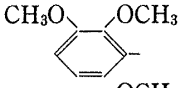
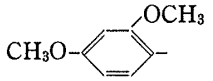
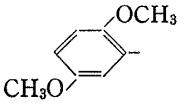
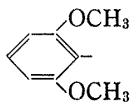
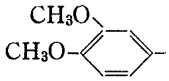
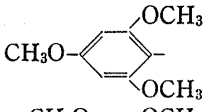
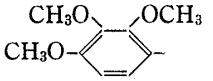
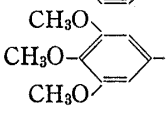
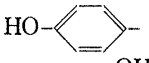
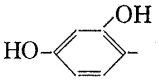
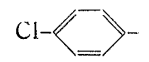
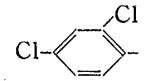
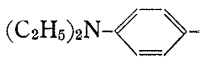
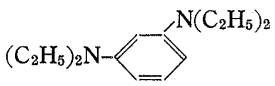
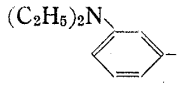
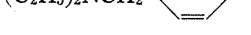


Fig. 1. Moieties of **2e** That were separately subjected to Chemical Modification

Investigation of the C moiety shown in Fig. 1 was next carried out. Compounds **18a—k** were synthesized by method A as shown in Chart 2. Table VI shows the structure-antitumor activity relationships *in vitro* and *in vivo* of **18a—k** and **12n**. Among **18a—g** and **12n**, **12n**, **18c**, and **18d**, in which the carbon number of the C moiety was 6, showed cytotoxicity. However, among these three compounds, only **12n** showed *in vivo* antitumor activity against EAC (*i.p.*—*i.p.*); the other two compounds showed no therapeutic effect. Among **18h—k**, **18h** showed cytotoxicity equal to that of **12n**, but no *in vivo* antitumor activity was found. Further, **18j** and **18k** showed lower cytotoxicities than **12n** but their *in vivo* antitumor activities were

Table III. Structure–Cytotoxicity Relationship of 1-Benzyl-4-*n*-hexyl-2,3-dioxopiperazine Derivatives

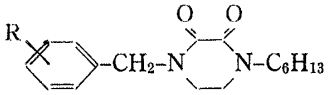


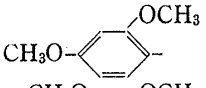
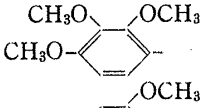
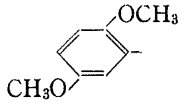
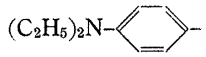
Compd. No.	R	MIC(μg/ml)[a] HeLaS3	MIC(μg/ml)[a] EAC
12a		50	25
12b		50	50
12c		100	100
2e		3.13	3.13
12d		6.25	6.25
12e		100	50
12f		50	50
12g		100	50
12h		3.13	3.13
12i		100	50
12j		100	100
12k		50	50
12l		100	50
12m		25	12.5
12n		3.13	3.13
12o		50	50
12p		50	50
12q		100	100

a) See the legend to Table I.

similar to that of **12n**. These results showed that the *in vitro* cytotoxicity did not correlate with the *in vivo* antitumor activity. This might be due to pharmacodynamic factors, so the metabolism of the basic compound **12n** in rats and mice was investigated (Fig. 2). At 5, 15, 30 min and 1 hr after intraperitoneal administration of **12n** to mice or rats, metabolites in

Table IV. Structure–Antitumor Activity Relationship of 1-Benzyl-4-*n*-hexyl-2,3-dioxopiperazine Derivatives



Compd. No.	R	*In vitro* MIC(μg/ml)[a] HeLaS3	*In vivo* LD_{50}[b] (mg/kg)	*In vivo* EAC(*i.p.*–*i.p.*)[c] Dose(mg/kg)	*In vivo* EAC(*i.p.*–*i.p.*)[c] T/C(%)
2e		3.13	200	40×7	110
12h		3.13	200	40×7	107
12d		6.25	200	40×7	101
12n		3.13	100	40×7	157

a) See the legend to Table I.
b) Animals; SLC–ICR (♀) mice, 6 weeks old, 2 mice/group.
Treatment: *i.p.*
Observation period: 1 week.
c) Animals: SLC–ICR (♀) mice, 6 weeks old, 4 or 5 mice/group.
Treatment: from day 1 to day 7
Inoculum size: EAC 1×10^6 cells/mouse, *i.p.*

$$T/C(\%)=\frac{\text{mean survival days of treated}}{\text{mean survival days of control}}\times100$$

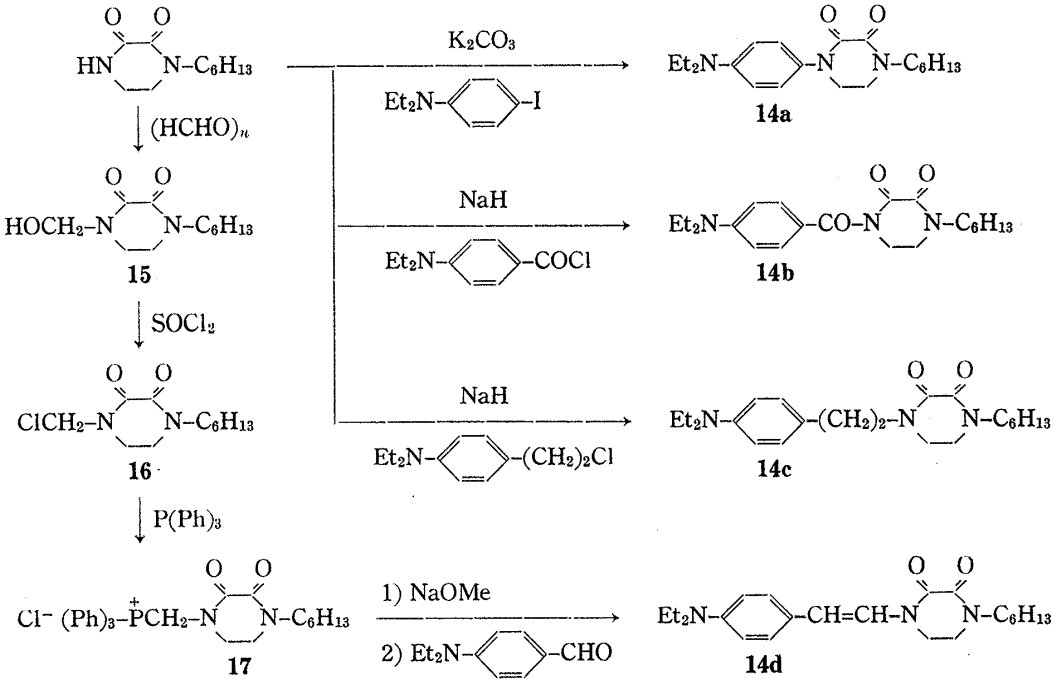
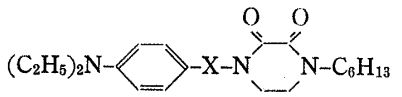


Chart 3

the blood, intraperitoneal cavity, liver, urine and feces were qualitatively studied by thin-layer chromatography (TLC). The metabolites of **12n** in rats were the same as those in mice. **12n** disappeared from the blood at 30 min after administration. **12n** could not be detected in urine and feces at 15 min after administration, and three metabolites (**19**, **20**, **21**) were obse-

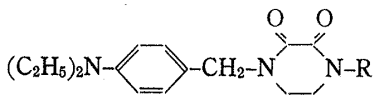
Table V. Structure–Cytotoxicity Relationship of 1-*n*-Hexyl-2,3-dioxopiperazine Derivatives

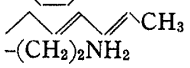
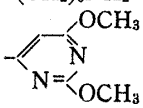
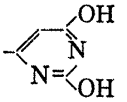
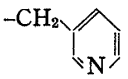
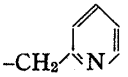


Compd. No.	–X–	MIC(μg/ml)[a] HeLaS3	MIC(μg/ml)[a] EAC
14a	—	12.5	50
14b	–CO–	100	100
12n	$-CH_2-$	3.13	3.13
14c	$-CH_2CH_2-$	12.5	50
14d	–CH=CH–	25	25

a) See the legend to Table I.

Table VI. Structure–Antitumor Activity Relationship of 1-(4-Diethylaminobenzyl)-2,3-dioxopiperazine Derivatives



Compd. No.	R	*In vitro*[a] MIC(μg/ml) HeLaS3	*In vivo* LD_{50}[b] (mg/kg)	*In vivo* EAC(*i.p.*–*i.p.*)[c] Dose (mg/kg)	*In vivo* EAC(*i.p.*–*i.p.*)[c] T/C (%)
18a	H	100	—	—	—
18b	C_2H_5	100	—	—	—
12n	C_6H_{13}	3.13	100	40×7	157
18c		12.5	—	40×7	102.6
18d		6.25	—	40×7	107.7
18e	$-(CH_2)_2NH_2$	25	200—300	40×7	134
18f	$-(CH_2)_3NH_2$	>100	—	40×7	116.7
18g	$-(CH_2)_6NH_2$	12.5	100	40×7	113
18h		1.56—3.13	—	40×7	121
18i		100	—	—	—
18j		25	500	100×7	159.5
18k		25	500	100×7	164.5

a) See the legend to Table I.
b,c) See the legend to Table IV.

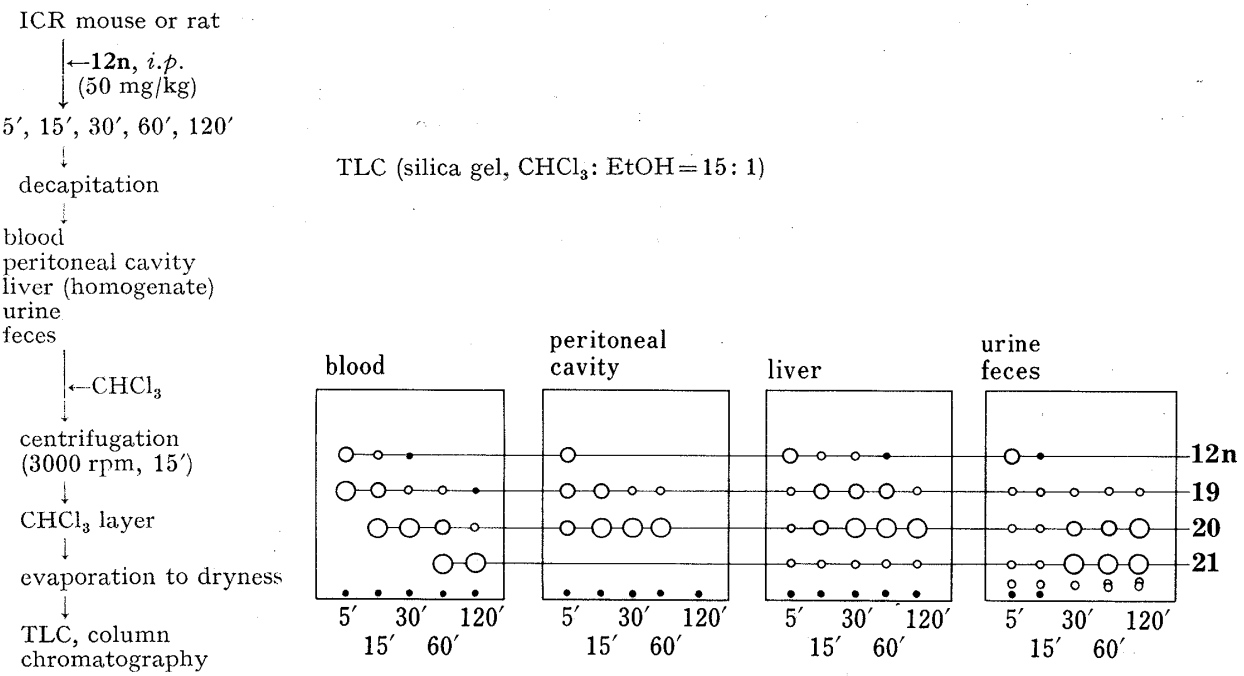


Fig. 2. Metabolism of **12n** in Rats and Mice

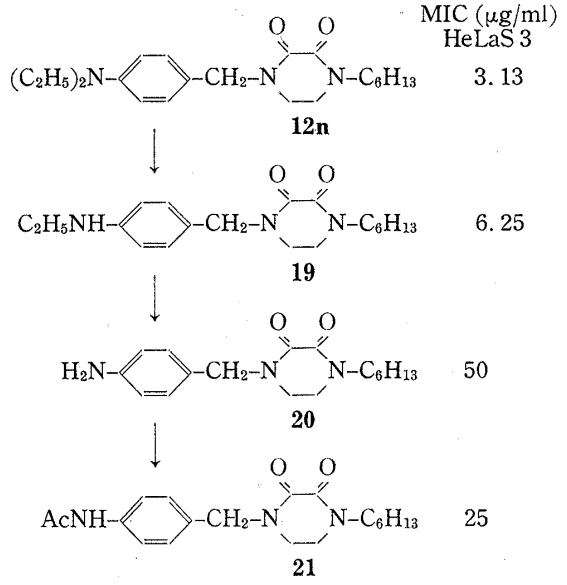


Chart 4

rved. These metabolites were separated and purified by column chromatography. It was found that **19** was an EtNH compound, **20** was an NH_2 compound and **21** was an AcNH compound. Their structures were confirmed by comparison of the infrared (IR) and nuclear magnetic resonance (NMR) spectra, and the *Rf* values on TLC of **19**, **20**, and **21** with those of authentic samples synthesized independently. These metabolites showed lower cytotoxicity than **12n**. It may be concluded that **12n** was easily metabolized to **21** through the pathway shown in Chart 4, inactivated and excreted.

The results of this investigation suggest that a more potent antitumor agent which possesses high *in vivo* antitumor activity reflecting the *in vitro* cytotoxicity can be developed if the metabolism of the Et_2N-moiety of **12n** can be suppressed. Studies along this line are in progress.

Experimental[6]

Cell Culture of HeLa S3 and Ehrlich Cells[7]——In each case 5×10^4 cells/bottle were inoculated in a 5 ml culture bottle containing 3 ml of Eagle's minimum essential medium (MEM) supplemented with 10% calf serum and cultured at 37° in a CO_2-incubator for 5 days. Cells which grew on a glass surface, after removal of the culture solution, in PBS (−) (Ca^{2+} and Mg^{2+} are free) containing 0.02% ethylenediaminetetraacetic acid (EDTA) were incubated for 5—7 min and then an equal volume of 0.2% trypsin was added. The clusters of cells were dissociated by pipetting. The cell suspension was added to a centrifuge tube containing 5 ml of Eagle's MEM and then centrifuged at 1000 rpm for 5 min. The precipitate was added to fresh medium. The resulting cell suspension was used for tests.

Cytotoxic Effects on HeLa and Ehrlich Cells in Microplate Tests[7]——Microplates (Sumitomo Bakelite

Co.) having 3×12 chambers per plate flattened on the bottom were used. Test compounds were dissolved in 20% dimethyl sulfoxide (DMSO) and sterilized by filtration through a Millipore filter, and then they were diluted with culture medium in 2 fold serial dilutions (12 steps). The concentrations of the compounds were from 100 μg/ml to 0.05 μg/ml. Test compounds dissolved in culture medium (0.1 ml) and cell suspension (0.1 ml, 2×10^4 cells/ml) in one chamber were cultured at 37° in a CO_2-incubator for 5 days. The culture medium was removed and the cells were washed twice with Hanks' salt solution. The cells were fixed for 5 min with 95% EtOH and stained with Giemsa solution for 15 min. The inhibition of cell growth could be observed by measuring the degree of cell staining on microplates macroscopically, because chambers in which the cell growth was inhibited were not stained. MIC values (minimum inhibitory concentration) were determined as the minimum concentrations of the compounds at which the cell growth was inhibited.

Acute Toxicity to Mice——One group, consisting of two 5-week-old female SLC–ICR mice weighing of 20 ± 1 g, received test compounds dissolved or suspended in saline or saline containing 0.3% carboxymethyl cellulose (CMC) intraperitoneally. At 7 days after administration, the number of deaths was noted and the LD_{50} was calculated.

Antitumor Activity against Ehrlich Ascites Carcinoma (*i.p.-i.p.*)——EAC cells (1×10^6 cells/head/0.2 ml saline) were intraperitoneally transplanted into one group containing of five 6-week-old female ICR mice weighing of 22 ± 1 g. Test compounds suspended in 0.3% CMC saline or dissolved in saline were intraperitoneally administered daily from the day after transplantation for 7 days. Antitumor activity (T/C%) was evaluated from the following formula.

$$\text{T/C}(\%) = \frac{\text{mean survival days of treated group}}{\text{mean survival days of control group}} \times 100$$

1-(2,4-Dimethoxybenzyl)-4-(2,4-hexadien-1-yl)-2,3-dioxopiperazine (2b)——A solution of 1-(2,4-dimethoxybenzyl)-2,3-dioxopiperazine[5] (**2a**) (10 g) in dimethylformamide DMF (70 ml) was added dropwise to a suspension of NaH (purity 50%, 1.8 g) in DMF (20 ml) over a period of 10 min at room temperature. The mixture was stirred at 50—60° for 30 min, then a solution of 2,4-hexadien-1-yl bromide (7.0 g) in DMF (20 ml) was added. The reaction mixture was stirred at 50—60° for 1 hr and evaporated to dryness *in vacuo*. The residue was dissolved in $CHCl_3$ (50 ml), washed with H_2O and dried over $MgSO_4$. Removal of the solvent *in vacuo* left a brown oil, which was chromatographed on silica gel with $CHCl_3$ to give **2b** as a liquid. Yield 10.8 g (81.7%). IR ν_{max}^{neat} cm^{-1}: 1668 (C=O). NMR ($CDCl_3$) δ: 1.72 (3H, d, $J=5.5$ Hz, CH_3), 3.38 (4H, bs, piperazine ring 5 and 6 CH_2), 3.77 (6H, s, $2 \times OCH_3$), 4.01 (2H, d, $J=6.5$ Hz, CH_2), 4.54 (2H, s, CH_2), 5.23—6.14 (4H, m, –CH=CH–CH=CH–), 6.27—6.66 (2H, m, benzene ring 3 and 5 CH), 7.15 (1H, d, $J=9$ Hz, benzene ring 6 CH).

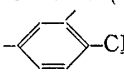
1-(2,4-Hexadien-1-yl)-2,3-dioxopiperazine (3)——Method A: CF_3COOH (50 ml) was added to a solution of **2b** (5.0 g) in anisole (50 ml) at room temperature. After being stirred at room temperature for 1 hr and at 50—60° for 1 hr, the whole was evaporated to dryness *in vacuo*. The residue was washed with iso-Pr_2O and dissolved in $CHCl_3$ (50 ml). The solution was washed with H_2O and dried over anhydrous $MgSO_4$. After removal of the solvent, the residue was chromatographed on silica gel with $CHCl_3$–EtOH to give **3** (0.2 g, 7.1%) as colorless crystals of mp 138—139° (iso-PrOH). IR ν_{max}^{KBr} cm^{-1}: 1695, 1665 (C=O). NMR (DMSO-d_6) δ: 1.71 (3H, d, $J=4.2$ Hz, CH_3), 3.42 (4H, bs, piperazine ring 5 and 6 CH_2), 4.00 (2H, d, $J=6.6$ Hz, CH_2), 5.20—6.30 (4H, m, –CH=CH–CH=CH–), 8.42 (1H, bs, NH). *Anal.* Calcd for $C_{10}H_{14}N_2O_2$: C, 61.83; H, 7.27; N, 14.22. Found: C, 61.68; H, 7.19; N, 14.08.

Method B: 2,4-Hexadien-1-yl bromide (14.9 g) was added dropwise to a solution of ethylenediamine (22.2 g) in EtOH (200 ml) over a period of 30 min at −30°, and the mixture was refluxed for 4 hr. After removal of the solvent *in vacuo*, H_2O (20 ml) and NaOH (10 g) were added to the residue and the separated oil was distilled to give N-(2,4-hexadien-1-yl)ethylenediamine (**5a**) (6.3 g, 48.7%) as a pale yellow liquid of bp 200—230° (15 mmHg). This liquid (6.3 g) and diethyl oxalate (6.56 g) were added dropwise at the same time to EtOH (60 ml) under ice-cooling over a period of 20 min. The reaction mixture was refluxed for 5 hr. Removal of the solvent *in vacuo* left a pale brown solid, which was chromatographed on silica gel with $CHCl_3$–EtOH to give **3** (3.5 g, 40%) as colorless crystals of mp 140° (iso-PrOH). The IR and NMR spectra and *Rf* value on TLC were identical with those of the product obtained by method A.

Compounds **5b** and **6** were similarly synthesized, as described in an earlier paper.[8]

1-*n*-Octyl-4-(2,4-hexadien-1-yl)-2,3-dioxopiperazine (1b)——Compound **1b** was obtained from 1-*n*-octyl-2,3-dioxopiperazine (**6**) and 2,4-hexadien-1-yl bromide by the procedure used to prepare **2b**. mp 81—83° (iso-Pr_2O–petr. ether). IR ν_{max}^{KBr} cm^{-1}: 1659 (C=O). NMR ($CDCl_3$) δ: 0.86 (3H, m, CH_3), 1.00—1.80 (12H, m, $6 \times CH_2$), 1.73 (3H, d, $J=4.2$ Hz, CH_3), 3.31—3.53 (6H, m, piperazine ring 5 and 6 CH_2 and $\overset{}{\underset{}{\text{—}}}\!\!\diagdown\!\!\text{NCH}_2\text{–}$), 4.07 (2H, d, $J=6.0$ Hz, $\overset{}{\underset{}{\text{—}}}\!\!\diagdown\!\!\text{NCH}_2\text{CH=}$), 5.10—6.40 (4H, m, –CH=CH–CH=CH–). *Anal.* Calcd for $C_{18}H_{30}N_2O_2$: C, 70.55; H, 9.87; N, 10.94. Found: C, 70.68; H, 9.93; N, 11.10.

Compound **1a** was similarly obtained. mp 136—138° (AcOEt). IR ν_{max}^{KBr} cm^{-1}: 1660, 1640 (C=O). *Anal.* Calcd for $C_{16}H_{22}N_2O_2$: C, 70.05; H, 8.80; N, 10.21. Found: C, 70.21; H, 8.11; N, 10.30.

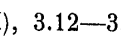
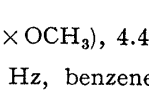
1-(2,4-Dimethoxybenzyl)-4-*n*-hexyl-2,3-dioxopiperazine (2e)——Compound **2e** was obtained from 2,4-dimethoxybenzaldehyde by the procedure used to prepare **2b**. mp 77—79° (AcOEt–iso-Pr_2O). IR ν_{max}^{KBr} cm^{-1}: 1658 (C=O). NMR ($CDCl_3$) δ: 0.81 (3H, m, CH_3), 1.00—1.80 (8H, m, $4\times CH_2$), 3.20—3.60 (6H, m, piperazine ring 5 and 6CH_2 and $-NCH_2-C_5H_{11}$), 3.78 (6H, s, $2\times OCH_3$), 4.56 (2H, s, ), 6.25—6.45 (2H, m, benzene ring 3 and 5 CH), 7.17 (1H, d, $J=8.5$ Hz, benzene ring 6 CH). *Anal.* Calcd for $C_{19}H_{28}N_2O_4$: C, 65.49; H, 8.10; N, 8.04. Found: C, 65.26; H, 8.13; N, 7.94.

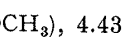
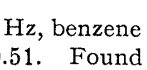
The following compounds (Table II) were similarly synthesized (Table VII).

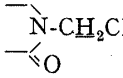
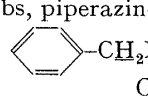
Table VII

Compd. No.	mp (°C) (Recryst. solv.)	Formula	Analysis(%) Calcd (Found) C	H	N	NMR (solvent) δ (ppm)
2c	115 —116.5 (AcOEt)	$C_{16}H_{20}N_2O_4$	63.14 (62.92	6.62 6.60	9.21 9.02)	($CDCl_3$) 3.42 (4H, bs), 3.79 (6H, s), 4.04 (2H, d), 4.60 (2H, s), 5.05 (1H, m), 5.27 (1H, m), 5.70 (1H, m), 6.30—6.47 (2H, m), 7.18 (1H, d)
2d	76 — 78 (AcOEt–iso-Pr_2O)	$C_{17}H_{24}N_2O_4$	63.73 (63.87	7.55 7.58	8.74 8.77)	($CDCl_3$) 0.85 (3H, m), 1.00—1.80 (4H, m), 3.35—3.55 (6H, m), 3.78 (6H, s), 4.59 (2H, s), 6.30—6.48 (2H, m), 7.18 (1H, d)
2f	81.5— 83.5 (AcOEt–iso-Pr_2O)	$C_{20}H_{30}N_2O_4$	66.27 (65.95	8.34 8.34	7.73 7.70)	($CDCl_3$) 0.84 (3H, t), 1.00—1.68 (10H, m), 3.11—3.58 (6H, m), 3.74 (6H, s), 4.52 (2H, s), 6.18—6.48 (2H, m), 7.10 (1H, d)
2g	75 — 76 (AcOEt–iso-Pr_2O)	$C_{21}H_{32}N_2O_4$	66.99 (66.91	8.57 8.75	7.44 7.49)	($CDCl_3$) 0.84 (3H, m), 1.00—1.80 (12H, m), 3.30—3.50 (6H, m), 3.76 (6H, s), 4.55 (2H, s), 6.26—6.45 (2H, m), 7.17 (1H, d)
2h	Oil					($CDCl_3$) 0.67—1.02 (3H, m), 1.07—1.50 (4H, m), 1.59 (3H, s), 1.67 (3H, s), 3.44 (6H, bs), 3.76 (3H, s), 3.79 (3H, s), 3.91—4.27 (1H, m), 4.58 (2H, s), 4.87—5.27 (1H, m), 6.23—6.54 (2H, m), 7.19 (1H, d)
2i	93 — 95 (AcOEt–iso-Pr_2O)	$C_{20}H_{28}N_2O_4$	66.64 (66.64	7.83 7.86	7.77 7.68)	(DMSO-d_6) 1.00—2.10 (11H, m), 3.22 (2H, d), 3.46 (4H, bs), 3.79 (3H, s), 3.80 (3H, s), 4.49 (2H, s), 6.43—6.56 (2H, m), 7.12 (1H, d)
2j	108 —110 (EtOH)	$C_{21}H_{24}N_2O_4$	68.46 (68.02	6.57 6.53	7.60 7.53)	($CDCl_3$) 2.81 (2H, t), 3.16 (4H, s), 3.59 (2H, t), 3.70 (6H, s), 4.47 (2H, s), 6.26—6.69 (2H, m), 6.89—7.19 (6H, m)
2k	75 — 76 (iso-PrOH–iso-Pr_2O)	$C_{19}H_{27}BrN_2O_4$	53.40 (53.59	6.36 6.48	6.55 6.59)	($CDCl_3$) 1.10—2.10 (8H, m), 3.22—3.57 (8H, m), 3.75 (6H, s), 4.54 (2H, s), 6.24—6.43 (2H, m), 7.12 (1H, d)

1-(2,4-Dimethoxybenzyl)-4-(6-hydroxy-1-hexyl)-2,3-dioxopiperazine (21)——Compound **2k** (2.0 g) and AcONa (0.6 g) were added to DMF (10 ml) and the reaction mixture was refluxed for 3 hr. After removal of the solvent *in vacuo*, the residue was dissolved in AcOEt (50 ml) and the solution was washed with H_2O and dried over $MgSO_4$. The solid obtained by removing the solvent was dissolved in MeOH (10 ml). NaOMe (0.4 g) was added to the above solution and the whole was stirred for 12 hr at room temperature. After removal of the solvent *in vacuo*, $CHCl_3$ was added to the residue and the extract was washed with H_2O and dried over $MgSO_4$. The oil obtained by removing the solvent was chromatographed on silica gel with $CHCl_3$–

EtOH to afford **21** as a liquid. Yield 1.13 g (66%). IR ν_{max}^{neat} cm^{-1}: 1650 (C=O). NMR ($CDCl_3$) δ: 1.06—1.82 (8H, m, $4\times CH_2$), 2.82 (1H, s, OH), 3.12—3.58 (8H, m, piperazine ring 5 and 6 CH_2,  –$(CH_2)_5$–CH_2OH), 3.70 (6H, s, $2\times OCH_3$), 4.49 (2H, s, ), 6.18—6.40 (2H, m, benzene ring 3 and 5 CH), 7.08 (1H, d, $J=9$ Hz, benzene ring 6 CH).

1-(6-Amino-1-hexyl)-4-(2,4-dimethoxybenzyl)-2,3-dioxopiperazine Hydrochloride (2m·HCl)——**2m·HCl** was similarly obtained by the method described in the previous paper,[3b] *i.e.*, Gabriel's primary amine synthetic method. mp 59—62°. IR ν_{max}^{KBr} cm^{-1}: 1650 (C=O). NMR ($CDCl_3$–DMSO-d_6) δ: 0.85—1.95 (8H, m, $4\times CH_2$), 2.65—3.02 (2H, m, $-(CH_2)_5CH_2NH_2$), 3.02—3.60 (6H, m, piperazine ring 5 and 6 CH_2 and ), 3.75 (6H, s, $2\times OCH_3$), 4.43 (2H, s, ), 6.29—6.70 (2H, m, benzene ring 3 and 5 CH), 7.05 (1H, d, $J=8.5$ Hz, benzene ring 6 CH), 7.70—8.65 (2H, bs, NH_2). *Anal.* Calcd for $C_{19}H_{30}ClN_3O_4$: C, 57.06; H, 7.56; N, 10.51. Found: C, 56.74; H, 7.80; N, 10.17.

1-(5-Carboxy-2,4-hexadien-1-yl)-4-(2,4-dimethoxybenzyl)-2,3-dioxopiperazine (2n)——NaH (purity 50%, 0.55 g) was added to a solution of **2a** (3.0 g) in DMF (30 ml) and after the mixture had been stirred for 1 hr at 50—60°, 1-bromo-2,2-diethoxyethane (2.9 g) was added dropwise at room temperature. After removal of the solvent *in vacuo*, the residue was dissolved in CH_2Cl_2 (50 ml) and the solution was washed with H_2O and dried over $MgSO_4$. After removal of the solvent under reduced pressure, 6 N HCl (50 ml) was added to the residue and the whole was stirred for 2 hr at 50—60°. The reaction mixture was extracted with AcOEt (50 ml), washed with satd. aq. NaCl and dried over $MgSO_4$. Removal of the solvent by evaporation gave 1-(2,4-dimethoxybenzyl)-4-formylmethyl-2,3-dioxopiperazine (2.1 g) as crystals. The crystals were added to a solution of 3-ethoxycarbonyl-2-buten-1-yltriphenylphosphonium bromide (3.0 g) and NaOMe (0.38 g) in abs. CH_2Cl_2 (30 ml) and the reaction mixture was refluxed for 4 hr. H_2O (30 ml) was added. The separated organic layer was washed with satd. aq. NaCl and dried over $MgSO_4$. After removal of the solvent, the residue was chromatographed on silica gel with $CHCl_3$ to give an oil. 6 N HCl (20 ml) was added to the oil and the whole was refluxed for 5 hr. The reaction mixture was adjusted to pH 8 with $NaHCO_3$ and washed with AcOEt (30 ml). The H_2O layer was adjusted to pH 1 with 6 N HCl and extracted with AcOEt (30 ml). The extract was washed with satd. aq. NaCl and dried over $MgSO_4$. After removal of the solvent, the residue was recrystallized from iso-PrOH. Yield 1.41 g (32%). mp 153—155°. IR ν_{max}^{KBr} cm^{-1}: 1670 (C=O). NMR ($CDCl_3$) δ: 1.93 (3H, s, CH_3), 3.43 (4H, bs, piperazine ring 5 and 6 CH_2), 3.77 (6H, s, $2\times OCH_3$), 4.00—4.45 (2H, m, ), 4.59 (2H, s, ), 5.45—6.20 (3H, m, $-CH=CH-CH=C(CH_3)-$), 6.20—6.65 (2H, m, benzene ring 3 and 5 CH), 7.23 (1H, d, $J=9$ Hz, benzene ring 6 CH), 9.04 (1H, bs, COOH). *Anal.* Calcd for $C_{20}H_{24}N_2O_6$: C, 61.85; H, 6.23; N, 7.21. Found: C, 61.62; H, 6.46; N, 6.81.

1-*n*-Hexyl-4-(4-methoxybenzyl)-2,3-dioxopiperazine (12b)——Compound **12b** was synthesized from 1-*n*-hexyl-2,3-dioxopiperazine and 4-methoxybenzyl chloride by method B shown in Chart 2. mp 137—138° (AcOEt). IR ν_{max}^{KBr} cm^{-1}: 1660 (C=O). *Anal.* Calcd for $C_{18}H_{26}N_2O_3$: C, 67.90; H, 8.23; N, 8.80. Found: C, 67.87; H, 8.27; N, 8.76.

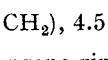
The following compounds were similarly obtained (Table VIII).

Table VIII

Compd. No.	mp (°C) (Recryst. solvent)	Formula	Analysis (%) Calcd (Found) C	H	N	NMR (solvent) δ (ppm)
12a	75 — 76 (AcOEt–iso-Pr_2O)	$C_{18}H_{26}N_2O_3$	67.90 (67.74	8.23 8.13	8.80 8.64)	($CDCl_3$) 0.85 (3H, t), 1.01—1.81 (8H, m), 3.21—3.61 (6H, m), 3.79 (3H, s), 4.63 (2H, s), 4.66—6.99 (2H, m), 7.04—7.41 (2H, m)
12c	72 — 74 (AcOEt–iso-Pr_2O)	$C_{19}H_{28}N_2O_4$	65.49 (65.57	8.10 7.99	8.04 8.03)	($CDCl_3$) 0.86 (3H, t), 1.04—1.84 (8H, m), 3.14—3.54 (6H, m), 3.80 (3H, s), 3.83 (3H, s), 4.67 (2H, s), 6.60—6.97 (3H, m)
12d	61 — 64 (AcOEt–iso-Pr_2O)	$C_{19}H_{28}N_2O_4$	65.49 (65.54	8.10 8.02	8.04 7.63)	($CDCl_3$) 0.86 (3H, m), 1.00—1.90 (8H, m), 3.30—3.50 (6H, m), 3.71 (3H, s), 3.76 (3H, s), 4.63 (2H, s), 6.76—6.91 (3H, m)

Compd. No.	mp (°C) (Recryst. solvent)	Formula	Analysis (%) Calcd (Found) C	H	N	NMR (solvent) δ (ppm)
12e	101 —104 (AcOEt–iso-Pr_2O)	$C_{19}H_{28}N_2O_4$	65.49 (65.24	8.10 8.02	8.04 7.78)	($CDCl_3$) 0.87 (3H, m), 1.00—1.70 (8H, m), 3.10—3.70 (6H, m), 3.79 (6H, s), 4.77 (2H, s), 6.50 (2H, d), 7.19 (1H, d)
12f	85 — 87 (AcOEt–iso-Pr_2O)	$C_{19}H_{28}N_2O_4$	65.49 (65.59	8.10 8.23	8.04 8.11)	($CDCl_3$) 0.85 (3H, m), 1.10—1.80 (8H, m), 3.20—3.60 (6H, m), 3.83 (6H, s), 4.55 (2H, s), 6.80 (3H, bs)
12h	111.5—113 (AcOEt–iso-Pr_2O)	$C_{20}H_{30}N_2O_5$	63.47 (63.31	7.99 7.83	7.40 7.20)	($CDCl_3$) 0.86 (3H, t), 1.03—1.81 (8H, m), 3.11—3.64 (6H, m), 3.83 (3H, s), 3.86 (3H, s), 3.88 (3H, s), 4.61 (2H, s), 6.64 (1H, d), 7.03 (1H, d)
12i	97 — 98 (AcOEt–iso-Pr_2O)	$C_{20}H_{30}N_2O_5$	63.47 (63.52	7.99 8.10	7.40 7.37)	($CDCl_3$) 0.86 (3H, m), 1.00—1.80 (8H, m), 3.38—3.64 (6H, m), 3.82 (9H, s), 4.58 (2H, s), 6.48 (2H, s)
12l	103 —105 (AcOEt)	$C_{17}H_{23}ClN_2O_2$	63.25 (63.40	7.18 7.32	8.68 8.86)	($CDCl_3$) 0.88 (3H, m), 1.05—1.85 (8H, m), 3.31—3.65 (6H, m), 4.62 (2H, s), 7.25 (4H, s)
12m	106 —108 (EtOH)	$C_{17}H_{22}Cl_2N_2O_2$	57.15 (57.09	6.21 6.27	7.84 7.87)	($CDCl_3$) 0.85 (3H, m), 1.05—1.85 (8H, m), 3.32—3.60 (6H, m), 4.72 (2H, s), 7.10—7.45 (3H, m)
12p	Oil					($CDCl_3$) 1.04 (6H, t), 0.71—1.82 (11H, m), 3.30 (4H, q), 3.39—3.95 (6H, m), 6.63—7.11 (4H, m)
12q·HCl	188 —190 (MeOH)	$C_{22}H_{36}ClN_3O_2$	62.24 (63.74	9.40 9.06	10.89 10.09)	(DMSO-d_6) 0.85 (3H, m), 1.01—1.83 (8H, m), 1.12 (6H, t), 3.21—3.68 (10H, m), 4.62 (2H, s), 4.48 (2H, s), 6.50 (2H, d), 7.10 (2H, d)

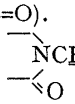
1-(4-Diethylaminobenzyl)-4-*n*-hexyl-2,3-dioxopiperazine (12n)——4-Diethylaminobenzaldehyde (22.2 g) and N-acetylethylenediamine (12.8 g) in benzene (130 ml) were refluxed azeotropically for 4 hr. After removal of the solvent *in vacuo*, the residue was dissolved in MeOH (160 ml) and $NaBH_4$ (6.2 g) was added in portions under ice-cooling. The mixture was stirred for 3 hr under ice-cooling, then the solvent was removed. The residual oil in $CHCl_3$ (100 ml) was washed with H_2O and dried over $MgSO_4$. After removal of the solvent, the residual oil was taken up in 20% HCl (110 ml) and refluxed for 1 hr. The reaction mixture was made strongly basic with KOH and extracted with benzene (100 ml). The organic layer was dried over $MgSO_4$ and evaporated to dryness to give an oil, which was distilled to afford N-(4-diethylaminobenzyl)-ethylenediamine (**10n**) as a liquid of 165—176° (2—3 mmHg). Yield 26.8 g (96.6%). Solutions of **10n** (26.8 g) in EtOH (20 ml) and of diethyl oxalate (17.7 g) in EtOH (20 ml) were added dropwise to EtOH (180 ml) over a period of 1 hr under ice-cooling and the whole was refluxed for 3 hr. Next, 80 ml of EtOH was removed and remaining reaction mixture was left to stand at room temperature. Separated crystals were collected and recrystallized from EtOH to give 1-(4-diethylaminobenzyl)-2,3-dioxopiperazine (**11n**) as colorless crystals of mp 214.5—215.5° (18.9 g, 56.7%). IR ν_{max}^{KBr} cm^{-1}: 3180, 3125 (NH), 1650 (C=O). *Anal.* Calcd for $C_{15}H_{21}N_3O_2$: C, 65.43; H, 7.69; N, 15.26. Found: C, 65.46; H, 7.61; N, 15.22.

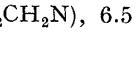
N-Hexylation of **11n** by the usual method gave **12n**. mp 73—75° (AcOEt-iso-Pr_2O). IR ν_{max}^{KBr} cm^{-1}: 1650 (C=O). NMR ($CDCl_3$) δ: 0.86 (3H, m, CH_3), 1.00—1.80 (8H, m, $4\times CH_2$), 1.14 (6H, t, $J=7.2$ Hz, $2\times CH_3$), 3.10—3.60 (10H, m, $5\times CH_2$), 4.51 (2H, s, ), 6.56 (2H, d, $J=9$ Hz, benzene ring 2 and 6 CH), 7.08 (2H, d, $J=9$ Hz, benzene ring 3 and 5 CH). **12n** was taken up in HCl–EtOH and recrystallized from EtOH to give **12n·HCl** of mp 194—195°. *Anal.* Calcd for $C_{21}H_{33}N_3O_2\cdot HCl$: C, 63.70; H, 8.67; N, 10.61. Found: C, 63.93; H, 8.86; N, 10.90.

The following compounds were similarly obtained. **12g**: mp 91—92° (AcOEt–iso-Pr_2O). NMR ($CDCl_3$)

δ: 0.86 (3H, t), 1.00—1.69 (8H, m), 3.00—3.56 (6H, m), 3.73 (6H, s), 3.76 (3H, s), 4.64 (2H, s), 6.04 (2H, s). *Anal.* Calcd for $C_{20}H_{30}N_2O_5$: C, 63.47; H, 7.99; N, 7.40. Found: C, 63.31; H, 8.00; N, 7.31. **12o**: oil. NMR ($CDCl_3$) δ: 0.85 (3H, m), 1.11 (12H, t), 1.00—1.70 (8H, m), 2.90 (8H, q), 3.20—3.60 (6H, m), 4.67 (2H, s), 6.36 (2H, m), 7.00 (1H, m).

1-(2,4-Dihydroxybenzyl)-4-*n*-hexyl-2,3-dioxopiperazine (12k)——Compound **2e** (2.0 g) in abs. CH_2Cl_2 (20 ml) was treated with BBr_3 (4.32 g) at -20 to $-30°$ and the solution was stirred for 1 hr at room temperature. MeOH (5 ml) was added to the reaction mixture and the solvent was evaporated off. The residue was dissolved in AcOEt (30 ml) and the solution was washed with satd. aq. NaCl and dried over $MgSO_4$. After removal of the solvent *in vacuo*, the residue was recrystallized from AcOEt. Yield 0.77 g (42%). mp 131—133°. IR ν_{max}^{KBr} cm^{-1}: 1620 (C=O). *Anal.* Calcd for $C_{17}H_{24}N_2O_4$: C, 63.73; H, 7.55; N, 8.74. Found: C, 63.64; H, 7.54; N, 8.62. Compound **12j** was similarly obtained. mp 82—87° ($CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1653 (C=O). NMR (DMSO-d_6) δ: 0.86 (3H, t), 1.02—1.79 (8H, m), 3.09—3.69 (6H, m), 4.47 (2H, s), 6.71 (2H, d), 7.11 (2H, d), 9.34 (1H, s). *Anal.* Calcd for $C_{17}H_{24}N_2O_3$: C, 67.08; H, 7.95; N, 9.20. Found: C, 66.96; H, 8.12; N, 9.16.

1-(4-Diethylaminophenyl)-4-*n*-hexyl-2,3-dioxopiperazine (14a)——1-*n*-Hexyl-2,3-dioxopiperazine (1.7 g), 4-diethylaminoiodobenzene (3.1 g), K_2CO_3 (1.5 g), and activated Cu[9] (as a catalyst) were added to DMF (20 ml) and the mixture was refluxed for 5 hr. H_2O (50 ml) was added and the reaction mixture was extracted with AcOEt. The organic layer was washed with satd. aq. NaCl and dried over $MgSO_4$. After removal of the solvent *in vacuo*, the residue was washed with iso-Pr_2O and recrystallized from AcOEt. Yield 1.5 g (50.7%). mp 114—115.5°. IR ν_{max}^{KBr} cm^{-1}: 1680 (C=O). NMR ($CDCl_3$) δ: 3.32 (4H, q, J=7 Hz, 2×CH_2), 3.39—3.97 (6H, m, piperazine ring 5 and 6 CH_2 and ), 6.56 (2H, d, J=9 Hz, benzene ring 3 and 5 CH), 7.09 (2H, d, J=9 Hz, benzene ring 2 and 6 CH). *Anal.* Calcd for $C_{20}H_{31}N_3O_2$: C, 69.53; H, 9.05; N, 12.16. Found: C, 69.43; H, 9.02; N, 12.28.

1-[2-(4-Diethylaminophenyl)ethyl]-4-*n*-hexyl-2,3-dioxopiperazine (14c)——Compound **14c** was obtained by a method similar to that used to prepare **12b**. mp 201—204° (iso-PrOH) (**14c·HCl**). IR ν_{max}^{KBr} cm^{-1}: 1660 (C=O). NMR ($CDCl_3$) δ: 0.88 (3H, m, CH_3), 1.13 (6H, t, J=7 Hz, 2×CH_3), 1.10—1.80 (8H, m, 4×CH_2), 2.79 (2H, t, J=7 Hz, CH_2), 3.31 (4H, q, J=7 Hz, 2×$CH_3C\underline{H}_2$–), 3.20—3.50 (6H, m, piperazine ring 5 and 6 CH_2 and ), 3.36 (2H, t, J=7 Hz, N–  –$C\underline{H}_2CH_2N$), 6.57 (2H, d, J=9 Hz, benzene ring 3 and 5 CH), 7.01 (2H, d, J=9 Hz, benzene ring 2 and 6 CH). *Anal.* Calcd for $C_{22}H_{35}N_3O_2$·HCl: C, 64.45; H, 8.85; N, 10.25. Found: C, 64.23; H, 8.83; N, 10.03.

Compound **14b** was similarly obtained. mp 115—116.5° (AcOEt–iso-Pr_2O). IR ν_{max}^{KBr} cm^{-1}: 1680, 1650 (C=O). NMR ($CDCl_3$) δ: 0.89 (3H, m), 1.16 (6H, t), 3.40 (4H, q), 3.46 (2H, t), 3.80 (4H, m), 6.72 (2H, d), 7.59 (2H, d). *Anal.* Calcd for $C_{21}H_{31}N_3O_3$: C, 67.53; H, 8.37; N, 11.25. Found: C, 67.33; H, 8.32; N, 11.12.

1-[2-(4-Diethylaminophenyl)vinyl]-4-*n*-hexyl-2,3-dioxopiperazine (14d)——1-*n*-Hexyl-2,3-dioxopiperazine (10.0 g) in 37% formalin (50 ml) was refluxed for 30 min. The reaction mixture was evaporated to dryness and the residue was extracted with MeOH. After removal of the solvent, the residual oil was added to $SOCl_2$ (30 ml) and the mixture was refluxed for 30 min. The residue obtained by removal of excess $SOCl_2$ was dissolved in benzene (100 ml) and a solution of Ph_3P (13.2 g) in benzene (30 ml) was added to the above solution, then the whole was refluxed for 3 hr. The separated oil was isolated by decantation and washed with iso-Pr_2O to afford (4-*n*-hexyl-2,3-dioxo-1-piperazinyl)methyl triphenylphosphonium chloride (**17**) as hygroscopic crystals (11.0 g, 43%). NaOMe (1.9 g) was added to **17** (8.6 g) in CH_2Cl_2 (100 ml) at $-10°$. The mixture was stirred at $-10°$ for 10 min, then a solution of 4-diethylaminobenzaldehyde (3.0 g) in CH_2Cl_2 (10 ml) was added and the whole was refluxed for 2 hr. The reaction mixture was extractde with 1 N HCl (30 ml). The extract was neutralized with $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with H_2O and dried over $MgSO_4$. After removal of the solvent, the residue was chromatographed on silica gel to give **14d** as an oil (2.82 g, 45%). IR ν_{max}^{neat} cm^{-1}: 1660 (C=O). NMR ($CDCl_3$) δ: 0.87 (3H, m, CH_3), 1.25 (6H, t, J=6.5 Hz, 2×$C\underline{H}_3CH_2N$–), 1.10—1.80 (8H, m, 4×CH_2), 3.32 (4H, q, J=6.5 Hz, 2×$CH_3C\underline{H}_2N$), 3.37—3.70 (6H, m, piperazine ring 5 and 6 CH_2 and ), 6.05 (1H, d, J=9.5 Hz,  –CH=C$\underline{H}$N), 6.60 (2H, m, benzene ring 3 and 5 CH), 6.69 (1H, d, J=9.5 Hz, –  –C$\underline{H}$=CH), 7.37 (2H, m, benzene ring 2 and 6 CH).

1-(4-Diethylaminobenzyl)-4-(2-pyridylamino)-2,3-dioxopiperazine (18k)——Compound **18k** was obtained by a method similar to that described for **12n**. mp 95° (AcOEt–iso-Pr_2O). IR ν_{max}^{KBr} cm^{-1}: 1670 (C=O). NMR ($CDCl_3$) δ: 1.14 (6H, t, J=7 Hz, 2×CH_3), 2.70—3.00 (8H, m, 4×CH_2), 4.52 (2H, s, CH_2), 4.72 (2H, s, CH_2), 6.57 (2H, d, J=8 Hz, benzene ring 2×CH), 6.90—7.45 (4H, m, benzene ring 2×CH and pyridine ring 2×CH), 7.65 (1H, m, pyridine ring CH), 8.45 (1H, m, pyridine ring CH). *Anal.* Calcd for $C_{21}H_{26}N_4O_2$: C, 68.83; H, 7.15; N, 15.29. Found: C, 68.64; H, 7.16; N, 15.15.

The following compounds were similarly obtained (Table IX).

Table IX

Compd. No.	mp°C (Recryst. solvent)	Formula	Analysis (%) Calcd (Found) C	H	N	NMR (solvent) δ (ppm)
18b	134—135 (AcOEt–iso-Pr_2O)	$C_{17}H_{25}N_3O_2$	67.30 (67.56	8.31 8.45	13.85 13.69)	($CDCl_3$) 1.15 (9H, t), 3.32 (4H, q), 3.40 (4H, s), 3.46 (2H, q), 4.50 (2H, s), 6.55 (2H, d), 7.07 (2H, d)
18c	159—161 (AcOEt)	$C_{21}H_{31}N_3O_2$	70.55 (70.57	8.74 8.64	11.75 11.92)	($CDCl_3$) 1.14 (6H, t), 1.24—1.94 (10H, m), 3.00—3.79 (8H, m), 4.16—4.66 (1H, m), 4.55 (2H, s), 6.59 (2H, d), 7.13 (2H, d)
18d	Oil					($CDCl_3$) 1.14 (6H, t), 1.76 (3H, d), 3.10—3.50 (8H, m), 4.01 (2H, d), 4.50 (2H, s), 5.00—6.30 (4H, m), 6.53 (2H, d), 7.05 (2H, d)
18h	160—161 (EtOH)	$C_{21}H_{27}N_5O_4$	61.00 (61.15	6.58 6.63	16.94 16.79)	($CDCl_3$) 1.14 (6H, t), 3.30 (4H, q), 3.30—3.67 (2H, m), 3.90 (6H, s), 4.15—4.40 (2H, m), 4.57 (2H, s), 6.56 (2H, d), 7.12 (2H, d), 7.37 (1H, s)
18j	150—152 (iso-PrOH)	$C_{21}H_{26}N_4O_2$	68.83 (68.81	7.15 7.17	15.29 15.30)	($CDCl_3$) 1.13 (6H, t), 3.31 (4H, q), 3.35 (4H, bs), 4.50 (2H, s), 4.61 (2H, s), 6.53 (2H, d), 7.05 (2H, d), 7.24 (1H, m), 7.63 (1H, m), 8.45 (2H, m)

1-(2-Aminoethyl)-4-(diethylaminobenzyl)-2,3-dioxopiperazine Hydrochloride (18e·2HCl)——**18e·HCl** was obtained by the method described in the previous paper,[3b] *i.e.*, Gabriel's primary amine synthetic method. mp 225—226° (EtOH). IR ν_{max}^{KBr} cm^{-1}: 1665 (C=O). *Anal.* Calcd for $C_{17}H_{26}N_4O_2 \cdot 2HCl$: C, 52.18; H, 7.21; N, 14.32. Found: C, 52.02; H, 7.19; N, 14.30.

The following compounds were similarly obtained. **18f·HCl**: mp 174—175° (EtOH). NMR (DMSO-d_6) δ: 0.85—1.87 (8H, m), 2.71—3.15 (2H, m), 3.15—3.70 (10H, m), 4.30 (2H, s), 6.51 (2H, d), 7.08 (2H, d). *Anal.* Calcd for $C_{19}H_{30}N_4O_2 \cdot 2HCl$: C, 54.41; H, 7.69; N, 13.36. Found: C, 54.18; H, 7.62; N, 13.22. (**18g**): amorphous solid. NMR ($CDCl_3$) δ: 0.88—1.90 (14H, m), 2.60—3.10 (2H, m), 3.10—3.62 (10H, m), 4.33 (2H, s), 6.54 (2H, d), 7.10 (2H, d). *Anal.* Calcd for $C_{21}H_{34}N_4O_2$: C, 67.34; H, 9.15; N, 14.96. Found: C, 67.25; H, 9.13; N, 14.62.

1-(4-Diethylaminobenzyl)-4-(2,6-dihydroxy-4-pyrimidinyl)-2,3-dioxopiperazine (18i)——A solution of **18h** (1.0 g) in abs. CH_2Cl_2 (20 ml) was added dropwise to a solution of BBr_3 (0.6 g) in abs. CH_2Cl_2 (20 ml) at −30° over a period of 10 min. After being stirred at room temperature for 2 hr and refluxed for 30 min, the reaction mixture was cooled to 0—5°. MeOH (30 ml) was added under ice-cooling and the reaction mixture was stirred for 30 min at the same temperature. After removal of the solvent, $CHCl_3$ (30 ml) and H_2O (30 ml) were added to the residue. The H_2O layer was neutralized with satd. aq. $NaHCO_3$ and extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and evaporated to dryness. The residue was chromatographed on silica gel with EtOH–$CHCl_3$ (1: 5) to give **18i** (0.2 g, 21.5%) of mp>270°. IR ν_{max}^{KBr} cm^{-1}: 1685, 1655 (C=O). NMR ($CDCl_3$) δ: 1.11 (6H, t, J=7.5 Hz, 2×CH_3), 3.22 (4H, q, J=7.5 Hz, 2×CH_2), 3.40—3.70 (2H, m, piperazine ring CH_2), 4.10—4.40 (2H, m, piperazine ring CH_2), 4.96 (2H, s, CH_2), 6.49 (2H, d, J=8.5 Hz, benzene ring 3 and 5 CH), 7.00 (1H, s, pyrimidine ring CH), 7.20 (2H, d, J=8.5 Hz, benzene ring 2 and 6 CH), 8.14 (1H, bs, OH), 8.71 (1H, bs, OH). *Anal.* Calcd for $C_{19}H_{23}N_5O_4$: C, 59.21; H, 6.02; N, 18.17. Found: C, 59.02; H, 5.98; N, 19.97.

The Metabolism of 12n in Mice——Compound **12n** (50 mg/kg) was suspended in 0.3% CMC saline and intraperitoneally administered to 6-week-old ICR female mice in which the urethral meatus was closed. Six groups, consisting of 2 mice per group, were used. At 5, 15, 30, 60, and 120 min after the administration, mice were decapitated and the blood, peritoneal cavity, liver, and urine were treated as follows. $CHCl_3$ (2 ml) and 10% $NaHCO_3$ were added to 0.4 ml of the blood. After centrifugation at 3000 rpm for 15 min, the $CHCl_3$ layer was evaporated to dryness and the residue was subjected to thin-layer chromatography with $CHCl_3$–MeOH (15: 1). In the case of the peritoneal cavity, after being washed with H_2O (5 ml), it was treated by the same method as the blood. In the case of the liver, H_2O (3 ml) was added and the mixture was homogenized. The procedure was the same as that for the blood. Urine was taken from the bladder and extracted with 3 ml of $CHCl_3$. The $CHCl_3$ layer was evaporated to dryness and the residue was tested by

thin-layer chromatography on silica gel with $CHCl_3$–MeOH (15: 1). One group was treated in the same way without the administration of test compounds; this was used as a control group.

Separation of the Metabolites of 12n——The first group of Wistar strain rats, consisting of 7 rats, received **12n** (**10** mg/ml/head) suspended in 0.3% carboxymethyl cellulose (CMC) saline intraperitoneally 3 times at 3 hr intervals and urine and feces were collected at 17 hr after administration. The second group, consisting of 14 Wistar rats, received **12n** (20 mg/ml/head) suspended in 0.3% CMC saline intraperitoneally at 4 hr intervals and urine and feces were collected at 17 hr after administration. The third group, consisting of 15 rats, received **12n** (10 mg/ml/head) suspended in 0.3% CMC saline intraperitoneally at 4 hr intervals and urine and feces were collected at 17 hr after administration. The above urine and feces of groups 1—3 were combined and H_2O was added. They were adjusted to pH 8.0 with 10% $NaHCO_3$ and extracted with $CHCl_3$. The $CHCl_3$ layer was dried over $MgSO_4$ and the solvent was removed. The residue was chromatographed on silica gel. **19** was eluted with $CHCl_3$ and **20** and **21** (52 mg) were eluted with $CHCl_3$–MeOH (50: 1). The physical properties of these products were as follows: **19**; mp 109—110°. IR ν_{max}^{KBr} cm^{-1}: 3340 (NH), 1660 (C=O). **20**; mp 99—101°. IR ν_{max}^{KBr} cm^{-1}: 3345, 3420 (NH), 1665 (C=O). **21**; mp 178—179° (AcOEt). IR ν_{max}^{KBr} cm^{-1}: 1660 (C=O). NMR ($CDCl_3$) δ: 0.84 (3H, m, CH_3), 1.06—1.75 (8H, m, $4\times CH_2$), 2.16 (3H, s, $COCH_3$), 3.16—3.56 (6H, m, piperazine ring 5 and 6 CH_2 and $\underline{NCH_2}$–), 4.49 (2H, s, CH_2), 6.99 (2H, d, $J=9$ Hz, benzene ring $2\times CH$), 7.39 (2H, d, $J=9$ Hz, benzene ring $2\times CH$), 8.86 (1H, bs, NH).

1-(4-Aminobenzyl)-4-*n*-hexyl-2,3-dioxopiperazine (20)——1-*n*-Hexyl-4-(4-nitrobenzyl)-2,3-dioxopiperazine (4.5 g), obtained from 4-nitrobenzyl bromide and 1-*n*-hexyl-2,3-dioxopiperazine by the method described for **12b**, was suspended in 50% H_2O–EtOH (150 ml) and Zn powder (22.5 g) was added. A solution of $CaCl_2$ (4.5 g) in H_2O (5 ml) was added to the mixture and the whole was refluxed for 2 hr. The reaction mixture was filtered off and the filtrate was evaporated to dryness *in vacuo*. The residue was extracted with AcOEt (50 ml) and the extract was washed with H_2O and dried over $MgSO_4$. Removal of the solvent gave a solid which was recrystallized from iso-PrOH. Yield 2.7 g (65.9%). mp 99—101.5°. The IR spectrum of the synthesized product **20** was identical with that of one of the metabolites of **12n**. *Anal.* Calcd for $C_{17}H_{25}N_3O_2$: C, 67.30; H, 8.31; N, 13.85. Found: C, 67.40; H, 8.36; N, 13.68. NMR ($CDCl_3$) δ: 0.86 (3H, m, CH_3), 1.00—1.82 (8H, m, $4\times CH_2$), 3.36 (6H, m, $3\times CH_2$), 3.57—3.92 (2H, m, NH_2), 4.48 (2H, s, CH_2), 6.54 (2H, d, $J=8$ Hz, benzene ring $2\times CH$), 6.99 (2H, d, $J=8$ Hz, benzene ring $2\times CH$).

1-(4-Ethylaminobenzyl)-4-*n*-hexyl-2,3-dioxopiperazine (19)——A suspension of **20** (2.7 g), EtI (1.1 ml), and Na_2CO_3 (1.4 g) in H_2O (30 ml) and EtOH (50 ml) was refluxed for 5 hr, then evaporated to dryness *in vacuo*. The residue was extracted with $CHCl_3$ (50 ml) and the extract was dried over $MgSO_4$. Removal of the solvent left a solid, which was chromatographed on silica gel with benzene–AcOEt (3: 1), and then recrystallized from AcOEt–iso-Pr_2O to give colorless crystals of mp 109—110.5°. Yield 1.5 g (52%). The IR spectrum was identical with that of the metabolite **19**. *Anal.* Calcd for $C_{19}H_{29}N_3O_2$: C, 68.85; H, 8.82; N, 12.68. Found: C, 68.82; H, 8.96; N, 12.62.

1-(4-Acetylaminobenzyl)-4-*n*-hexyl-2,3-dioxopiperazine (21)——Ac_2O (0.56 ml) was added to a solution of **20** (1.5 g) in MeOH (30 ml). The mixture was stirred for 3 hr at room temperature, then the solvent was evaporated off under reduced pressure and the residue was recrystallized from iso-PrOH–iso-Pr_2O to give **21** (1.0 g, 59%). mp 178—179°. The IR and NMR spectra of the product were identical with those of one of the metabolites of **12n**. *Anal.* Calcd for $C_{19}H_{27}N_3O_3$: C, 66.06; H, 7.88; N, 12.16. Found: C, 66.20; H, 7.95; N, 12.03.

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