(24) E. A. Bauer, A. Z. Eisen, and J. J. Jeffrey, *Biochim. Biophys. Acta*, **206**, 152 (1970).
(25) L. L. Espey and P. Rondell, *J. Appl. Physiol.*, **23**, 757 (1967).
(26) G. Gries, H. Buresch, and L. Strauch, *Experientia*, **26**, 31 (1970).
(27) H. G. Heidrich, D. Prokopova, and K. Hannig, *Hoppe-Seyler's Z. Physiol. Chem.*, **350**, 1430 (1969).
(28) E. Koren and S. Milkovic, *J. Reprod. Fertil.*, **32**, 349 (1973).
(29) G. A. Levidikova and V. N. Orekhovich, *Clin. Chim. Acta*, **42**, 205 (1972).
(30) P. B. Robertson, R. B. Ryel, R. E. Taylor, and K. W. Shyu, *Science*, **177**, 64 (1972).
(31) L. Strauch, H. Vencelj, and K. Hannig, *Hoppe-Seyler's Z. Physiol. Chem.*, **349**, 171 (1968).
(32) L. Strauch and H. Vencelj, *Hoppe-Seyler's Z. Physiol. Chem.*, **348**, 465 (1967).
(33) E. Wojtecka-Lukasik, *Reumatologia*, **9**, 194 (1971).
(34) *J. Biol. Chem.*, **247**, 977 (1972).
(35) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Am. Chem. Soc.*, **86**, 1839 (1964).
(36) C. Ressler and V. du Vigneaud, *J. Am. Chem. Soc.*, **76**, 3103 (1954).
(37) W. Grassmann and E. Wünsch, *Chem. Ber.*, **91**, 449 (1958).
(38) L. Bernardi, G. Bosisio, R. DeCastiglione, O. Goffredo, and F. Chillami, *Gazz. Chim. Ital.*, **94**, 853 (1964).
(39) J. L. Everett and W. C. J. Ross, *J. Chem. Soc.*, 1972 (1949).
(40) M. Szekerke, R. Wade, and F. Bergel, *J. Chem. Soc.*, 1907 (1965).

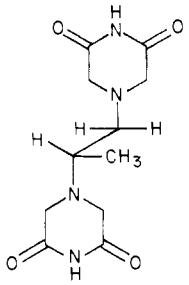
Stereoselective Effects of *cis*- and *trans*-Cyclopropylbis(dioxopiperazines) Related to ICRF-159 on Metastases of a Hamster Lung Adenocarcinoma[1]

Donald T. Witiak,* Hee J. Lee, H. Duane Goldman, and Bruce S. Zwilling

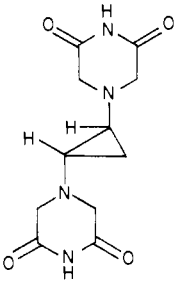
Division of Medicinal Chemistry, College of Pharmacy, and Department of Microbiology, College of Biological Sciences and Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio 43210. *Received June 16, 1978*

The synthesis for *cis*-4,4'-(1,2-cyclopropanediyl)bis(2,6-piperazinedione) (*cis*-**3**) is discussed. Stereoselective effects on metastases of *cis*-**3** and the previously reported *trans*-**2** isomer were compared to conformationally mobile ICRF-159 using a Syrian hamster lung adenocarcinoma (LG1002). Whereas ICRF-159 and *cis*-**3** significantly inhibited lung metastases, the *trans*-**2** isomer significantly increased the number of metastatic nodules in the lung. Thus, these studies have revealed that, at least in one tumor model, antimetastatic activity can be separated from metastatic potentiating activity by controlling drug geometry.

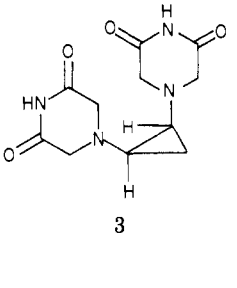
A previous report from these laboratories described the comparative effects of ICRF-159 (**1**), the *trans*-cyclopropyl



1



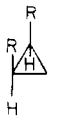
2



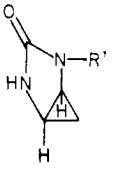
3

analogue **2**, and certain related tetraacids and esters on cytotoxicity, mutagenicity, and scheduled and unscheduled DNA synthesis in tissue culture.[2] However, perhaps of greater significance is the observation that ICRF-159 inhibits metastases in the Lewis lung tumor (3LL) animal model without impeding the growth of the primary implant.[3-13] Histological examination of blood, lungs, and primary tumors indicated that antimetastatic activity is likely due to normalization of the developing blood vessels at the invading margins of the primary tumors.[4,9] Whereas this angiometamorphic effect is not unique to 3LL infected animals,[8,14,15] histological features suggested that ICRF-159 antimetastatic effects in an experimental transplanted murine squamous carcinoma did not depend upon morphological changes in vascularity.[16] Although ICRF-159 does not reduce metastases in all tumor models,[17] it is particularly interesting to note the results of Lazo et al.[18] These investigators have observed that incubation of exponentially growing B16 melanoma cells with ICRF-159 significantly increased their in vivo lung colony-forming efficiency.[18] Concurrently, we have been investigating the antimetastatic effects of ICRF-159 and the *trans*- and *cis*-cyclopropyl analogues (**2** and **3**, respectively) in the allogeneic hamster lung adenocarcinoma model. A priori we discuss the synthesis of *cis*-**3** and our preliminary biological results revealing the stereoselective actions of **2** and **3** on metastases in this animal model.

Synthetic Aspects. The synthesis for *cis*-**3** from *cis*-1,2-cyclopropanedicarboxylic acid (**4**) is similar, but not



4, R = CO_2H
5, R = COCl
6, R = CON_3
7, R = NCO
8, R = $NHCO_2$-*t*-Bu



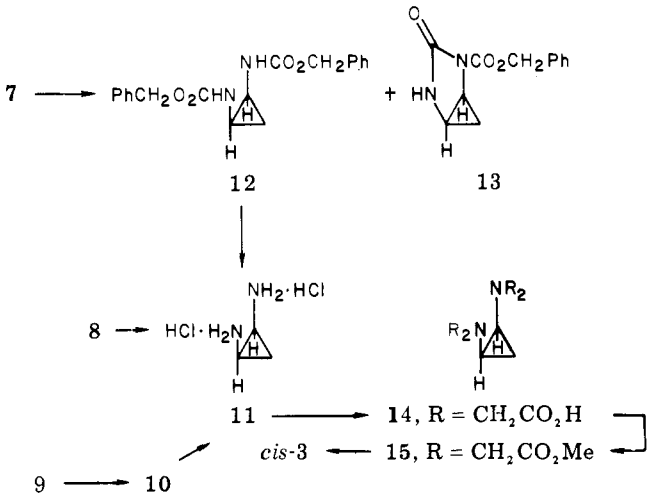
9, R' = CO_2-*t*-Bu
10, R' = H

identical, to the reported[2] preparation of *trans*-**2** from the corresponding *trans*-dicarboxylic acid. Starting *cis*-**4** was prepared according to the method of Payne[19a] and McCoy[19b] and readily converted to the diacid chloride **5** by treatment with PCl_5.[20] Reaction of **5** with NaN_3 in aqueous acetone afforded the white crystalline diazide, **6**, which underwent Curtius rearrangement upon heating in toluene affording crude diisocyanate **7**. Treatment of **7** with *tert*-butyl alcohol, unlike the *trans*-isocyanate,[2] gave less than 5% of the desired dicarbamate **8** and produced diazabicyclohexanes **9** and **10** as major products.

Dicarbamate **8** was rapidly hydrolyzed under acidic conditions to diamine **11**. Although carbamate **9** was easily converted to **10**, various attempts (both hydrolytic and reductive) to transform **10** to *cis*-diamine **11** failed. However, reaction of diisocyanate **7** with benzyl alcohol

0022-2623/78/1821-1194$01.00/0

gave a readily separable mixture (1:1 ratio) of the dibenzyl carbamate **12** and the bicyclic carbamate **13**. Hydrogenolysis of **12** over Pd/C in EtOH–AcOH generated **11** in high yield. Addition of *cis*-diamine **11** to an aqueous solution of bromoacetate,[21] under basic conditions, gave, upon acidification, crude tetraacid **14**, which was isolated as its tetramethyl ester **15** following treatment with 2,2-dimethoxypropane containing concentrated HCl. Saponification of **15** in methanolic NaOH gave tetraacid **14** in fair yield. Whereas attempts to cyclize *cis*-tetraester **15** under conditions similar to those used in the preparation of *trans*-**2** were unsuccessful,[2] treatment of **15** with NH_2CHO–NaH in DME[22,23] generated the desired bis(dioxopiperazine) **3** in modest yield.



Structure Confirmation. The 90-MHz proton resonance spectrum for cyclopropane ring protons in *trans*-**2**, *cis*-**3**, and their diamine dihydrochloride precursors confirmed the structural assignments. For *trans*-**2** and its diamine dihydrochloride precursor the expected AA'XX' pattern appeared as two deceptively simple triplets[24] having apparent J_{AX} = 6 Hz for *trans*-**2** and J_{AX} = 7 Hz for the *trans*-diamine dihydrochloride. For the respective cis isomers the proton resonance patterns were considerably more complex. The computer-simulated analysis for the ABX_2 spectrum attributable to the cyclopropane ring proton resonance signals for *cis*-**3** showed δ 0.56 for H_A, 0.81 for H_B, and 1.96 for H_{X_2} with J_{AB} = −5.7 Hz,[25] J_{AX_2} = 5.3 Hz, and J_{BX_2} = 7.7 Hz. Similarly, for the *cis*-diamine dihydrochloride precursor **11** the resonance signals were δ 1.27 for H_A, 1.52 for H_B, and 2.96 for H_{X_2} with J_{AB} = −8.65 Hz, J_{AX_2} = 5.82 Hz, and J_{BX_2} = 8.68 Hz. In both *cis*-**3** and *cis*-**11** the proton signal for H_A cis to the amino substituents appears at higher field than the geminal, trans H_B resonance signal. Interestingly, the $\Delta\delta$ (0.71) for the H_A resonance signals of the two cis isomers (**3** and **11**) is equal to the $\Delta\delta$ (0.71) for the H_B resonance signals in these compounds. Therefore, the $\Delta\delta$ for H_A vs. H_B in both compounds are identical (0.25 Hz). Although solvent effects (*cis*-**3** in Me_2SO-d_6 and **11** in D_2O) are expected to affect the chemical shifts for these proton resonances, the same relative downfield shift for both H_A and H_B in **11** when compared to *cis*-**3** likely is a reflection of the greater deshielding effect of the protonated diamino groups.

Biological Results. The effects of intraperitoneal administration of ICRF-159 (**1**) or the *trans*- and *cis*-cyclopropyl analogues (**2** and **3**, respectively) on metastases of bronchogenic adenocarcinoma (designated LG1002)[26] in inbred male Syrian golden hamsters are shown in Table I. The dose chosen (15 mg/kg) was previously observed to inhibit liver metastasis of a hamster lymphoma.[15] Thus, for these initial investigations no attempt was made to determine the optimal drug concentration nor the route of administration. Under conditions described in the Experimental Section both ICRF-159 (**1**) and *cis*-**3** significantly reduced the number of lung metastases when compared against the carboxymethylcellulose (CMC) vehicle control. Only *cis*-**3** showed a significant reduction in metastases when compared against the saline control. However, *trans*-**2** administration produced a significantly greater number of lesions than those found in the lungs of animals from the other treatment groups. Additionally, *trans*-**2** stimulated the growth of the primary tumor whereas ICRF-159 and *cis*-**3** had no effect on primary tumor growth. The tumor growth in the latter animals was not different than that observed in animals receiving saline. Tumors appeared 3 to 4 days earlier in the *trans*-**2** treated animals and grew to a larger size (>100 mm^2) by the time of excision on the 28th day.

Table I. Effect of ICRF-159 and Stereoisomeric Analogues **2** and **3** on Metastasis of a Hamster Lung Adenocarcinoma

treatment[a]	no. of animals	no. of metastases[b]
ICRF-159 (**1**)	10	174.9 ± 31.5[c]
cis-**3**	10	171.0 ± 26.7[d]
trans-**2**	10	264.8 ± 23.9[e]
CMC control	9	205 ± 24.4
saline control	9	211.6 ± 43.9

[a] Stock suspensions (100 mL) of drug (2.8 mg/mL) containing 3 drops of concentrated HCl and 5% carboxymethylcellulose. Solutions were warmed to 37 °C in a water bath prior to injection. Control solutions having no drug were utilized in a similar fashion. [b] Mean ± standard error. [c] $p < 0.005$ vs. CMC, ns vs. saline. [d] $p < 0.025$ vs. saline. [e] $p < 0.001$ vs. CMC, $p < 0.01$ vs. saline.

Discussion

To the best of our knowledge, these results are the first describing geometrical stereoselective control of metastases. Although Poggi et al.[27] have reported that (*R*)- and (*S*)-warfarin enantiomorphs show stereoselective antimetastatic properties, our studies have shown that one isomer (trans) stimulated and the other (cis) inhibited metastasis in the allogeneic hamster lung adenocarcinoma model. We are of the opinion that the results of this investigation are sufficiently encouraging to warrant further study of this family of compounds in several tumor systems with a view toward optimization of dose and route of administration and determination of the reasons why *trans*-**2** and *cis*-**3** show markedly different effects. Using Syrian hamster cells (V-79A) *trans*-**2** was considerably less mutagenic and cytotoxic than ICRF-159.[2] One wonders whether the potentiating effects of *trans*-**2** may be related to an effect on cell volume and glycosaminoglycan biosynthesis as proposed by Lazo et al.[18] for the effects of ICRF-159 on B16 melanoma cells, whereas *cis*-**3** may selectively cause normalization of developing blood vessels in the primary tumor and thus inhibits metastases by mechanisms proposed by Hellmann and his collaborators.[4,9]

Experimental Section

Chemistry. Melting points were determined in open, glass capillaries on a Thomas-Hoover apparatus and are not corrected. Spectra were recorded on either a Perkin-Elmer 257 or Beckman Model 4230 spectrophotometer and Varian A-60 or Bruker HX-90E spectrometer. GLC utilized a Hewlett-Packard 402 biomedical gas chromatograph and elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Analyses were within ±0.4% of the calculated values. Mass spectra were

determined using a Du Pont Model 21-491 instrument by direct probe insertion (EI mode, 70 eV).

***cis*-1,2-Cyclopropanedicarboxylic acid (4)** was prepared according to the method of Payne[19a] and purified by chromatography on silica gel 60 (70–250 mesh), eluting with EtOAc–toluene–HCO_2H (49:33:1). An analytical sample was prepared by recrystallization from nitromethane, mp 139–140 °C (lit.[19b] mp 139–142 °C).

***cis*-1,2-Cyclopropanedicarbonyl Dichloride (5).** Diacid *cis*-**4** (52.0 g, 0.40 mol) and PCl_5[20] (249.6 g, 1.20 mol) were mixed and warmed on a steam bath for 5 h. After the mixture was cooled, excess PCl_5 was filtered and washed with Et_2O. Fractional distillation of the filtrate afforded 61.2 g (91.6%) of dichloride **5**: bp 73–75 °C (1.5 mm); IR (neat) 1800 cm^{-1} (C=O); NMR ($CDCl_3$) δ 1.5–2.2 (m, 2, CH_2), 2.67–3.07 (m, 2, CH).

***cis*-1,2-Cyclopropanedicarbonyl Diazide (6).** Compound **5** (30.0 g, 0.18 mol) in acetone (60 mL) was added dropwise with stirring to a cooled (ice–salt bath) aqueous solution (100 mL) of NaN_3 (35.1 g, 0.54 mol). After complete addition, the reaction mixture was stirred at 0–5 °C for 2 h and then poured into ice–H_2O (400 mL). The aqueous phase was extracted with Et_2O (3 × 75 mL), and the combined organic layers were washed with cold H_2O (50 mL) and then dried ($MgSO_4$). Removal of volatiles under reduced pressure ($T \leq 35$ °C) gave an oil which crystallized upon trituration with hexane (200 mL) to afford 28.2 g (87%) of diazide **6**: mp 37–38 °C; IR (neat) 2140 (-N=N=N), 1720 cm^{-1} (C=O).

***cis*-1,2-Cyclopropane Diisocyanate (7).** Azide **6** (46.0 g, 0.25 mol) in Na-dried toluene (500 mL) was heated on a steam bath until N_2 evolution ceased (3 h). Removal of solvent under reduced pressure gave crude diisocyanate **7** (IR 2280 cm^{-1} for NCO) which was used in subsequent reactions without further purification.

Reaction of *cis*-Diisocyanate 7 with *tert*-Butyl Alcohol. Crude diisocyanate **7** (obtained from 46 g of diazide **6**) was refluxed in *t*-BuOH (500 mL) for 4 h, followed by removal of solvent under reduced pressure. The resulting residue was slurried in a small amount of ethyl acetate and filtered. Fractional crystallization of the precipitate from benzene gave 2-(2-methyl)propyl *cis*-3-oxo-2,4-diazabicyclo[3.1.0]hexane-2-carboxylate (**9**) [mp 123–124 °C; IR (KBr) 1770 cm^{-1} (C=O); NMR ($CDCl_3$) δ 0.33–1.05 (m, 2, CH_2), 1.56 [s, 9, $C(CH_3)_3$], 2.92–3.20 (m, 1, CHN), 3.53–3.85 [m, 1, $CHNCO_2C(CH_3)_3$], 7.35 (br s, 1, HN). Anal. ($C_9H_{14}N_2O_3$) C, H, N] and *cis*-2,4-diazabicyclo[3.1.0]hexan-3-one (**10**) [mp 204–205 °C (MeOH); IR (KBr) 1690 cm^{-1} (C=O); NMR (Me_2SO-d_6) δ 0.1–0.65 (m, 2, CH_2), 3.05 (q, 2, CHN), 6.93 (br s, 2, NH). Anal. ($C_4H_6N_2O$) C, H, N]. A single product could be obtained by refluxing the crude mixture in MeOH containing several drops of concentrated HCl for 2 h, followed by recrystallization from MeOH, generating 14.0 g (50%) of **10**.

Chromatography of the ethyl acetate filtrate on silica gel 60 using EtOAc–MeOH (19:1) as eluant gave 3.5 g (<5%) of di-*tert*-butyl *cis*-1,2-cyclopropanediylbis(carbamate) (**8**) which was recrystallized from benzene: mp 125–126 °C; IR (KBr) 3370 (NH), 1705 cm^{-1} (C=O); NMR ($CDCl_3$) δ 0.4–1.4 (m, 2, CH_2), 1.46 [s, 18, $C(CH_3)_3$], 2.5–2.8 (m, 2, CHN), 4.85 (br s, 2, HN). Anal. ($C_{13}H_{24}N_2O_4$) C, H, N.

***cis*-1,2-Diaminocyclopropane Dihydrochloride (11) from Dicarbamate 8.** Compound **8** (2.2 g, 8.0 mmol) was refluxed in MeOH (30 mL) containing concentrated HCl (3 mL) for 1 h. After removal of volatiles under reduced pressure, the resulting residue was recrystallized from MeOH–Et_2O to give 1.03 g (89%) of **11**: mp >220 °C dec; IR (KBr) 3000–2400 cm^{-1} (br, $^+NH_3$); NMR (D_2O[28]) δ 1.18–1.83 (m, 2, CH_2), 3.01 (q, 2, CHN). Anal. ($C_3H_{10}N_2Cl_2$) C, H, N.

***cis*-1,2-Diaminocyclopropane Dihydrochloride (11) from Dicarbamate 12.** Compound **12** (11.3 g, 0.033 mol) was dissolved in absolute EtOH (130 mL) containing AcOH (12 mL) and hydrogenated (40 psi, room temperature) in the presence of 10% Pd/C (1.0 g) for 2 h. After removal of catalyst, the solution was poured into ethereal HCl, cooled, and stirred for 0.5 h. The resulting precipitate was collected and washed thoroughly with absolute EtOH–Et_2O (1:4) to give 4.5 g (93.7%) of **11** which was used without further purification in subsequent reactions. An analytical sample could be prepared as previously described.

Dibenzyl *cis*-1,2-Cyclopropanediylbis(carbamate) (12). Crude diisocyanate **7** (from 38.5 g, 0.213 mol, of diazide **6**) was heated to 100 °C in benzyl alcohol (600 mL) for 2 h (IR absorption at 2280 cm^{-1} was absent) and then allowed to come to room temperature overnight. After filtering, the resulting precipitate was slurried in MeOH and filtered. Recrystallization from $CHCl_3$–hexane gave 30.2 g (41.8%) of dicarbamate **12**: mp 167–168 °C; IR (KBr) 3320 (NH), 1695 cm^{-1} (C=O); NMR ($CDCl_3$) δ 0.5–1.5 (m, 2, CH_2), 2.55–2.85 (m, 2, CHN), 5.10 (s, 4, $PhCH_2$), 7.35 (s, 10, aromatic). Anal. ($C_{19}H_{20}N_2O_4$) C, H, N.

Benzyl *cis*-3-Oxo-2,4-Diazabicyclo[3.1.0]hexane-2-carboxylate (13). Concentration of the filtrate and MeOH washings from the isolation of crude carbamate **12** afforded a residue which crystallized from $CHCl_3$–hexane to give 21 g (42.7%) of pure **13**: mp 102–103 °C; IR (KBr) 1770 cm^{-1} (C=O); NMR ($CDCl_3$) δ 0.4–1.0 (m, 2, CH_2), 2.9–3.23 (m, 1, CHN), 3.6–3.9 (m, 1, CHN), 5.30 (s, 2, $PhCH_2$), 7.38 (s, 5, aromatic). Anal. ($C_{12}H_{12}N_2O_3$) C, H, N.

***cis*-2,2′,2″,2‴-(1,2-Cyclopropanediyldinitrilo)tetrakis(acetic acid) (14).** Tetraester **15** (0.9 g, 2.5 mmol) was stirred overnight at 45–50 °C in a mixture of MeOH (20 mL) and 1 N aqueous NaOH (40 mL). After cooling, the pH was adjusted to 2–3 using dilute aqueous HCl. The volatiles were removed under reduced pressure and the solids were redissolved in H_2O (15 mL). The pH of this solution was adjusted to 1.5 with concentrated HCl, and the solution was kept at 4 °C for 3 days. The resulting crystals were collected and washed with cold H_2O, generating 0.48 g (63%) of **14**. Recrystallization from MeOH–H_2O (4:1) afforded transparent crystals: mp 166–168 °C dec; IR (KBr) 1750, 1700 cm^{-1} (CO_2H); NMR (D_2O[28]–NaOH, 90 MHz) δ 0.57–0.88 (m, 2, CH_2), 2.00 (q, 2, CHN), 3.62 (s, 8, CH_2). Anal. ($C_{11}H_{16}N_2O_8$) C, H, N.

Tetramethyl *cis*-2,2′,2″,2‴-(1,2-Cyclopropanediyldinitrilo)tetrakis(acetate) (15). Bromoacetic acid (8.34 g, 0.06 mol)[21] was dissolved in H_2O (16 mL), cooled in an ice bath, and neutralized (pH 7–8) by the dropwise addition of 6 N NaOH. To this solution (under N_2) was added **11** (1.45 g, 0.01 mol) in small portions. The solution was kept at pH 7–8 by concurrent dropwise addition of 6 N NaOH. After complete addition, the temperature was raised to 45–50 °C and the pH was adjusted and maintained at 10.5–11.5 by the addition of 6 N NaOH. After 2 h, the consumption of alkali decreased and only small amounts were required over the next 5 h. After stirring overnight at room temperature, the reaction mixture was cooled and acidified to pH 1.5 with concentrated HCl. Volatiles were removed under reduced pressure and the resulting residue was stirred overnight at room temperature in 2,2-dimethoxypropane (250 mL) containing concentrated HCl (10 mL). Upon removal of solvent under reduced pressure, the residue was dissolved in ice–H_2O and extracted with Et_2O (3 × 50 mL). The aqueous phase was made basic with Na_2CO_3 and extracted with Et_2O (3 × 50 mL). The organic layers were combined, dried ($MgSO_4$), and concentrated to afford 2.85 g (80%) of an oily product, **15**, which was used without further purification. Distillation gave an analytical sample: bp 120–130 °C (0.05 mm); IR (neat) 1740 cm^{-1} (ester); NMR ($CDCl_3$) δ 0.48–1.00 (m, 2, CH_2), 2.51 (q, 2, CHN), 3.68 (s, 20, CH_2 and CH_3). Anal. ($C_{15}H_{24}N_2O_8$) C, H, N.

***cis*-4,4′-(1,2-Cyclopropanediyl)bis(2,6-piperazinedione) (3).** To 57% NaH (1.48 g) dispersion in mineral oil in DME (50 mL, dried over $LiAlH_4$), heated to 95 °C under N_2, was added, dropwise with stirring, a mixture of **15** (2.78 g, 7.7 mmol) and formamide (1.4 g, 31 mmol) in DME (30 mL).[22,23] After complete addition, the reaction mixture was refluxed for 2 h, followed by removal of volatiles under reduced pressure. The resulting paste was suspended in Et_2O and ice–H_2O (50 mL) was slowly added with cooling. The aqueous phase was filtered, acidified (pH 4) with aqueous HCl, and cooled (4 °C) overnight to give a crude product which was crystallized from Me_2SO–MeOH affording 0.75 g (36.5%) of **3**: mp >230 °C; IR (KBr) 1730, 1690 cm^{-1} (imide); NMR (Me_2SO-d_6, 90 MHz) δ 0.46–0.97 (m, 2, CH_2), 1.94 (q, 2, CHN), 3.43 (s, 8, CH_2), 11.07 (s, 2, NH). Anal. ($C_{11}H_{14}N_4O_4$) C, H, N.

Comparative mass spectra of major ions [*m/e* (% ICRF-159, % **2**, % **3**)] for bis(dioxopiperazines) show 268 (M^+) (0, 1.2, 3.4), 267 (0.5, 5.7, 10.2), 154 (5.8, 4.5, 6.3), 153 (71.5, 52.7, 63.2), 141 (68.1, 1.5, 2.3), 140 (6.5, 5.0, 6.0), 127 (100.0, 100.0, 100.0), 113 (5.3, 2.3, 3.0).

Biology. Inbred male Syrian golden hamsters, strain LSH/LAK, were obtained from Charles River Laboratories (Lakeview,

N.J.). Animals were housed five per cage under standard laboratory conditions and were used when 7–12 weeks of age.

The tumor cell line used in this study was a broncogenic adenocarcinoma, designated LG1002.[26] This line was induced in an outbred Syrian golden hamster by the intratracheal instillation of benzo[a]pyrene adsorbed to ferric oxide particles and suspended in saline.[29] The tumor was maintained by serial passage in the hamster cheek pouch. Tumor cell inocula were prepared by digesting small tumor pieces with trypsin and suspending the cells at the desired concentration in Hank's balanced salt solution (BSS).

Groups of hamsters averaging approximately 93 g each were injected intraperitoneally with 15 mg/kg of the compound being tested (see Table I, footnote *a*). Animals were treated every 48 h for 4 weeks and the last treatment occurred 48 h prior to excision of tumor. Growth of tumor cell inocula, given intradermally in the back, was monitored by measuring the greatest and the least diameters of the tumor nodule with calipers. Tumor size was expressed as the product of the two diameters.

Intradermal tumors were excised 4 weeks after implantation. The wound was inspected for the presence of subcutaneous tumor growth. The wound edges were apposed and fastened with sterile skin clips (Clay Adams, Parsippany, N.J.). Animals were checked daily for regrowth of the tumor at the excision site and sacrificed 7 days later. To enumerate the number of metastases, the lungs were cleared of blood by severing the dorsal aorta and injecting 3–5 mL of saline into the right ventricle. They were then removed, fixed as described by Williams and Nettesheim,[30] and stained and cleared according to the method of Yuhas.[31] Mean tumor sizes and number of metastasis were compared using Student's *t* test.

Acknowledgment. The authors are indebted to Scott Lewellyn and Jeffrey Resnick for technical assistance.

References and Notes

(1) The authors gratefully acknowledge partial support of this work by National Cancer Institute Grant Nos. CA-16058 and CA-16432.
(2) D. T. Witiak, H. J. Lee, R. W. Hart, and R. E. Gibson, *J. Med. Chem.*, **20**, 630 (1977).
(3) K. Hellmann and K. Burrage, *Nature (London)*, **224**, 273 (1969).
(4) A. J. Salsbury, K. Burrage, and K. Hellmann, *Br. Med. J.*, 4, 344 (1970).
(5) K. Burrage, K. Hellmann, and A. J. Salsbury, *Br. J. Pharmacol.*, **39**, 205P (1970).
(6) K. Hellmann in "Advances in Antimicrobial and Antineoplastic Chemotherapy", Vol. 2, M. Hejzlav, M. Semonsky, and S. Masak, Eds., University Park Press, Baltimore, Md., 1972, p 769.
(7) A. W. LeServe, *Br. J. Pharmacol.*, **43**, 457P (1971).
(8) A. W. LeServe and K. Hellmann, *Br. Med. J.*, 1, 597 (1972).
(9) S. E. James and A. J. Salsbury, *Cancer Res.*, **34**, 839 (1974).
(10) A. J. Salsbury, K. Burrage, and K. Hellmann, *Cancer Res.*, **34**, 843 (1974).
(11) K. Hellmann, A. J. Salsbury, K. S. Burrage, A. W. LeServe, and S. E. James in "Chemotherapy of Cancer Dissemination and Metastasis", S. Garattini and G. Franchi, Eds., Raven Press, New York, N.Y., 1973, pp 355–359.
(12) H. C. Hoover, Jr., and A. S. Ketcham, *Cancer*, **35**, 5 (1975).
(13) K. Hellmann, S. E. James, and A. S. Salsbury, *Br. J. Cancer*, **30**, 179 (1974).
(14) T. C. Stephens and A. M. Creighton, *Br. J. Cancer*, **29**, 99 (1974).
(15) A. Atherton, *Eur. J. Cancer*, 11, 383 (1975).
(16) L. J. Peters, *Br. J. Cancer*, **32**, 355 (1975).
(17) M. V. Pimm and R. W. Baldwin, *Br. J. Cancer*, 31, 62 (1975).
(18) J. S. Lazo, D. E. Ingber, and A. C. Sartorelli, *Cancer Res.*, in press. We wish to thank these authors for providing us with a copy of their very interesting manuscript prior to its publication.
(19) (a) G. B. Payne, *J. Org. Chem.*, **32**, 3351 (1967); (b) L. L. McCoy, *J. Am. Chem. Soc.*, **80**, 6568 (1958).
(20) H. A. Hoffman and A. Burger, *J. Am. Chem. Soc.*, **74**, 5485 (1952).
(21) H. Okaku, K. Toyoda, Y. Moriguchi, and K. Uens, *Bull. Chem. Soc. Jpn.*, **40**, 2326 (1967).
(22) J. F. Wolfe and G. B. Trimitsis, *J. Org. Chem.*, **33**, 894 (1968).
(23) E. L. Allred and M. D. Hurwitz, *J. Org. Chem.*, **30**, 2376 (1965).
(24) Certain combinations of parameters, notably in ABX and AA'XX' cases, often lead to spectra which contain fewer lines than expected and are termed deceptively simple because they resemble certain first-order spectra (L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", 2nd ed., Pergamon Press, New York, N.Y., 1969). A simple six-line spectrum is seen in some AA'XX' cases, but the maximum number of lines is 24. In some trans-disubstituted cyclopropanes, where $J_{cis} \neq J_{trans}$, deceptively simple spectra are not observed [T. Shono, T. Marikawa, A. Oku, and R. Oda, *Tetrahedron Lett.*, 791 (1964)].
(25) Considerable data obtained from substituted cyclopropanes show that the geminal coupling constants are in the range of −3.1 to −9.1 Hz (see Jackman and Sternhell in ref 24).
(26) A. P. Bakaletz and B. S. Zwilling, *Cancer Res.*, submitted for publication.
(27) A. Poggi, L. Mussoni, L. Kornblihtt, E. Ballabio, G. DeGaetano, and M. B. Donati, *Lancet*, 163 (1978).
(28) D_2O + 1% (w/w) sodium 2,2-dimethyl-2-silapentane-5-sulfonate; 98.8% D.
(29) U. Safiotte, F. Cefis, and L. H. Kolb, *Cancer Res.*, **28**, 104 (1968).
(30) M. L. Williams and P. Nettesheim, *J. Natl. Cancer Inst.*, **51**, 1513 (1973).
(31) J. M. Yuhas, *J. Natl. Cancer Inst.*, **50**, 69 (1973).