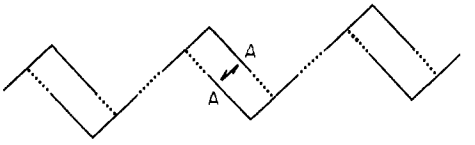
Table III. Atomic Deviations (Å) from Least-Squares Planes[a]

| ICRF-159 | | | | ICRF-187 | | | |
|---|---|---|---|---|---|---|---|
| atom | deviation | atom | deviation | atom | deviation | atom | deviation |
| C2 | 0.015 | C8 | -0.019 | C2 | -0.002 | C8 | 0.013 |
| C3 | -0.015 | C9 | 0.019 | C3 | 0.002 | C9 | -0.012 |
| C5 | 0.015 | C11 | -0.019 | C5 | -0.002 | C11 | 0.012 |
| C6 | -0.015 | C12 | 0.019 | C6 | 0.002 | C12 | -0.013 |
| N1 | 0.660 | N7 | -0.585 | N1 | 0.667 | N7 | -0.659 |
| N4 | 0.067 | N10 | -0.115 | N4 | 0.095 | N10 | -0.103 |
| O16 | -0.046 | O18 | 0.131 | O16 | -0.060 | O18 | 0.060 |
| O17 | -0.013 | O19 | 0.038 | O17 | -0.101 | O19 | 0.153 |

[a] Lower group of atoms not included in calculation of planes.

The bond distances and angles in the two molecules are in agreement with accepted values. The six $sp^3$ C-N bonds average 1.46 Å and the four $sp^2$ C-N distances average 1.37 Å for both compounds. The average bond angles of 125-126° at the trigonal nitrogen atoms agree well with the value in planar 2,5-piperazinedione,[12] and the average value of 112° for the angles around N1 and N7 is typical for $sp^3$ N hybridization.

**B. Intermolecular Interactions.** Stereoscopic drawings of the molecular packing in the crystals of both compounds are given in Figure 3. As noted previously, enantiomeric ICRF-187 is dramatically more soluble than the racemic material, and it is of interest to seek the basis for the differing solubilities in terms of intermolecular attractions in the crystals of the two forms. In the case of the soluble ICRF-187 (Figure 3b), the linear molecules are hydrogen-bonded end-to-end by two N-H···O links (N···O distances are 2.86 and 2.96 Å), forming parallel ribbons of molecules through the crystal. The only interactions between ribbons are normal van der Waals approaches. The arrangement in the racemate is more complex (Figure 3a). One end of each molecule is hydrogen-bonded to the next in the same manner as for the enantiomeric structure (N···O distances = 2.94 Å), while the other ring in the molecule (labeled A in Figure 3a) is involved in a stacking interaction and reciprocal hydrogen bonding with a similar heterocycle of another molecule. This latter system involves the trigonal nitrogen atom as H-bond acceptor (N···N distances = 2.97 Å), and the A···A parallel ring separation is such to allow interaction of $\pi$-electron systems (N4···N4 = 3.36 Å, O16···O17 = 3.55 Å, C3···C5 = 3.38 Å). The results of these interactions are ribbons of dimeric cis-conformation molecules throughout the crystal, schematically as shown below. In addition,




there is partial overlapping of $\pi$-electron systems between the ribbons, indicated by B···B labeling in Figure 3a (O19···N10 = 3.46 Å, C11···C11 = 3.28 Å), in addition to the normal van der Waals approaches. Thus both qualitatively and quantitatively the intermolecular network of forces in crystals of the racemic compound significantly exceeds that existing in crystals of the pure enantiomer and can reasonably account for the widely differing solubilities and melting points.

**Acknowledgment.** Support was from the Medical Research Council of Canada and from U.S. Public Health Service Grant CA15879.

**Registry No.** ICRF-159, 21416-87-5; ICRF-187, 24584-09-6.

**Supplementary Material Available:** A listing of observed and calculated structure factors, hydrogen atom fractional coordinates, and heavy-atom anisotropic thermal parameters for both structures (21 pages). Ordering information is given on any current masthead page.

(12) Degeilh, R.; Marsh, R. E. *Acta Crystallogr.* **1959**, *12*, 1007-1014.

# Stereochemistry of Conformationally Restricted Analogues of the Antitumor Agent ICRF-159: Crystal and Molecular Structures of *cis*- and *trans*-Cyclopropylbis(dioxopiperazine)

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**Abstract:** Crystal structure determinations of *cis*- and *trans*-cyclopropylbis(dioxopiperazine), fixed-conformation analogues of the cytostatic agent ICRF-159, have confirmed their geometries. Comparisons of their stereochemical characteristics with those of the cis and trans conformations of ICRF-159 have been performed; the cis analogue closely resembles the observed cis conformation of ICRF-159 but the trans analogue and trans ICRF-187 (enantiomeric ICRF-159) differ somewhat. These observations support the concept that cytostatic activity resides in the cis conformation in these compounds. Crystals of the cis analogue are orthorhombic, space group *Pnam*, $a = 9.731$, $b = 7.080$, $c = 18.208$ Å, with four molecules per cell; those of the trans analogue are monoclinic, space group $C2/c$, with $a = 19.172$, $b = 6.650$, $c = 9.854$ Å, $\beta = 109.43°$, with four molecules per unit cell.

## Introduction

The antitumor agent ICRF-159 [(±)-4,4′-(1,2-propanediyl)-bis(4-piperazine-2,6-dione)] (**1**) possesses rotational mobility about the inter-ring bonds and could adopt a variety of conformations with different arrangements of the piperazinedione rings relative to each other. Crystal structure determinations[2] of racemic ICRF-159 and a pure enantiomer have shown that both a cis "face-to-face" conformation of the rings and an extended trans conformation, with a parallel arrangement of ring planes, are

(1) (a) University of Toronto. (b) University of Washington.

(2) Hempel, A.; Camerman, N.; Camerman, A. *J. Am. Chem. Soc.*, preceding paper in this issue.

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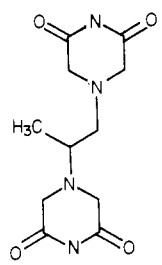
Table I. Crystal Data

| | *cis*-2 | *trans*-3 |
|---|---|---|
| formula | $C_{11}H_{14}N_4O_4$ | $C_{11}H_{14}N_4O_4$ |
| mol wt | 266.26 | 266.26 |
| crystal system | orthorhombic | monoclinic |
| space group | *Pnam* (or $Pna2_1$) | *C2/c* (or *Cc*) |
| *a* (Å) | 9.731 (5) | 19.172 (8) |
| *b* (Å) | 7.080 (5) | 6.650 (4) |
| *c* (Å) | 18.208 (7) | 9.854 (5) |
| α (deg) | 90 | 90 |
| β (deg) | 90 | 109.43 (8) |
| γ (deg) | 90 | 90 |
| no. of molecules in cell | 4 | 4 |
| density, calcd (g $cm^{-3}$) | 1.41 | 1.49 |
| linear absorption coeff. ($cm^{-1}$) (Cu Kα radiation) | 9.36 | 9.90 |

stable. It was shown[3] in the initial description of ICRF-159 that biological activity varied markedly upon minor chemical modification, indicating that stereochemical and conformational specificities were important in its cellular interactions. Witiak et al.[4] synthesized cyclopropyl ring analogues of ICRF-159 in which the piperazinedione rings were restricted to be cis (**2**) or trans (**3**) to each other and found that the two conformational isomers pos-



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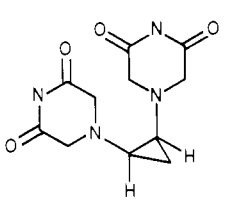

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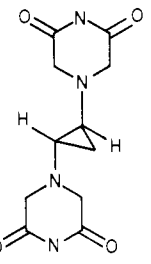

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sessed dramatically different biological properties. Intraperitoneal injection of both ICRF-159 and *cis*-**2** significantly inhibited development of lung metastases and bronchogenic adenocarcinoma in hamsters, with no effect on primary tumor growth, while the *trans*-[3] isomer appeared to stimulate the growth of lung metastases and of the primary tumor.[4] Thus antimetastatic activity seems to reside in the cis conformation of ICRF-159 and analogues, while the trans conformation demonstrates metastatic stimulation. We have crystallized and elucidated the three-dimensional structures of *cis*-2 and *trans*-**3** in order to definitively establish their conformational features and to compare them with the previously determined molecular conformations of racemic and enantiomeric ICRF-159. We report here the results of our studies.

## Experimental Section

Both *cis*-**2** and *trans*-**3** isomers were supplied by D. T. Witiak.

**A. *cis*-2.** Small colorless plates were obtained by evaporation of cold aqueous ethanol. Crystal data are given in Table I. Intensity data were collected from a crystal of dimensions 0.3 × 0.3 × 0.07 mm on an automated diffractometer using Cu Kα radiation and $2\theta/\theta$ scan. Of 1110 independent reflections in the range $0 < 2\theta < 130°$, 785 had $I > 3\sigma(I)$ and were used in the structure refinement. The data were corrected for background, an empirical φ correction for absorption was applied, and structure amplitudes were derived in the normal manner.

The structure was solved in space group $Pna2_1$ using the direct phasing program MULTAN 78. Input consisted of 176 reflections with $|E| > 1.4$, and the *E*-map based on the best set of phases allowed identification of all nonhydrogen atoms. Full-matrix least-squares refinement and difference electron density calculations led to positions for all hydrogen atoms and a discrepancy index $R = 0.041$ for the observed reflections (all parameters refined except hydrogen-atom temperature factors which were fixed at $B = 2.8$ Å$^2$). Chemically equivalent bond lengths and angles calculated at this point showed unacceptably large deviations throughout the molecule (e.g., C2–C3 = 1.57 Å, C5–C6 = 1.43 Å), and an analysis of the atomic coordinates indicated the existence of a molecular mirror plane perpendicular to the crystallographic *c* axis. Refinement was therefore continued in space group *Pnam* with half the molecule as the asymmetric unit, and resulted in a final discrepancy factor $R = 0.049$, and much improved bond parameters. Scattering factors were as cited in the preceding paper. Table II lists the fractional coordinates for the nonhydrogen atoms; anisotropic thermal parameters, hydrogen atom coordinates, and tables of observed and calculated structure factors are available.[5]

Table II. Fractional Atomic Coordinates (×$10^4$)

| | cis | | | trans | | |
|---|---|---|---|---|---|---|
| atom | *x* | *y* | *z* | *x* | *y* | *z* |
| N1 | 6905 (2) | 4056 (3) | 1685 (1) | 9193 (1) | 3787 (3) | 3120 (3) |
| C2 | 7158 (3) | 3019 (4) | 1008 (1) | 9205 (2) | 1598 (4) | 3153 (3) |
| C3 | 8362 (3) | 3816 (4) | 597 (1) | 8807 (2) | 769 (4) | 4097 (3) |
| N4 | 8706 (2) | 5650 (3) | 739 (1) | 8284 (1) | 1996 (4) | 4346 (3) |
| C5 | 7927 (3) | 6875 (4) | 1154 (2) | 8034 (1) | 3768 (5) | 3645 (3) |
| C6 | 6675 (3) | 6023 (4) | 1503 (2) | 8437 (1) | 4522 (4) | 2668 (3) |
| C7 | 5752 (3) | 3281 (4) | 2086 (1) | 9589 (1) | 4485 (4) | 2186 (3) |
| C8 | 5971 (4) | 1481 (6) | 2500 | 10000 | 6441 (6) | 2500 |
| O9 | 9013 (2) | 2889 (3) | 147 (1) | 8918 (1) | −906 (3) | 4614 (3) |
| O10 | 8247 (3) | 8518 (3) | 1215 (1) | 7508 (1) | 4640 (3) | 3798 (2) |

**B. *trans*-3.** Colorless needles were obtained from dimethyl sulfoxide by solvent evaporation. Crystal data are given in Table I. A crystal of dimensions 0.06 × 0.3 × 0.1 mm was used for data collection by the procedures described above. A total of 1078 independent reflections were recorded, of which 757 had $I > 3\sigma(I)$ and were classified as observed. The structure was solved in space group *Cc* and refined as described for the *cis*-**2** isomer; full-matrix least-squares refinement of all parameters except hydrogen atom temperature factors (fixed at $B = 2.5$ Å$^2$) converged at $R = 0.039$ for the observed data. Again, as was the case with the cis isomer, bond lengths and angles calculated at this point had unacceptable deviations among chemically equivalent bonds (e.g., C–O carbonyl distances of 1.11 and 1.30 Å). Analysis of atomic coordinates indicated the two halves of the molecule were related by a twofold axis parallel to *b*; accordingly refinement was continued in space group *C2/c* with half the molecule as the asymmetric unit. The final *R* index was 0.046, and bond parameters were greatly improved. Heavy atom coordinates are given in Table II; other parameters are available.[5]

## Results and Discussion

The molecular conformations of the *cis*- and *trans*-cyclopropyl analogues of ICRF-159 are shown stereoscopically in Figure 1, and the atomic numbering scheme and bond parameters are given in Figure 2. The results confirm the geometries of the two isomers. The molecular mirror plane results in an N7–C13–C14–N1 torsion angle in the cis isomer of 0° and eclipsed positioning of N1 ring atoms; the orientation of the ring planes is roughly "face-to-face", but not as much so as in the cis conformation observed for racemic ICRF-159. The same torsion angle in the trans isomer is 138°, somewhat smaller than the value of 177° in the trans conformation of ICRF-187 (enantiomeric ICRF-159), and, unlike ICRF-187, the planes of the piperazinedione rings are not parallel (angle between plane perpendiculars is 54°). The conformations of the rings in both compounds are similar, and the same as observed in both racemic and enantiomeric ICRF-159; they form slightly bowed half-chairs, with N1 lying 0.65–0.69 Å out of the plane of the carbon atoms, and N4 being 0.07–0.09 Å from the plane in the same direction.

The bond distances and angles in the two compounds (Figure 2) agree well with each other and with accepted values. Thus, for example, the average $sp^3$ C–N length is 1.455 Å, the mean $sp^2$ c–N length is 1.375 Å, and $sp^3$ C–$sp^2$ C is 1.50 Å for both.

The biological properties of *cis*-**2** and *trans*-**3** are markedly different, with the former inhibiting and the latter appearing to stimulate development of metastases. It is therefore of interest to compare these conformationally fixed analogues stereochemically with the cytostatic, conformationally mobile parent compound ICRF-159. Figure 3 is a stereoscopic superposition of the structure of *cis*-**2** and the observed[2] cis conformation of ICRF-159, with the two piperazine rings in each maximally fitted. The fit

(3) Creighton, A. M.; Hellmann, K.; Whitecross, S. *Nature (London)* **1969**, *222*, 384–385.

(4) Witiak, D. T.; Lee, H. J.; Goldman, H. D.; Zwilling, B. S. *J. Med. Chem.* **1978**, *21*, 1194–1197.

(5) See paragraph at end of paper regarding supplementary material.

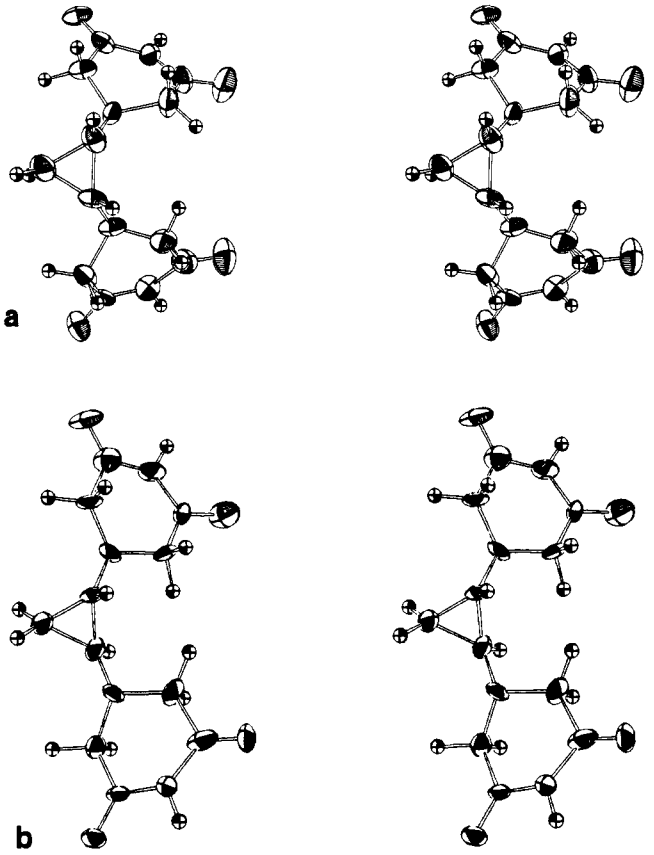


Figure 1. Stereoscopic drawings of the structures of (a) *cis*-**2** and (b) *trans*-**3**.

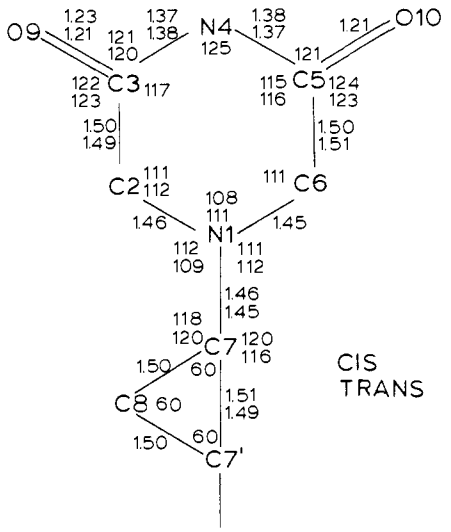


Figure 2. Atomic numbering and interatomic bond distances and angles in *cis*-**2** (upper figures) and *trans*-**3**. Estimated standard deviations are 0.003–0.004 Å and 0.2–0.3°.

is excellent: the nitrogen atoms of each compound occupy similar positions, and small rotations of the *cis*-**2** rings about the C(cyclopropane)–N bonds would bring the oxygen atoms into almost exact coincidence with those of ICRF-159. Thus these two cis conformations possess almost identical stereochemistry, especially with respect to their functional groups, and since the *cis*-**2** analogue exhibits similar biological functioning to ICRF-159, these structural results strongly support the conclusion[4] that the antimetastatic activity of ICRF-159 is expressed through cis-conformation interactions.

The question then arises as to why ICRF-159, also able to adopt a stable trans conformation,[2] does not exhibit the stimulation of metastatic growth demonstrated by *trans*-[3]. This may be considered by comparing the trans conformations of the two compounds (Figure 4), superimposed so that atoms of the piperazine rings in each are maximally fitted. The fit is not nearly as good as for the cis conformation compounds: three of the oxygen atoms lie 2 Å or more from their counterparts and rotation of the rings in *trans*-**3** about the C(cyclopropane)–N bonds will not reduce these distances. The only way the two compounds may be made to compare more closely stereochemically is through rather large alterations in the observed ICRF-187 (trans ICRF-159) conformation. Thus one might speculate that the reason that conformationally mobile ICRF-159 does not cause the biological effects seen with the fixed-conformation trans analogue is that to do so it would have to adopt a conformation rather less stable than those observed for it in the two crystal structures described.[2]

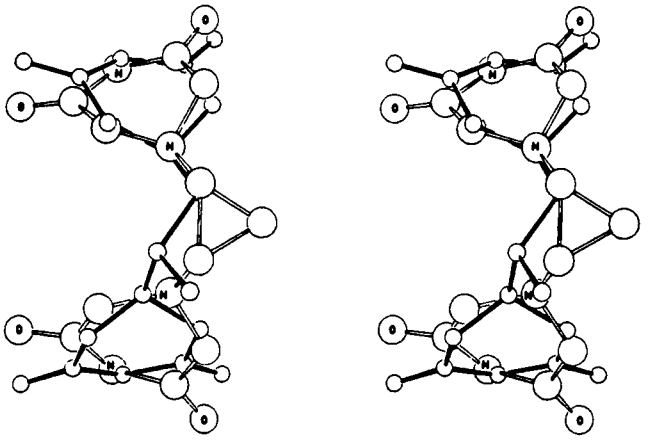

Figure 3. Stereoscopic drawing of *cis*-**2** (large circles, light bonds) and ICRF-159 superimposed. Hydrogen atoms are omitted.

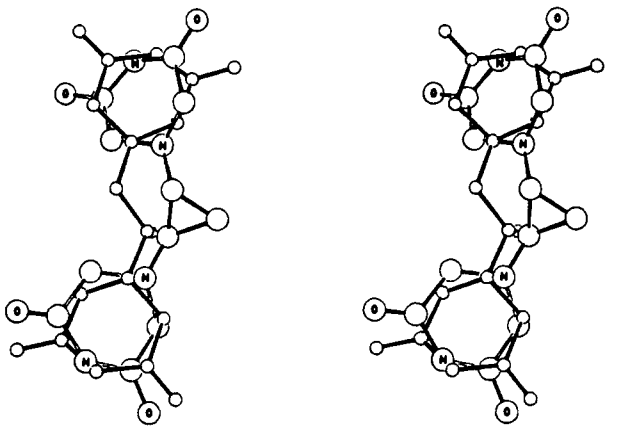

Figure 4. Stereoscopic superposition of *trans*-**3** (large circles, light bonds) and ICRF-187. Hydrogen atoms are omitted.

Crystal packing schemes for both cis and trans analogues have been deposited as supplementary material. In both cases molecules are linked in chains via two N—H···O bonds at each end, the N···O distances ranging from 2.93 to 2.95 Å. In *cis*-**2** this results in a sinusoidal pattern of chains and in *trans*-**2** a more densely packed ribbon pattern with more van der Waals contacts between chains than in *cis*-**2**. In accordance with this latter feature we have observed that the trans analogue is less soluble than the cis compound.

**Acknowledgment.** We thank D. T. Witiak for supplying the title compounds. Support was from the Medical Research Council of Canada and from U.S. Public Health Service Grant CA15879.

**Registry No.** **2**, 66054-21-5; **3**, 66054-22-6.

**Supplementary Material Available:** A listing of observed and calculated structure factors, hydrogen atom coordinates, heavy-atom anisotropic thermal parameters, and stereoscopic drawings of the crystal packing schemes for both structures (15 pages). Ordering information is given on any current masthead page.