Table IV. Estimated PA's (kcal/mol) of N-Arylazacycloalkanes[a]

substituent	ring size 3	4	5	6
Ph	218	222	226	226
o-MePh		223	226	228
o,p-Me_2Ph	221	225	228	230
o,o'-Me_2Ph	220	223	232	232

[a] Estimated from the equation: PA (kcal/mol) = 296 − 8.13IP (eV) − 11.7 cos θ.

For perpendicular N-arylazacycloalkanes, we can use the correlation given above to estimate PA's, while for planar species, the PA's are expected to be ~11.7 kcal/mol lower for a given IP. In order to provide a single estimate of PA as a function of IP and rotational angle, we have defined the following equation:

$$PA(est) = 296 - 8.13IP_1 - 11.7 \cos \theta$$

where IP_1 is the first IP of the N-aryl azacycloalkane, and θ is the angle of rotation ($\theta = 0°$ for a "planar" species). Using the values of θ listed in Table III, the PA's listed in Table IV are predicted.

These predicted PA's qualitatively follow the order of measured solution pK_a's (±1 pK_a unit) except for three notable exceptions, **5-Ph**, **4-o,p-Me_2Ph**, and **6-o,o'-Me_2Ph**, all of which have pK_a's ≈ 2 pK_a units too low. The remaining compounds seem to have maximum pK_a's of about 5.5, even when the estimated PA's are quite high, which we attribute to steric hindrance to solvation of the ammonium cations.[19b] Of the three compounds noted above with especially low pK_a's, only the last would seem to provide especially high steric hindrance to solvation.

Conclusion

The PES studies have shown that the conformations of N-arylazacycloalkanes may be quite different for different amine ring sizes. Furthermore, the conformations of the aryl group with respect to the amine influences not only the IP, but are predicted to influence gas-phase proton affinities as well. Solution pK_a's are influenced by aryl conformations through the effect on IP's and variations in steric hindrance to the solvation of ammonium cations.

Acknowledgment. We are grateful to National Science Foundation and National Institute on Drug Abuse grants to K.N.H. for financial support of this work.

Registry No. 3-Ph, 696-18-4; 3-o,p-Me_2Ph, 78376-89-3; 3-o,o'-Me_2Ph, 78376-90-6; 4-Ph, 3334-89-2; 4-o-MePh, 19198-94-8; 4-o,p-Me_2Ph, 81506-10-7; 4-o,o'-Me_2Ph, 19199-06-5; 4-o,o'-Et_2Ph, 81506-11-8; 5-Ph, 4096-21-3; 5-o-MePh, 41378-30-7; 5-o,p-Me_2Ph, 81506-12-9; 5-o,o-Me_2Ph, 64175-53-7; 5-o,o'-Et_2Ph, 81506-13-0; 6-Ph, 4096-20-2; 6-o-MePh, 7250-70-6; 6-o,p-Me_2Ph, 81506-14-1; 6-o,o'-Me_2Ph, 81506-15-2; N,N-dimethylaniline, 121-69-7; N,N,2,4-tetramethylaniline, 769-53-9; N,N,2,6-tetramethylaniline, 769-06-2; N-ethylaniline, 103-69-5; 2,6-diethylaniline, 579-66-8; N,N-O,O'-tetraethylaniline, 81506-16-3.

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Stereochemistry of the Antitumor Agent 4,4'-(1,2-Propanediyl)bis(4-piperazine-2,6-dione): Crystal and Molecular Structures of the Racemate (ICRF-159) and a Soluble Enantiomer (ICRF-187)

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Abstract: Crystal structure elucidations of racemic 4,4'-(1,2-propanediyl)bis(4-piperazine-2,6-dione) (ICRF-159) and the S-(+) enantiomer (ICRF-187) have shown that both the cis and trans arrangements of the heterocyclic rings within the molecules represent stable conformations. In addition, analysis of the crystal packing in the two compounds has led to a plausible explanation for their very different solubilities. Crystals of ICRF-159 are triclinic, space group $P\bar{1}$, $a = 6.931$, $b = 11.930$, $c = 8.581$ Å, $\alpha = 101.06$, $\beta = 108.40$, $\gamma = 97.40°$, with two molecules per cell; those of ICRF-187 are monoclinic, $P2_1$, with $a = 10.578$, $b = 9.459$, $c = 6.594$ Å, $\beta = 95.02°$, with two molecules per cell.

Introduction

ICRF-159 [(±)-4,4'-(1,2-propanediyl)bis(4-piperazine-2,6-dione)] (NSC 129943) is a cytostatic agent[2] which has demonstrated significant in vitro and in vivo antitumor activity against a number of tumor types. Its effects appear to be antimetastatic, rather than cytotoxic, with a mechanism of action probably involving changes in tumor vasculature and inhibition of release of tumor cells into the circulation.[3,4] Antitumor activity varies markedly with chemical modification,[2] indicating that specific stereochemical and conformational characteristics are required for interactions with cell components.

Pharmacokinetic studies[5] have shown that orally administered ICRF-159 is poorly absorbed, especially at high doses; this is probably due to the compound's low solubility. In order to increase the bioavailability of the drug, use was made of the observations that the enantiomers of ICRF-159 are biologically active[2] and are more soluble than the racemate[6,7] to perform a systematic study

(1) (a) University of Toronto. (b) University of Washington.
(2) Creighton, A. M.; Hellmann, K; Whitecross, S. *Nature (London)* **1969**, *222*, 384-385.
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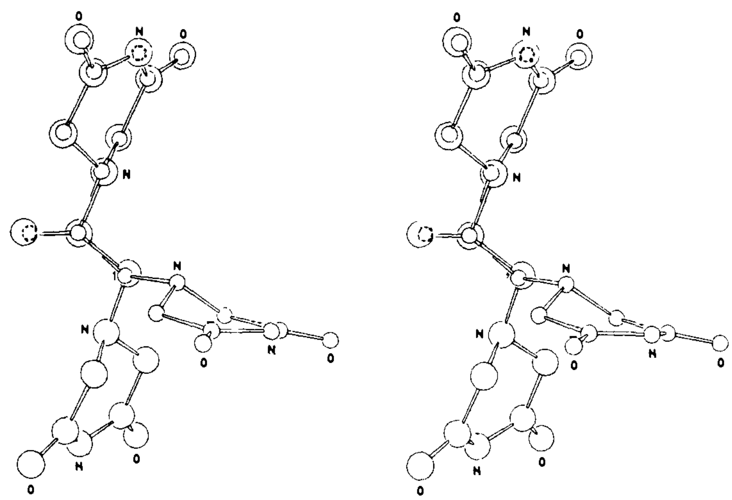

Figure 1. Stereoscopic drawings of ICRF-159 (small circles) and ICRF-187 (large circles) superimposed. The *S* enantiomer of racemic ICRF-159 is shown to facilitate comparisons. Hydrogen atoms are omitted.

Table I. Crystal Data

	ICRF-159	ICRF-187
formula	$C_{11}H_{16}N_4O_4$	$C_{11}H_{16}N_4O_4$
mol wt	268.28	268.28
crystal system	triclinic	monoclinic
space group	$P\bar{1}$	$P2_1$
a (Å)	6.931 (5)	10.578 (5)
b (Å)	11.930 (7)	9.459 (5)
c (Å)	8.581 (6)	6.594 (4)
α (deg)	101.06 (8)	90
β (deg)	108.40 (9)	95.02 (5)
γ (deg)	97.40 (7)	90
no. of molecules in cell	2	2
density, calcd (g cm^{-3})	1.37	1.35
linear absorption coeff. (cm^{-1}) (Cu Kα radiation)	9.05	8.92

which resulted in a formulation of enantiomeric ICRF-159 with five times the solubility of the racemate, suitable for parenteral or oral use.[7] (For a discussion of the ways in which the optical purity of a compound can affect its solubility see ref 7.)

Such marked differences in solubility (and also melting point, 41 °C difference) of (±) ICRF-159 and its enantiomers imply the existence of radically different intermolecular forces in these compounds. We have undertaken crystal and molecular structure determinations of (racemic) ICRF-159 and the *S*-(+) enantiomer (ICRF-187; NSC 169780) in order to establish their stereochemical and conformational features, and to investigate the intermolecular forces responsible for the different physical properties of the two crystalline forms.

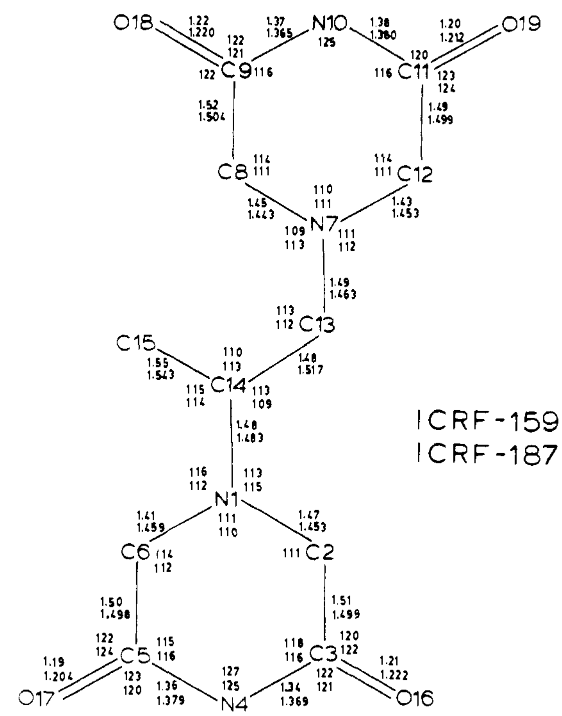

Figure 2. Atomic numbering and interatomic distances and angles in ICRF-159 (upper figures) and ICRF-187. Estimated standard deviations are 0.01 Å and 1° for the racemate and 0.005 Å and 1° for the enantiomeric ICRF-187.

Experimental Section

ICRF-159 and ICRF-187 were supplied by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute.

A. ICRF-159. Small crystals were obtained from ethanol by solvent evaporation. Crystal data are given in Table I. A needle, 0.2 × 0.03 × 0.05 mm, was selected and the X-ray intensities of all independent reflections having 2θ (Cu Kα) < 130° were measured on an automated four-circle diffractometer using nickel-filtered Cu Kα radiation and the $2\theta/\theta$ scan technique. The intensities were corrected for background, an empirical ϕ absorption correction was applied, and structure amplitudes were derived in the usual way. Only 883 of a total of 2310 independent reflections were considered to be observed [$I > 3\sigma(I)$] and were used in the structure refinement. Numerous attempts at achieving better quality crystals were not successful.

The structure was solved using the direct phasing program MULTAN 78. Input consisted of 176 reflections with $[E] > 1.6$, 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 final discrepancy index $R = 0.094$ for the observed reflections (all parameters refined except hydrogen atom temperature factors which were held constant at $B = 3.0$ Å^2). Scattering factors were as cited for the heavy[8] and hydrogen atoms.[9] 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.[10]

B. ICRF-187. Crystals were obtained from aqueous methanol. Crystal data are given in Table I. A block of dimensions 0.35 × 0.20 × 0.45 mm was used for data collection by the procedures described above. A total of 1309 independent data were measured, of which 1126 were classified as observed. The structure was solved and refined as outlined for ICRF-159 above; refinement of all parameters except hydrogen atom thermal factors (fixed at $B = 2.9$ Å^2) converged at $R = 0.041$ for the observed data. An attempt to verify the S absolute configuration of ICRF-187 using anomalous scattering failed; R values for both possible chiralities were identical. Heavy atom coordinates are in

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

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

	ICRF-159			ICRF-187		
atom	*x*	*y*	*z*	*x*	*y*	*z*
N1	6871 (13)	3834 (8)	11717 (11)	236 (2)	2026	1275 (4)
C2	8447 (18)	4816 (11)	11775 (16)	−175 (3)	838 (6)	−16 (6)
C3	7519 (17)	5884 (10)	11586 (15)	−1315 (3)	1214 (5)	−1427 (5)
N4	5764 (13)	5928 (8)	11918 (12)	−2068 (3)	2276 (5)	−797 (5)
C5	4856 (18)	5186 (11)	12629 (15)	−1953 (3)	2872 (5)	1118 (6)
C6	5858 (18)	4164 (11)	12881 (14)	−796 (3)	2465 (5)	2462 (5)
N7	6100 (14)	2010 (8)	8635 (13)	3741 (3)	1495 (4)	2426 (5)
C8	4499 (21)	1132 (11)	8724 (15)	4206 (4)	2670 (6)	3670 (7)
C9	2594 (19)	742 (10)	7120 (16)	5374 (3)	2256 (6)	5005 (6)
N10	2855 (16)	1006 (8)	5704 (12)	6085 (3)	1186 (5)	4305 (5)
C11	4743 (21)	1354 (10)	5546 (17)	5903 (3)	611 (5)	2378 (6)
C12	6580 (18)	1671 (10)	7126 (17)	4705 (3)	1022 (6)	1137 (6)
C13	7983 (17)	2218 (11)	10167 (15)	2530 (3)	1783 (6)	1254 (5)
C14	7645 (17)	2736 (10)	11755 (15)	1432 (3)	1805 (5)	2586 (5)
C15	9608 (22)	2817 (11)	13297 (17)	1408 (3)	493 (6)	3972 (6)
O16	8289 (13)	6623 (8)	11047 (12)	−1559 (2)	620 (5)	3060 (4)
O17	3380 (13)	5328 (7)	13016 (10)	−2761 (2)	3655 (5)	1642 (4)
O18	935 (13)	253 (8)	7118 (10)	5684 (2)	2849 (5)	6613 (4)
O19	4850 (14)	1395 (7)	4187 (11)	6702 (2)	156 (5)	1757 (5)

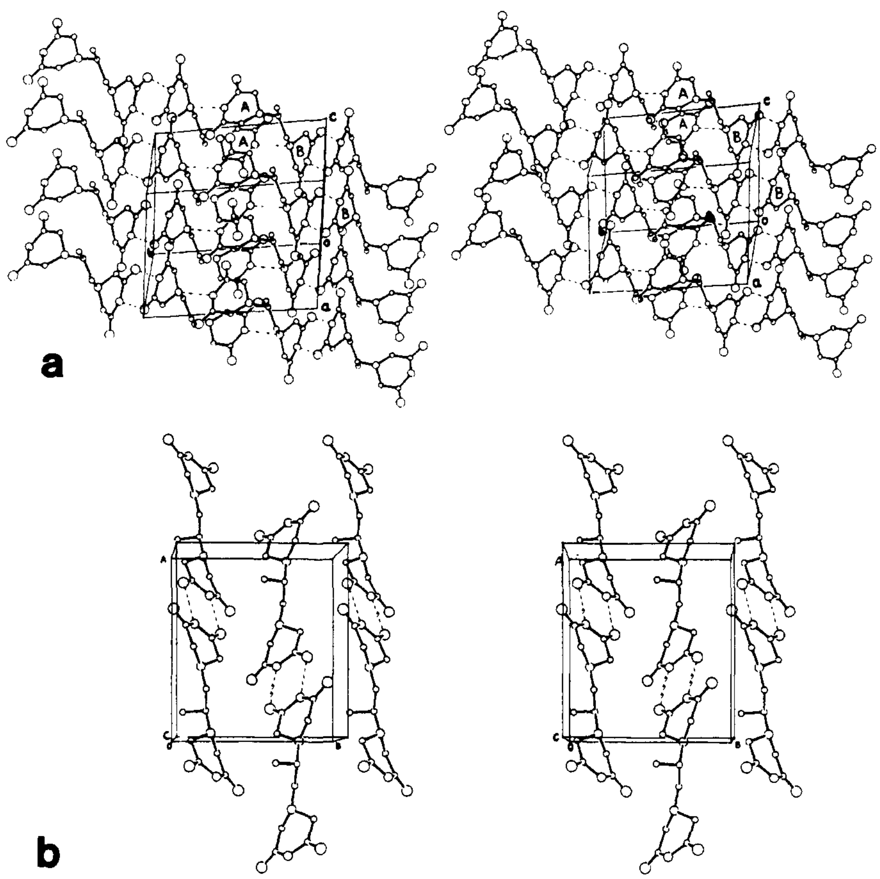

Figure 3. Stereoscopic representation of the molecular packing in the crystal of (a) ICRF-159 and (b) ICRF-187.

Table II; other parameters are available.[10]

Results and Discussion

A. Molecular Conformations and Geometry. The molecular structures of ICRF-159 and ICRF-187 in the crystals are compared stereoscopically in Figure 1, and atomic numbering and bond distances and angles are given in Figure 2. The conformations in the two cases are different; the racemic ICRF-159 adopts a cis arrangement of the two rings (N7–C13–C14–N1 torsion angle is 55.5°), while the molecular conformation in the crystal of the optically active ICRF-187 is trans (N7–C13–C14–N1 torsion angle is 177°). The conformations of the four piperazinedione rings in the two molecules are virtually identical: all are slightly bowed half-chairs with the tetrahedral nitrogen atom approximately 0.6 Å and the trigonal nitrogen atom 0.1 Å away from the plane of the carbon atoms (Table III). The planes of the two rings in trans ICRF-187 are parallel (angle between plane normals is 2°); in the cis conformation ICRF-159 the plane normals are 70° apart and the ring planes are oriented roughly "face-to-face" (torsion angles C8–N7–C13–C14 and C2–N1–C14–C13 are 65 and 71°, respectively). In addition to revealing the apparent consistency of the piperazinedione ring conformation, these structure determinations also indicate that both cis and trans arrangements of the rings are equally available; this may have significance biologically as experiments with fixed-conformation analogues have indicated dramatically different pharmacological properties for the two arrangements.[11]

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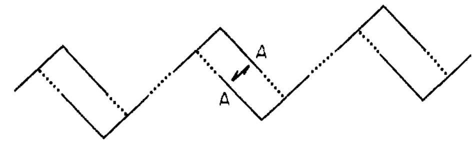
Table III. Atomic Deviations (Å) from Least-Squares Planes[a]

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 π-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 π-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, β = 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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