

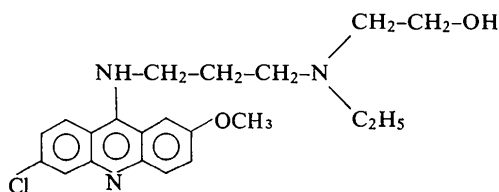
The Crystal Structure of ICR-170-OH, an Analogue of an Antitumor Agent and Mutagen

BY HELEN M. BERMAN AND JENNY PICKWORTH GLUSKER

The Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania 19111, U.S.A.

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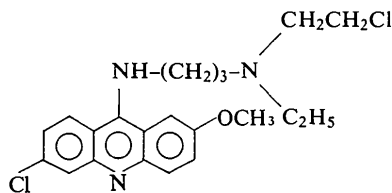
The crystal structure of the hydroxy-derivative of a nitrogen mustard, ICR-170-OH, an antitumor agent and a mutagen for *Neurospora*, has been determined. The formula of the molecule is



The crystals are monoclinic, space group $P2_1/c$ with four molecules in a cell of dimensions $a = 13.644$ (3), $b = 11.587$ (3), $c = 12.613$ (2) Å, $\beta = 100.13$ (2)°, $Z = 4$, observed density 1.31 g.cm^{-3} , calculated density 1.31 g.cm^{-3} . The intensities of 3223 reflections were measured with an automatic diffractometer with Cu $K\alpha$ radiation. Of these reflections the intensities of 1257 were too weak to measure. The structure was solved by the symbolic addition procedure. Atomic parameters were refined by block-diagonal and full-matrix least-squares methods to a final R value of 0.068 for observed data. All hydrogen atoms were located from a difference map and were refined. In this structure, there is an intramolecular hydrogen bond between nitrogen atoms in the side chain, as well as an intermolecular hydrogen bond between the hydroxyl group at the end of the side chain of one molecule and the ring nitrogen atom of another molecule. The ring system is not planar but buckled by about 10.7° . The molecules stack so that two out of three rings overlap in adjacent pairs of molecules.

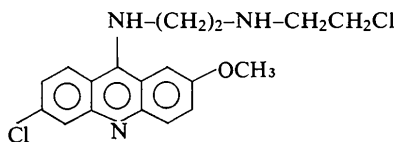
Introduction

ICR-170-OH is the hydroxy derivative of one of a series of acridine nitrogen half-mustards, some of which show antitumor and mutagenic activity (Peck, Preston & Creech, 1961). The hydroxy-compound [in which the $-\text{CH}_2-\text{CH}_2-\text{Cl}$ group of the mustard (I) is replaced by $-\text{CH}_2-\text{CH}_2-\text{OH}$] was studied because this derivative could be readily crystallized. Formulae for similar compounds, ICR-171 (II) and ICR-191 (III), are shown.



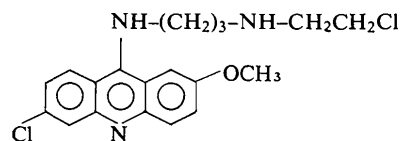
ICR-170

(I)



ICR-171

(II)



ICR-191

(III)

These differ only in their side chains, but have substantially different biological activities (Peck, *et al.* 1961; Ames & Whitfield, 1966; Malling, 1967). ICR-170 is a potent antitumor agent against ascites tumors and a strong mutagen for *Neurospora*, but a weak mutagen for *Salmonella*; ICR-191 is a powerful mutagen for *Salmonella*, a much less powerful one for *Neurospora*, and is slightly active against ascites tumors; ICR-171 has no antitumor activity but is a mild mutagen for *Salmonella*. Determinations of the crystal structures of ICR-170-OH, ICR-191-OH (Carrell, 1971), and ICR-171-OH (Glusker, Minkin & Orehowsky, 1971) were undertaken in order to find those conformational features in the molecules that might be responsible for the differing activities of such similar compounds.

Experimental

The crystals were provided by Drs Peck and Preston in the form of large, yellowish-orange prisms. Unit-cell

dimensions were measured on a Picker automatic diffractometer with copper $K\alpha$ radiation [$\lambda(K\alpha_1)$] = 1.54050 Å and were $a = 13.644$ (3), $b = 11.587$ (3), $c = 12.613$ (2) Å, $\beta = 100.13$ (2)°. The space group is determined to be $P2_1/c$ from the systematic absences $h0l$ with l odd, $0k0$ with k odd. The density, measured by flotation in potassium iodide solution, is 1.31 g.cm^{-3} , and that calculated for four units of $\text{C}_{21}\text{H}_{26}\text{N}_3\text{O}_2\text{Cl}$ is 1.31 g.cm^{-3} . The formula weight is 387.5 and the unit-cell volume is 1958.18 Å^3 . The chemical name is 2-methoxy-6-chloro-

9-[3-(ethyl-2-hydroxyethyl)aminopropylamino]acridine.

Three-dimensional data were collected with copper $K\alpha$ radiation on a Picker automatic diffractometer with the θ - 2θ scan technique. The crystal size was $0.20 \times 0.25 \times 0.25 \text{ mm}^3$. All data out to $2\theta = 135^\circ$ were scanned, and of these 3223 reflexions, 1966 were such that $I/\sigma(I) \geq 3$, and, therefore, were considered to be observed. The data were corrected for Lorentz and polarization factors with a program written by Craven & Chu (1967) and an absorption correction ($\mu = 18.9 \text{ cm}^{-1}$) was made assuming an ellipsoid of revolution (Johnson, 1963).

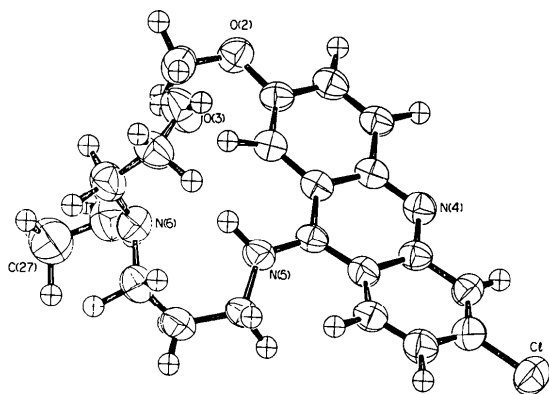


Fig. 1. ORTEP plot of one molecule showing thermal ellipsoids. The nitrogen, oxygen and chlorine atoms and the terminal methyl group are labeled.

Determination and refinement of the structure

The structure was solved by the symbolic addition procedure (Karle & Karle, 1963). An E synthesis, computed with all values of E greater than 1.8, revealed the entire acridine portion and all but eight atoms of the side chain. The rest of the structure appeared in the electron-density map, calculated on the phases of 19 atoms. Three cycles of isotropic block-diagonal least-squares refinement decreased the R value from 0.27 to 0.18. Four cycles of anisotropic least-squares refinement lowered the R value to 0.13, at which point a difference map was computed. All the hydrogen atoms were located in this map. The electron densities of H(), H(27-1), and H(27-2) were quite diffuse and paramete.

Table 1. Atomic parameters and their e.s.d.'s

Positional parameters are expressed as fractions of cell edges. Anisotropic temperature factors are expressed as:

$$\exp [-(b_{11}h^2 - b_{22}k^2 - b_{33}l^2 - b_{12}hk - b_{23}kl - b_{13}hl)].$$

Isotropic temperature factors are of the form $\exp(-B \sin^2 \theta/\lambda^2)$, and values of B are given in Å². E.s.d.'s, determined from the inverted full matrices, are listed in parentheses beside each parameter with respect to the last decimal place given.

Parameters for nonhydrogen atoms ($\times 10^4$).

	x	y	z	b_{11}	b_{22}	b_{33}	b_{12}	b_{13}	b_{23}
Cl	8853 (1)	5401 (2)	4179 (1)	71 (1)	189 (2)	103 (1)	0 (2)	0 (1)	-67 (2)
O(2)	2700 (3)	2900 (3)	-1339 (3)	78 (2)	97 (3)	84 (2)	8 (4)	11 (4)	-58 (4)
O(3)	2008 (4)	6178 (4)	-295 (4)	123 (3)	144 (4)	133 (4)	-67 (6)	118 (6)	-107 (7)
N(4)	6348 (3)	3546 (3)	1261 (3)	67 (2)	72 (3)	71 (2)	21 (4)	31 (4)	-4 (4)
N(5)	3854 (3)	5079 (3)	2250 (3)	69 (2)	92 (3)	61 (2)	15 (4)	33 (4)	-27 (4)
N(6)	1653 (3)	5400 (4)	1823 (3)	73 (3)	114 (4)	91 (3)	12 (5)	16 (5)	7 (6)
C(7)	3628 (3)	3806 (4)	269 (3)	71 (3)	71 (3)	67 (3)	2 (5)	28 (5)	1 (5)
C(8)	3553 (3)	3133 (4)	-628 (3)	73 (3)	72 (3)	61 (3)	7 (5)	20 (5)	-7 (5)
C(9)	4402 (4)	2603 (4)	-906 (3)	84 (3)	73 (3)	68 (3)	11 (5)	44 (5)	-2 (5)
C(10)	5307 (3)	2762 (3)	-274 (3)	73 (3)	72 (3)	60 (3)	14 (5)	32 (5)	-1 (5)
C(11)	7452 (3)	4409 (4)	2681 (4)	60 (2)	100 (4)	73 (3)	13 (5)	11 (4)	-7 (6)
C(12)	7649 (4)	5205 (4)	3482 (4)	73 (3)	104 (4)	75 (3)	-4 (6)	22 (5)	2 (6)
C(13)	6895 (4)	5909 (4)	3762 (4)	76 (3)	99 (4)	83 (4)	1 (6)	36 (5)	-37 (6)
C(14)	5941 (4)	5755 (4)	3258 (4)	76 (3)	88 (4)	67 (3)	2 (5)	33 (5)	-24 (5)
C(15)	4680 (3)	4668 (3)	1906 (3)	68 (2)	62 (3)	53 (2)	13 (4)	31 (4)	6 (4)
C(16)	4559 (3)	3988 (3)	950 (3)	69 (2)	53 (3)	63 (3)	9 (4)	36 (4)	9 (4)
C(17)	5421 (3)	3462 (3)	677 (3)	73 (3)	58 (3)	61 (3)	10 (4)	34 (4)	2 (4)
C(18)	6460 (3)	4252 (3)	2116 (3)	69 (3)	68 (3)	57 (3)	19 (5)	21 (4)	9 (5)
C(19)	5662 (3)	4883 (3)	2453 (3)	66 (3)	77 (3)	61 (3)	13 (5)	33 (4)	1 (5)
C(20)	1790 (4)	3346 (5)	-1109 (5)	71 (3)	114 (5)	101 (4)	8 (6)	9 (6)	-24 (7)
C(21)	3785 (3)	5516 (4)	3315 (3)	71 (3)	99 (4)	62 (3)	10 (5)	38 (5)	-23 (5)
C(22)	2763 (4)	5283 (5)	3594 (4)	82 (3)	129 (5)	73 (3)	20 (6)	47 (5)	-5 (7)
C(23)	1925 (4)	5906 (5)	2896 (4)	81 (3)	129 (5)	95 (4)	31 (7)	53 (6)	-22 (7)
C(24)	1054 (4)	6209 (6)	1076 (5)	83 (4)	147 (6)	104 (4)	55 (7)	58 (7)	62 (8)
C(25)	1699 (4)	6892 (5)	457 (4)	103 (4)	111 (5)	96 (4)	34 (7)	77 (7)	5 (7)
C(26)	1231 (5)	4207 (6)	1746 (6)	107 (5)	149 (6)	125 (6)	28 (9)	44 (9)	30 (10)
C(27)	318 (6)	4050 (7)	2213 (7)	122 (6)	222 (9)	140 (7)	52 (12)	57 (11)	66 (13)

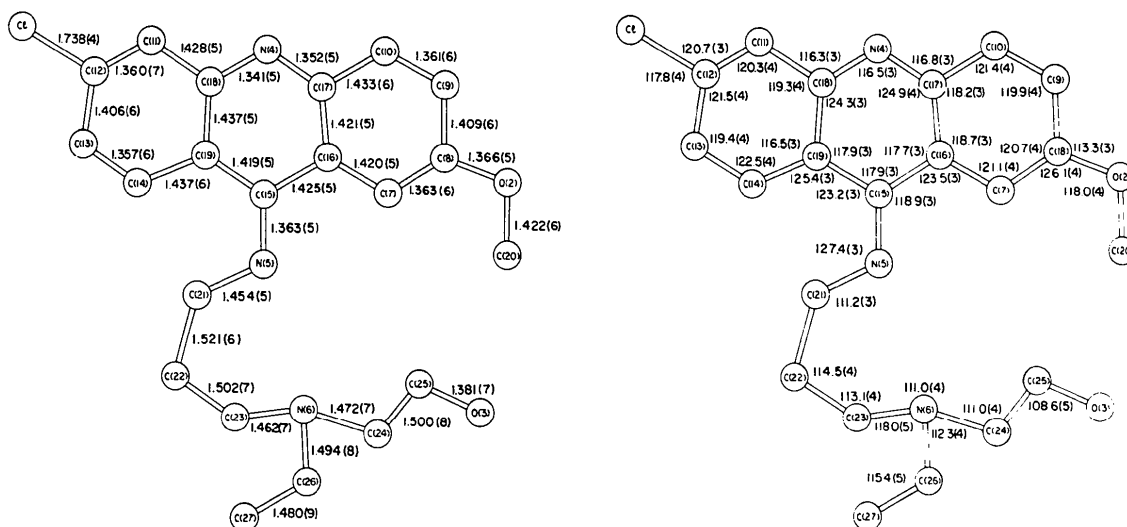


Fig. 2. Interatomic distances (Å) and interbond angles ($^{\circ}$). Numbers in parentheses are e.s.d.'s with respect to the last significant digit. Hydrogen atoms are not included.

Table 1 (cont.)

Parameters for hydrogen atoms.

Positional parameters are multiplied by 10^3 .

	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i>
*H(3)	240	660	- 60	10.0
H(5)	328	505	180	6.5
H(7)	293	423	21	7.4
H(9)	436	213	-150	7.3
H(10)	602	238	- 43	7.1
H(11)	797	395	246	5.5
H(13)	712	663	436	6.3
H(14)	541	634	352	5.9
H(20-1)	183	424	-100	4.5
H(20-2)	161	315	- 51	10.6
H(20-3)	126	316	-200	10.2
H(21-1)	396	625	339	6.7
H(21-2)	431	515	389	4.8
H(22-1)	255	436	360	8.0
H(22-2)	281	557	430	7.0
H(23-1)	218	683	277	8.4
H(23-2)	130	583	334	8.7
H(24-1)	57	694	148	10.7
H(24-2)	65	558	46	9.7
H(25-1)	246	715	96	11.5
H(25-2)	134	760	21	8.3
H(26-1)	96	414	95	16.6
H(26-2)	151	342	197	13.1
*H(27-1)	50	290	220	10.0
*H(27-2)	52	426	318	10.0
H(27-3)	- 21	462	177	9.2

* These hydrogen atoms were not refined but were included in the structure-factor calculation.

The average estimated standard deviation of the hydrogen positional parameters is 0.05 Å and of the temperature parameters 1.2 Å².

for these atoms did not refine well. Full-matrix isotropic refinement of the rest of the hydrogen atoms and anisotropic refinement of the heavier atoms resulted in a final agreement index of 0.068.

The final atomic parameters, together with the estimated standard deviations, are listed in Table 1. The observed and calculated structure factors are listed in Table 2.

Computations

The data reduction was done on an XDS computer at Smith, Kline and French Laboratories, Philadelphia, Pa., with programs written by Craven & Chu (1967). The extension of the phases determined by hand by the symbolic addition procedure, was done on a UNIVAC 1108 computer, *via* a DCT 2000 terminal, with the program written by Hall (1967). All the Fourier, least-squares, and molecular analysis programs were modified by H. L. Carrell from programs written

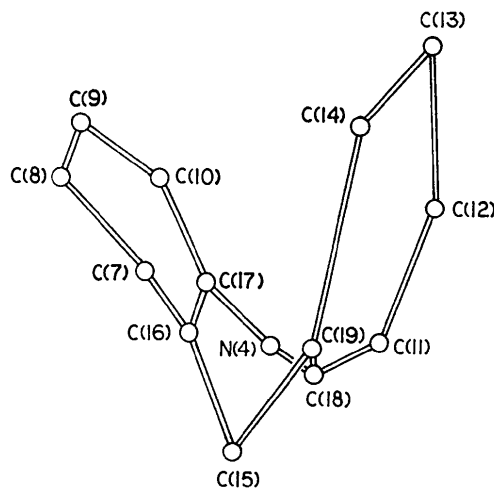


Fig. 3. Distorted view of the ring system of the molecule. The vertical axis is increased in scale by a factor of 20 over that for the horizontal axis.

at UCLA. The full-matrix least-squares refinement was done on a CDC 6600 computer at New York University. The quantity minimized in the least-squares cal-

culations was $\sum w(|F_o| - |F_c|)^2$, with the weights, w , calculated as described by Hughes (1941) (with $4 F_{min} = 5.19$). The atomic scattering factors were taken

Table 2. Observed and calculated structure factors

Each entry lists, in order, h , $|F_o|$ and F_c . Unobserved reflections are not included.

h	F_o	F_c	h	F_o	F_c	h	F_o	F_c	h	F_o	F_c	h	F_o	F_c	h	F_o	F_c	h	F_o	F_c	h	F_o	F_c	h	F_o	F_c	h	F_o	F_c	h	F_o	F_c	h	F_o	F_c	h	F_o	F_c																																																																																																																																																																																																																																																																					
1	100	0	2	100	0	3	100	0	4	100	0	5	100	0	6	100	0	7	100	0	8	100	0	9	100	0	10	100	0	11	100	0	12	100	0	13	100	0	14	100	0	15	100	0	16	100	0	17	100	0	18	100	0	19	100	0	20	100	0	21	100	0	22	100	0	23	100	0	24	100	0	25	100	0	26	100	0	27	100	0	28	100	0	29	100	0	30	100	0	31	100	0	32	100	0	33	100	0	34	100	0	35	100	0	36	100	0	37	100	0	38	100	0	39	100	0	40	100	0	41	100	0	42	100	0	43	100	0	44	100	0	45	100	0	46	100	0	47	100	0	48	100	0	49	100	0	50	100	0	51	100	0	52	100	0	53	100	0	54	100	0	55	100	0	56	100	0	57	100	0	58	100	0	59	100	0	60	100	0	61	100	0	62	100	0	63	100	0	64	100	0	65	100	0	66	100	0	67	100	0	68	100	0	69	100	0	70	100	0	71	100	0	72	100	0	73	100	0	74	100	0	75	100	0	76	100	0	77	100	0	78	100	0	79	100	0	80	100	0	81	100	0	82	100	0	83	100	0	84	100	0	85	100	0	86	100	0	87	100	0	88	100	0	89	100	0	90	100	0	91	100	0	92	100	0	93	100	0	94	100	0	95	100	0	96	100	0	97	100	0	98	100	0	99	100	0	100	100	0

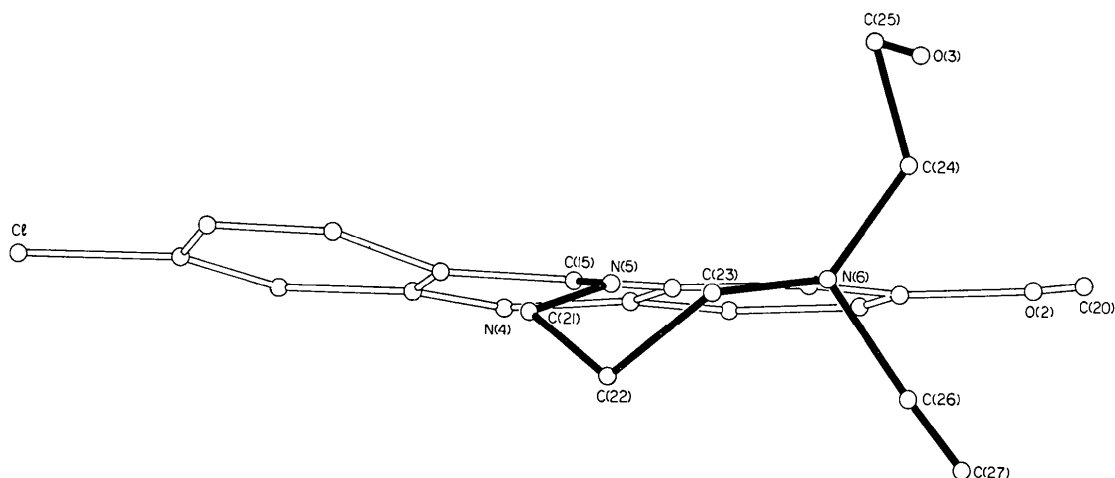


Fig. 4. Undistorted view of the molecule showing the conformation of the side chain.

from *International Tables for X-ray Crystallography* (1962), except those for hydrogen atoms for which the values listed by Stewart, Davidson & Simpson (1965) were used. The chlorine scattering-factor curve was corrected for anomalous dispersion with $\Delta f' = 0.3$.

Discussion of structure

A picture of the molecule, ICR-170-OH, is shown in Fig. 1 in which the thermal ellipsoids are illustrated (Johnson, 1965). In Fig. 2 the bond distances and inter-bond angles are shown. The only significant differences between distances in this molecule and those in ICR-171-OH (Glusker *et al.*, 1971), apart from the additional $-C_2H_5$ and $-CH_2-$ groups in the side chain, are the lengths of the bond C(15)–N(5) [1.363 (5) Å] which has less double-bond character in ICR-171-OH [1.336 (3)], and the short distances C(25)–O(3) [1.381 (7) Å] [1.409 (5) Å for C(24)–O(3) in ICR-171-OH]. The bond C(26)–C(27) of 1.480 (9) Å is 3σ shorter than an average single bond (C–C, 1.508 Å in this structure). This effect is probably a result of high thermal motion in the side chain. In addition, some of the angles in the ring to which the chlorine atom is attached vary significantly between the two structures. In the side chain of ICR-171-OH, the angles at atoms N(5) and C(24) are 130.2 (3) and 113.1 (3); and in this structure, the angles at N(5) and C(25) are 127.4 (3) and 108.6 (5).

Each fused six-membered ring in the acridine moiety is slightly puckered. The outer ring with the methoxy substituent shows the least deviation from planarity. The entire ring system both folds and twists about N(4)···C(15) as shown in Fig. 3. The angle between the best planes through the outer aromatic rings is 10.7° . This angle may be compared with values of 7.5° for ICR-171-OH and $0-2^\circ$ for acridine (Phillips, 1956; Phillips, Ahmed & Barnes, 1960). The angles and distances in the acridine portion of the molecule do not differ significantly from those for acridine except, possibly, C(15)–

C(19) and C(15)–C(16), with values 1.382–1.405 (6) Å for acridine, 1.425 and 1.435 (5) Å in ICR-171-OH, and 1.419 and 1.425 (6) Å in ICR-170-OH. The different lengths of the first bond to the side chain, C(15)–N(5), have already been mentioned. It seems that, while the angles around C(15) add up to 360° in both compounds, the differing bond lengths around it may account for most of the deviation from planarity in the ring system.

The side chain has an intramolecular close contact between N(5) and N(6) of 2.979 Å, with an N–H–N angle of 139° , similar to that present in ICR-171-OH.

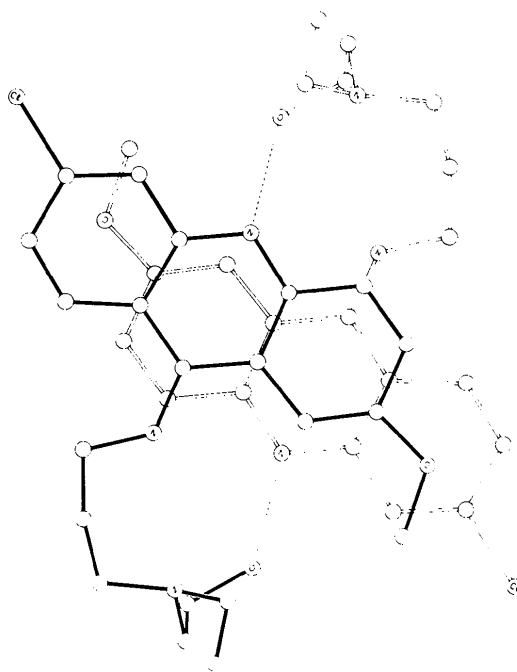


Fig. 5. Overlap of the ring systems of two molecules forming a hydrogen-bonded dimer. The least-squares plane of the ring system is in the plane of the page.

This is apparently a strong interaction since it is present in both ICR-171-OH, in which there are two $-\text{CH}_2-$ groups between the nitrogen atoms, and in ICR-170-OH, in which there are three.

Both structures have apparent intermolecular hydrogen bonds between O(3) and N(4). As shown in Fig. 4, the substituents attached to N(6) are perpendicular to the plane of the ring. Two of the six-membered rings of molecules that are related by a center of inversion partially overlap, and the hydroxyl group of one mol-

ecule hydrogen-bonds to the ring nitrogen atom of the other (Fig. 5). The hydrogen-bonding distance is 2.755 Å. This is a much more compact arrangement than was found for ICR-171-OH. The closest ring-ring distance is 3.50 Å between C(9) of one molecule and C(14) and C(19) of another molecule, overlapping the first and related by a center. There are many other such distances that are less than 3.8 Å. Another less dominant interaction occurs between the molecule at x, y, z and another related by the screw axis at $z = \frac{1}{4}$. Here, the

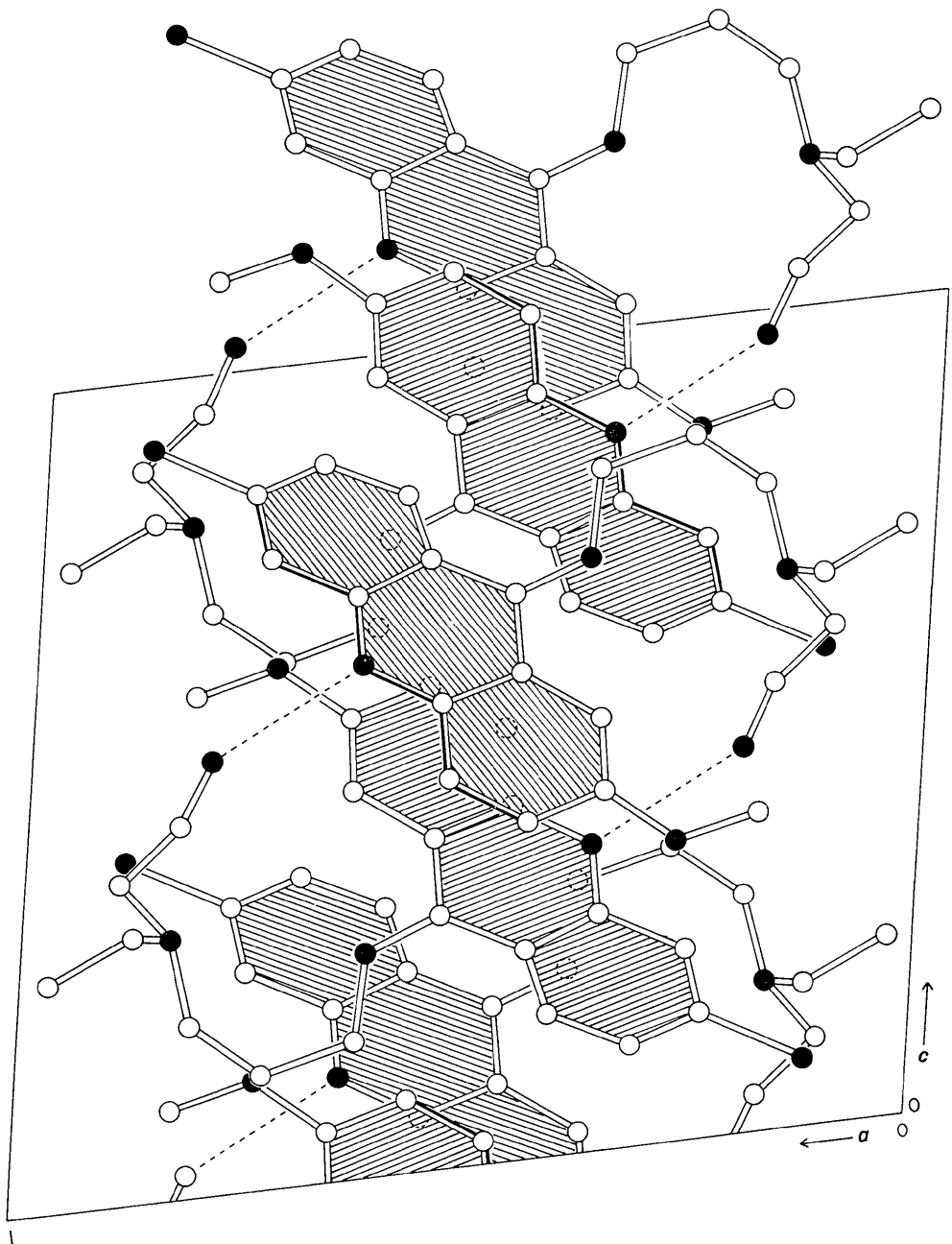


Fig. 6. Packing of molecules. The ring systems are shaded to indicate overlap. The chlorine, nitrogen, and oxygen atoms and the bonds of two molecules that form a hydrogen-bonded dimer are marked in solid black.

only close contact occurs between C(13), C(7), and C(8) of a symmetry-related molecule. A view of the crystal structure down the *b* axis is shown in Fig. 6.

We wish to thank Drs Creech, Peck and Preston for the crystalline sample on which these studies were done. We are grateful to Smith, Kline and French Research Laboratories, Philadelphia, Pa., and to Dr David Zacharias for the use of the Picker automatic diffractometer on which the data were collected, and for the use of their XDS computer for the data reduction. We are also grateful to Miss Joyce Dargay for help with the computing and to Dr H. L. Carrell for help in the preliminary stages of the work. This research was supported by U.S.P.H.S. grants CA-10925, CA-06927, and RR-05539 from the National Institutes of Health, and by an appropriation from the Commonwealth of Pennsylvania. A preliminary account of this work was presented at a meeting of the American Crystallographic Association, Ottawa, Canada, August 16–22, 1970 (Paper D-8).

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Molecular and Crystal Structure of the tRNA Minor Constituent Dihydrouridine

BY DIETRICH SUCK AND WOLFRAM SAENGER

*Max-Planck-Institut für Experimentelle Medizin, Abteilung Chemie, 34 Göttingen,
Hermann-Rein Strasse 3, Germany*

AND KLAUS ZECHMEISTER

*Max-Planck-Institut für Eiweiss- und Lederforschung, Abteilung für Röntgenstrukturforschung,
Schillerstrasse 42–44, 8 München 15, Germany*

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The tRNA minor constituent dihydrouridine crystallized from aqueous *n*-butanol as needles in the space group $P2_12_12_1$ with $a=11\cdot779$, $b=8\cdot150$, $c=23\cdot068$ Å, and two molecules of the nucleoside plus one molecule of water in the asymmetric unit. The structure was solved by direct methods using the tangent formula and refined to an *R* value of 5·1%. The oxygen atom O(5') of one molecule is threefold disordered with the occupational parameters 0·5:0·25:0·25 but generally bond distances and angles are similar in both molecules. Owing to the saturated C(5)–C(6) bond the two nucleobases are puckered so that atom C(6) is on opposite sides of the plane through atoms N(1), C(2), N(3), C(4), *i.e.* the two nucleosides within an asymmetric unit are conformational isomers. Both molecules exhibit the usual *anti* conformation; the conformation of the ribose residues is C(2')-*endo*. The packing of the molecules in the crystal is determined by a network of numerous hydrogen bonds and shows no base stacking.

Introduction

Transfer ribonucleic acids (tRNA's) are macromolecules of molecular weight 25000 to 30000 consisting of about 75 to 85 nucleosides. These nucleosides are not

only adenosine, guanosine, uridine, cytidine but about 10% are modified nucleosides such as dihydrouridine. Since tRNA's are synthesized in the cell on DNA-cistrons it is generally assumed that the 'virgin' tRNA does not contain modified nucleosides *a priori* but that