

## The Crystal Structure of an Analogue of a Mutagen, 2-[3-(6-Chloro-2-methoxy-9-acridinylamino)propylamino]ethanol (ICR-191-OH)

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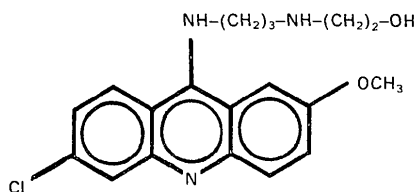
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The crystal structure of the hydroxy derivative of a nitrogen mustard, 2-[3-(6-chloro-2-methoxy-9-acridinylamino)propylamino]ethanol (ICR-191-OH), has been studied. The mustard itself is a strong mutagen for *Salmonella*, much less active for *Neurospora*, and is only slightly active as an antitumor agent in mice bearing ascites tumors. The crystals are monoclinic, space group  $A2/a$ , with eight molecules in a cell of dimensions  $a = 23.957 \pm 0.006$ ,  $b = 10.086 \pm 0.003$ ,  $c = 15.519 \pm 0.004$  Å,  $\beta = 110.92 \pm 0.05^\circ$ . The structure was solved by direct methods and refined by full-matrix least-squares methods, including hydrogen atoms, to  $R = 0.055$  using 2520 observed data measured with an automatic four-circle diffractometer. The acridine portion of the molecule is not planar. There is both a folding of the molecule about the line between the two central atoms of the acridine moiety and a twisting of the two outer rings. There is an intramolecular hydrogen bond between the nitrogen atoms in the side chain and an intermolecular hydrogen bond between the terminal hydroxyl group and the acridine nitrogen atom. There is also a weak intermolecular hydrogen bond between a nitrogen atom in the side chain of one molecule and the terminal hydroxyl group of another molecule.

### Introduction

The nitrogen mustard, ICR-191 (Peck, Preston & Creech, 1961), is a powerful mutagen for *Salmonella*, much less active for *Neurospora*, and is only slightly active as an antitumor agent for ascites tumors. The hydroxy precursor, ICR-191-OH, for which the formula is given below, was provided as yellow rectangular prisms by Drs Creech, Peck, and Preston of this Institute.



This study was undertaken in order to compare the results with those of other acridine analogues which are similar in chemical formulae but show different biological activity. The structures of two other acridine analogues have been reported. They are ICR-170-OH (Berman & Glusker, 1972) and ICR-171-OH (Glusker, Minkin & Orehowsky, 1972). The only chemical difference between the latter two hydroxy precursors of the acridine nitrogen mustard and ICR-191-OH is that the side chain  $-\text{NH}-(\text{CH}_2)_3-\text{NH}-(\text{CH}_2)_2-\text{OH}$  has been replaced by  $-\text{NH}-(\text{CH}_2)_3-\text{N}-(\text{C}_2\text{H}_4\text{OH}) (\text{C}_2\text{H}_5)$  (ICR-170-OH) and by  $-\text{NH}-(\text{CH}_2)_2-\text{NH}-(\text{CH}_2)_2-\text{OH}$  (ICR-171-OH). The biological activities of the three acridine mustards, however, are markedly different (Peck *et al.*, 1961; Ames & Whitfield, 1966; Malling, 1967).

### Experimental

The crystal data for ICR-191-OH are presented in Table 1. For the data collection, a crystal was cut to dimensions  $0.2 \times 0.2 \times 0.25$  mm with a razor blade. Three-dimensional data were collected on the Picker four-circle automatic diffractometer at the University of Pennsylvania. The data were collected with nickel-filtered  $\text{Cu K}\alpha$  radiation out to  $2\theta = 125^\circ$  by the  $\theta$ - $2\theta$  scan technique, using a scan rate of  $1^\circ \text{ min}^{-1}$  and a total background counting time of 40 sec for each reflection. 2804 independent data were measured, of which 2520 had an intensity greater than  $2.33 \times \sigma(I)$  and were considered to be observed. Lorentz and polarization corrections were applied as well as a correction for absorption assuming an ellipsoid of revolution (Johnson, 1963). The minimum and maximum values for  $A^*$  are 1.34 and 1.41 respectively. The standard deviations of the observed structure amplitudes,  $\sigma(F)$ , were calculated from counting statistics and measured instrumental uncertainties. The data were scaled and normalized structure factors,  $|E(hkl)|$ , were calculated. The values for the averages of  $|E|$ ,  $|E|^2$ , and  $|E^2 - 1|$  are 0.779, 0.999 and 1.034 respectively. The space group, therefore, is  $A2/a$ .

### Determination and refinement of the structure

The structure was solved by direct methods with the multiple-solution technique, using the program of Long (1965). The solution of the structure presented some difficulty due to the overall planarity of the molecule; however, the correct  $E$  map revealed the positions of the atoms of the acridine moiety and those

Table 1. *Crystal data for ICR-191-OH*

Formula: C<sub>19</sub>H<sub>22</sub>N<sub>3</sub>O<sub>2</sub>Cl F.W. 359.8  
 Crystal system: monoclinic  
 Cell dimensions:  $a = 23.957 \pm 0.006 \text{ \AA}$   
 $b = 10.086 \pm 0.003$   
 $c = 15.519 \pm 0.004$   
 $\beta = 110.92 \pm 0.05^\circ$   
 $\lambda(\text{Cu } K\alpha_1): 1.5405 \text{ \AA}$   
 Systematic absences:  $hkl$  when  $k+l=2N+1$   
 $h0l$  when  $h=2N+1$   
 $D_m: 1.36 \text{ g.cm}^{-3}$  (floatation)  $D_x: 1.365 \text{ g.cm}^{-3}$   
 $Z: 8$   $\mu(\text{Cu } K\alpha) = 20.8 \text{ cm}^{-1}$   
 Space group:  $A2/a$   
 General equivalent positions:  
 $\pm(x, y, z); \pm(x, \frac{1}{2} + y, \frac{1}{2} + z)$   
 $\pm(\frac{1}{2} + x, -y, z); \pm(\frac{1}{2} + x, \frac{1}{2} - y, \frac{1}{2} + z)$

atoms directly bonded to it. A Fourier synthesis based on these atoms gave the positions of the remainder of the atoms. The structure was refined initially by block-diagonal least-squares methods for only the nonhydrogen atoms with isotropic thermal parameters to  $R=0.14$  where  $R = \sum |F_o - F_c| / \sum |F_o|$ . This was followed by refinement with anisotropic thermal parameters to  $R=0.09$ . At this stage all of the hydrogen atoms were located in a difference map. The structure was ultimately refined by full-matrix least-squares methods, with anisotropic thermal parameters for the heavy atoms and isotropic thermal parameters for the hydrogen atoms, to an  $R$  of 0.055 for the 'observed' data and 0.067 for all data. The final nonhydrogen atomic parameters are given in Table 2 and the parameters for the hydrogen atoms are listed in Table 3. A comparison of the observed and calculated structure factors is presented in Table 4. Of the 284 unobserved data, 16 had calculated intensities greater than the threshold

value and only two had calculated intensities twice the threshold value of 2.33  $\sigma(I)$ .

Table 3. *Atomic positions and isotropic thermal parameters for the hydrogen atoms*

	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i>
H(3)	0.362 (1)	0.187 (2)	0.516 (1)	5.2 (7)
H(5)	0.121 (1)	0.066 (2)	0.258 (1)	3.3 (5)
H(6)	0.215 (1)	0.116 (3)	0.401 (2)	6.7 (9)
H(7)	0.125 (1)	0.261 (2)	0.275 (1)	4.9 (7)
H(9)	0.035 (1)	0.574 (2)	0.173 (1)	5.3 (7)
H(10)	-0.041 (1)	0.467 (2)	0.082 (1)	4.7 (6)
H(11)	-0.141 (1)	0.079 (2)	-0.038 (1)	4.8 (7)
H(13)	-0.060 (1)	-0.274 (2)	0.024 (1)	5.0 (7)
H(14)	0.024 (1)	-0.168 (2)	0.121 (1)	3.5 (6)
H(201)	0.205 (1)	0.529 (3)	0.383 (1)	5.9 (8)
H(202)	0.201 (1)	0.403 (3)	0.321 (2)	7.8 (9)
H(203)	0.176 (1)	0.384 (3)	0.398 (2)	7.6 (9)
H(211)	0.065 (1)	-0.144 (2)	0.266 (1)	4.8 (7)
H(212)	0.115 (1)	-0.168 (2)	0.221 (1)	5.2 (6)
H(221)	0.159 (1)	-0.218 (3)	0.376 (1)	5.8 (7)
H(222)	0.147 (1)	-0.068 (3)	0.399 (2)	6.9 (9)
H(231)	0.246 (1)	-0.108 (3)	0.411 (2)	6.4 (8)
H(232)	0.217 (1)	-0.112 (3)	0.300 (2)	7.6 (8)
H(241)	0.264 (1)	0.109 (3)	0.276 (2)	7.9 (8)
H(242)	0.262 (1)	0.237 (3)	0.330 (2)	7.5 (8)
H(251)	0.360 (1)	0.149 (3)	0.387 (2)	6.4 (8)
H(252)	0.340 (1)	0.008 (2)	0.412 (1)	5.3 (7)

### Computations

All computations were done on UNIVAC 1108 and CDC 6600 computers *via* a DTC 2000 remote terminal in this laboratory. The block-diagonal least-squares program was a modified version of that written by Harris (1966) and the full-matrix program

Table 2. *Atomic positions and thermal parameters for the nonhydrogen atoms*

The temperature factor is of the form:  $T = \exp(-h^2b^{11} - k^2b^{22} - l^2b^{33} - hkb^{12} - hlb^{13} - klb^{23})$ .

	<i>x</i>	<i>y</i>	<i>z</i>	10 <sup>4</sup> <i>b</i> <sup>11</sup>	10 <sup>4</sup> <i>b</i> <sup>22</sup>	10 <sup>4</sup> <i>b</i> <sup>33</sup>	10 <sup>4</sup> <i>b</i> <sup>12</sup>	10 <sup>4</sup> <i>b</i> <sup>13</sup>	10 <sup>4</sup> <i>b</i> <sup>23</sup>
Cl(1)	-0.17176 (3)	-0.18354 (7)	-0.08811 (5)	18.8 (1)	124 (1)	61.2 (3)	-27 (1)	6.7 (3)	-27 (1)
O(2)	0.1296 (1)	0.5219 (2)	0.2895 (1)	23.9 (4)	74 (2)	71 (1)	-18 (1)	26 (1)	-30 (2)
O(3)	0.3303 (1)	0.1569 (2)	0.4898 (1)	19.3 (3)	159 (2)	52 (1)	-44 (1)	21 (1)	-32 (2)
N(4)	-0.0588 (1)	0.2263 (2)	0.0607 (1)	15.8 (3)	74 (2)	47 (1)	11 (1)	16 (1)	7 (2)
N(5)	0.0935 (1)	0.0230 (2)	0.2338 (1)	14.4 (3)	64 (2)	51 (1)	-1 (1)	6 (1)	9 (2)
N(6)	0.2146 (1)	0.0769 (2)	0.3523 (1)	15.4 (4)	98 (2)	45 (1)	2 (2)	9 (1)	-10 (3)
C(7)	0.0904 (1)	0.3026 (2)	0.2353 (2)	16.6 (4)	71 (2)	47 (1)	0 (2)	20 (1)	0 (3)
C(8)	0.0867 (1)	0.4382 (2)	0.2340 (2)	20.3 (4)	70 (2)	53 (1)	-11 (2)	31 (1)	-17 (3)
C(9)	0.0358 (1)	0.5008 (2)	0.1739 (2)	26.8 (5)	53 (2)	74 (2)	7 (2)	35 (1)	8 (3)
C(10)	-0.0104 (1)	0.4306 (2)	0.1181 (2)	19.9 (5)	70 (2)	60 (1)	18 (2)	19 (1)	16 (3)
C(11)	-0.1078 (1)	0.0278 (2)	-0.0032 (2)	14.8 (4)	95 (3)	43 (1)	4 (2)	14 (1)	5 (3)
C(12)	-0.1085 (1)	-0.1051 (3)	-0.0122 (2)	16.8 (4)	97 (3)	41 (1)	-17 (2)	19 (1)	-10 (3)
C(13)	-0.0588 (1)	-0.1826 (2)	0.0358 (2)	19.0 (5)	71 (2)	51 (1)	-9 (2)	17 (1)	0 (3)
C(14)	-0.0095 (1)	-0.1228 (2)	0.0962 (2)	16.4 (4)	73 (2)	45 (1)	4 (2)	10 (1)	13 (3)
C(15)	0.0444 (1)	0.0824 (2)	0.1756 (1)	14.7 (4)	69 (2)	36 (1)	6 (2)	18 (1)	10 (2)
C(16)	0.0426 (1)	0.2252 (2)	0.1763 (1)	16.9 (4)	64 (2)	39 (1)	3 (2)	23 (1)	3 (3)
C(17)	-0.0094 (1)	0.2896 (2)	0.1174 (1)	17.3 (4)	67 (2)	43 (1)	10 (2)	23 (1)	11 (3)
C(18)	-0.0563 (1)	0.0927 (2)	0.0589 (1)	15.1 (4)	76 (2)	39 (1)	3 (2)	19 (1)	3 (3)
C(19)	-0.0060 (1)	0.0157 (2)	0.1131 (1)	14.9 (4)	65 (2)	37 (1)	-1 (2)	17 (1)	5 (2)
C(20)	0.1823 (1)	0.4627 (3)	0.3513 (2)	20.9 (5)	106 (3)	75 (2)	-23 (2)	24 (1)	-44 (4)
C(21)	0.1040 (1)	-0.1131 (2)	0.2658 (2)	17.5 (5)	62 (2)	52 (1)	7 (2)	10 (1)	7 (3)
C(22)	0.1552 (1)	-0.1207 (3)	0.3576 (2)	22.9 (5)	79 (3)	55 (1)	13 (2)	8 (1)	19 (3)
C(23)	0.2139 (1)	-0.0684 (3)	0.3574 (2)	18.2 (5)	105 (3)	63 (2)	24 (2)	1 (1)	-10 (4)
C(24)	0.2661 (1)	0.1314 (3)	0.3345 (2)	19.7 (5)	156 (4)	49 (1)	-5 (2)	20 (1)	0 (4)
C(25)	0.3266 (1)	0.1040 (3)	0.4036 (2)	18.9 (5)	124 (3)	55 (1)	-7 (2)	23 (1)	-28 (3)



was that of Gantzel, Sparks, Long & Trueblood (UCLALS4). The molecular geometry calculations were carried out using a program written by the author and A. Caron. The quantity minimized in the least-squares calculations was  $w(|kF_o| - |F_c|)^2$  with weights,  $w$ , being  $1/[\sigma(F)]^2$  for the observed data and zero for the unobserved data. The atomic scattering factors for the nonhydrogen atoms were taken from *International Tables for X-ray Crystallography* (1962) and for the hydrogen atoms, the values of Stewart, Davidson & Simpson (1965) were used. The chlorine scattering factor curve was corrected for the real component of anomalous dispersion, with  $Af' = 0.3$  (*International Tables for X-ray Crystallography*, 1962).

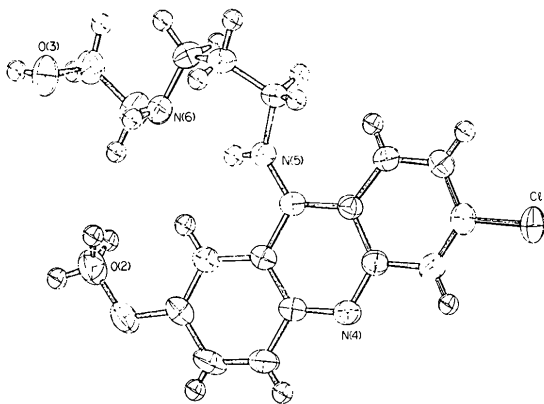


Fig. 1. ORTEP drawing of the molecule showing the thermal ellipsoids, which are drawn at 50% probability.

## Discussion

A diagram of the molecule, ICR-191-OH, showing the thermal ellipsoids (Johnson, 1965) is presented in Fig. 1. The bond lengths and angles are shown in Fig. 2. Within the acridine moiety itself, the only significant difference found between ICR-191-OH and the two previously reported acridine analogues, ICR-171-OH (Glusker *et al.*, 1972) and ICR-170-OH (Berman & Glusker, 1972), is that the C(15)–C(16) bond length (1.441 Å) appears to be significantly longer than the C(15)–C(19) bond length (1.421 Å), whereas the two lengths are the same in both ICR-170-OH (1.422 ± 0.005 Å) and ICR-171-OH (1.430 ± 0.007 Å). In a comparison within acridine (Phillips, 1956; Phillips, Ahmed & Barnes, 1960), the only significant differences in the bonding with the acridine moiety are found in the slightly longer C(15)–C(16) and C(15)–C(19) bonds, which for acridine range from 1.382–1.405 Å, and the C(8)–C(9) (1.395 Å) and C(12)–C(13) (1.396 Å) bond lengths, which are shorter than those found for acridine (1.415–1.436 Å). The C(15)–N(5) bond length, the link of the side chain to the acridine moiety, is 1.342 Å which is similar to that (1.332 Å) found in ICR-171-OH. At the same time, the angle C(15)–N(5)–C(21) of 131.1° found in ICR-191-OH is similar to that found in ICR-171-OH (130.2°) but greater than that found in ICR-170-OH (127.4°).

The side chain  $-\text{NH}-(\text{CH}_2)_3-\text{NH}-(\text{CH}_2)_2-\text{OH}$  in the ICR-191-OH molecule is curled rather than extended in a zigzag pattern. The side chain also curls in and out of the plane of the acridine. The acridine moiety itself is not planar (Table 5), the best description of the non-

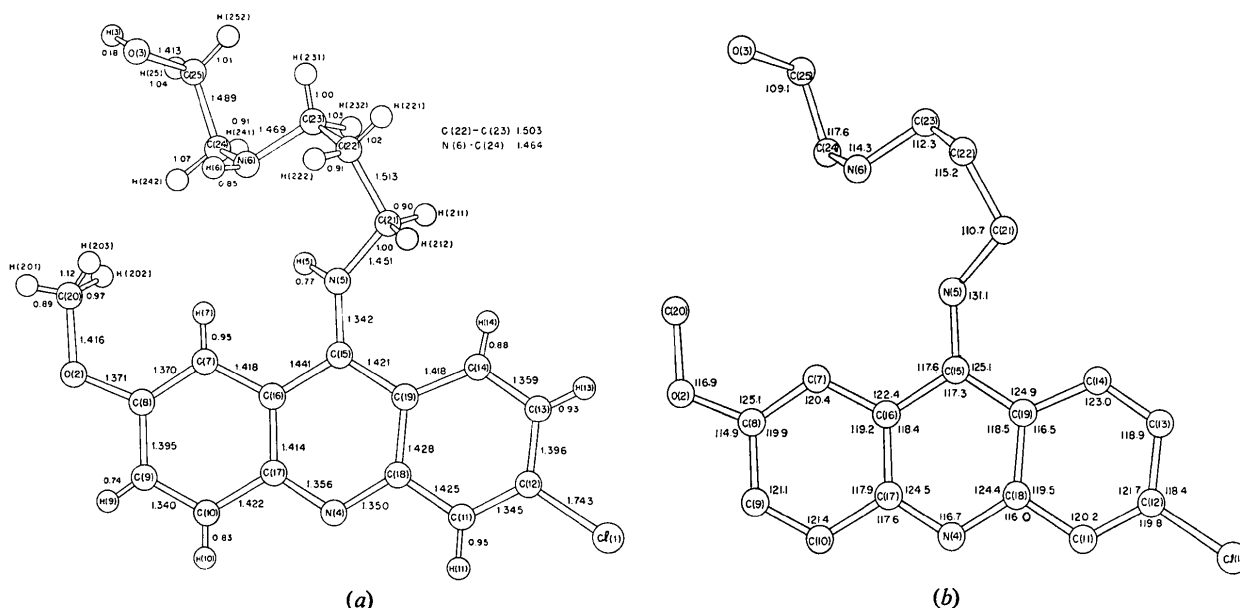


Fig. 2. (a) Bond lengths and (b) bond angles for ICR-191-OH. The e.s.d. for C–C, C–O, C–N, and C–Cl bonds is 0.003 Å, and for C–H, N–H, and O–H bonds the e.s.d. is 0.03 Å.

planarity of the acridine moiety being that the fused six-membered rings both fold and twist about the C(15)···N(4) line (Fig. 3). The overall nonplanarity of the acridine moiety is somewhat less pronounced than that found for the acridine systems of ICR-170-OH and ICR-171-OH. The angle between the best least-squares planes of the two outer rings of the acridine system is  $4.9^\circ$ , while for ICR-170-OH and ICR-171-OH this angle has values of  $10.7$  and  $7.5^\circ$  respectively. Taken separately, neither of the two outer fused rings is planar, each having a puckered conformation; however, the ring having the methoxy sub-

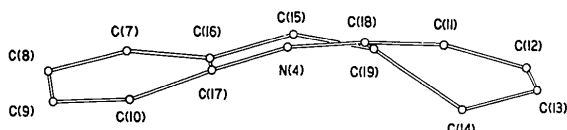


Fig. 3. A distorted view of the acridine portion of the molecule. The vertical scale has been increased eightfold to show the nature of the nonplanarity more clearly.

stituent demonstrates a higher degree of planarity than the ring having the chlorine substituent.

The molecular packing is illustrated in Fig. 4. Chains of molecules are linked by hydrogen-bonding of the terminal hydroxyl, O(3)–H(3), with the acridine nitrogen N(4), the O(3)–H···N(4) separation being  $2.75 \text{ \AA}$  with an angle O(3)–H(3)···N(4) of  $170^\circ$ . There is also a weak intermolecular hydrogen bond formed between N(6)–H(6) on one molecule and the terminal hydroxyl oxygen atom, O(3), of the side chain of another. The N(6)–H···O(3) separation is  $3.11 \text{ \AA}$  with an N–H···O angle of  $148^\circ$ . In addition, each molecule has an intramolecular hydrogen bond between the two nitrogen atoms of the side chain. The N(5)–H···N(6) distance is  $2.88 \text{ \AA}$  and the N(5)–H(5)···N(6) angle is  $148^\circ$ .

A feature of the molecular packing is the stacking of the molecules which is illustrated in Fig. 5. The stacking is such that each molecule has two near neighbors, each related to the first by a center of symmetry. The separation of the best least-squares planes of the acridine moiety are  $3.34 \text{ \AA}$  and  $3.47 \text{ \AA}$ , the first related

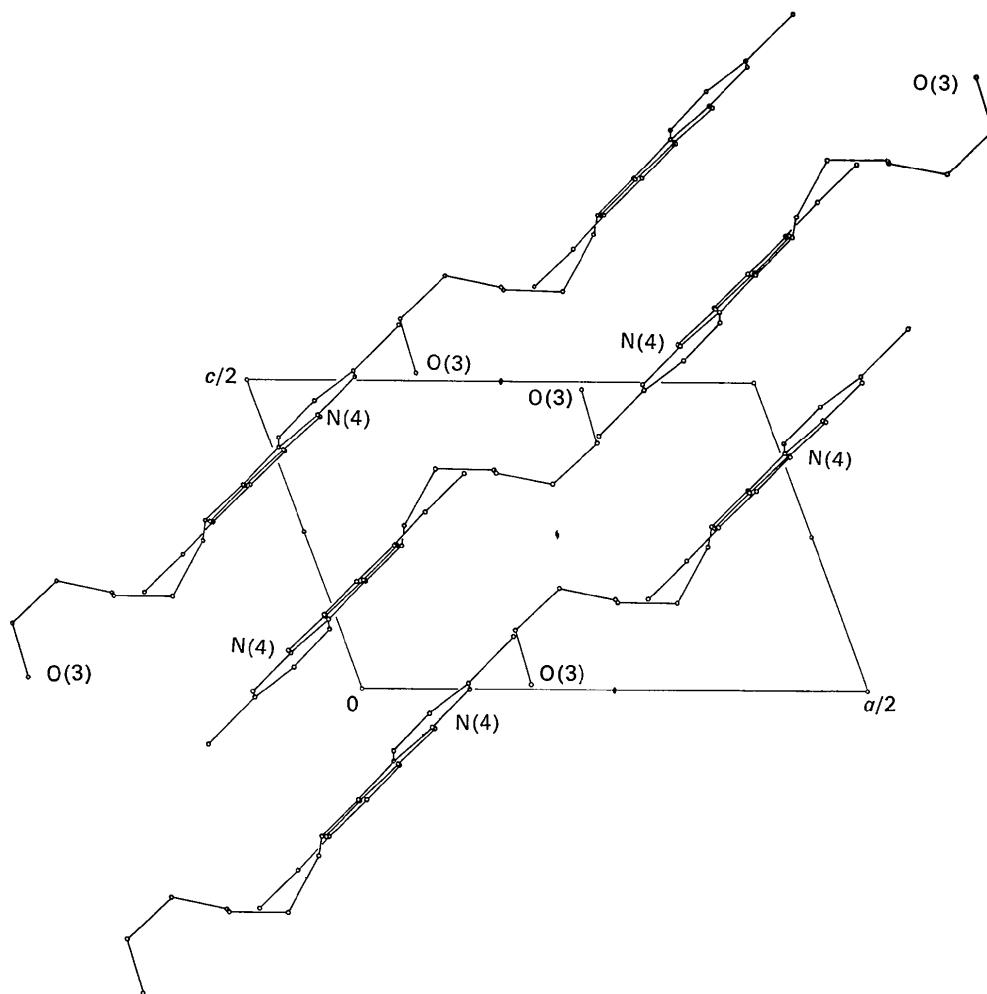


Fig. 4. Molecular packing diagram as viewed down the *b* axis.

Table 5. Deviations (in Å) from the best least-squares planes for (a) the acridine moiety (the atoms included in the acridine moiety are marked with an asterisk), (b) the ring containing C(7), C(8), C(9), C(10), C(16), C(17), and (c) the ring containing C(11), C(12), C(13), C(14), C(18), and C(19)

	(a)	(b)	(c)
C(1)	0.007		
O(2)	0.033		
O(3)	-0.209		
N(4)	0.039*		
N(5)	0.115		
N(6)	-0.220		
C(7)	0.029*	0.001	
C(8)	-0.005*	0.007	
C(9)	-0.060*	-0.006	
C(10)	-0.057*	-0.003	
C(11)	0.045*		-0.005
C(12)	0.004*		0.021
C(13)	-0.086*		-0.013
C(14)	-0.070*		-0.011
C(15)	0.060*		
C(16)	0.019*	-0.010	
C(17)	-0.002*	0.011	
C(18)	0.047*		0.019
C(19)	0.037*		0.027
C(20)	0.081		
C(21)	0.475		
C(22)	0.948		
C(23)	-0.053		
C(24)	-1.355		
C(25)	-1.350		

Equations of the planes (a), (b), and (c) with respect to the crystal axes, where  $x$ ,  $y$  and  $z$  are fractional coordinates, are:

$$\begin{aligned} (a) \quad & -16.4985x - 0.5658y + 14.2932z - 1.6711 = 0 \\ (b) \quad & -16.8352x - 0.2780y + 14.1974z - 1.7335 = 0 \\ (c) \quad & -16.1975x - 1.0757y + 14.3127z - 1.6741 = 0 \end{aligned}$$

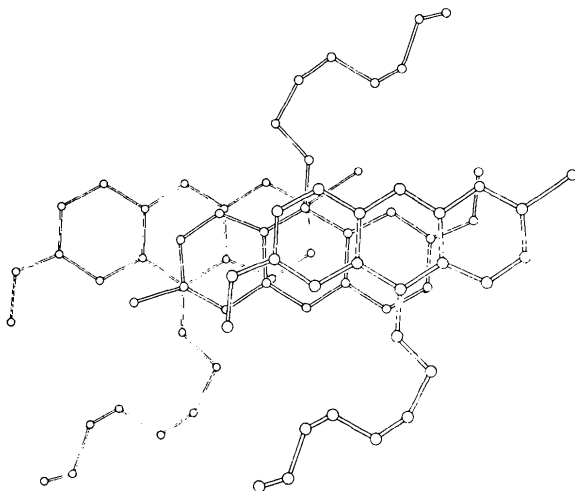


Fig. 5. Molecular overlap packing diagram projected onto the plane of the molecule.

to the molecule at  $(x, y, z)$  by the center at the origin and the latter by the center at  $(0, \frac{1}{4}, \frac{1}{4})$ . In the case of the molecules at  $(x, y, z)$  and  $(\bar{x}, \bar{y}, \bar{z})$ , the closest interatomic approaches occur between C(14)···C(18) and C(12)···C(15) where the distances are 3.33 Å and 3.41 Å respectively. Relative to the stacking planes, these atoms are very nearly superposed. The closest interatomic approach between the molecules at  $(x, y, z)$  and  $(-x, \frac{1}{2} - y, \frac{1}{2} - z)$  is 3.43 Å between C(11) and C(20). As seen in the diagram (Fig. 5), the area of overlap of the molecules related by the center at the origin is somewhat less than the overlap area of the molecules related by the center at  $(0, \frac{1}{4}, \frac{1}{4})$ . As might be expected, the area of overlap is greater for the more planar portion of the molecule.

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