

Proflavine Dichloride Dihydrate (3,6-Diaminoacridine Dihydrochloride Dihydrate)

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(Received 30 November 1973; accepted 18 January 1974)

Proflavine dichloride dihydrate (3,6-diaminoacridine dihydrochloride dihydrate), $C_{13}N_3H_{11} \cdot 2HCl \cdot 2H_2O$, crystallizes in the monoclinic space group $P2_1/a$, $a=17.960$ (2), $b=7.156$ (1), $c=12.615$ (1) Å, $\beta=109.47^\circ$ (1), $V=1528.6$ (3) Å³, F.W. 318.21, $Z=4$, $D_x=1.38$, $D_{obs}=1.37$ g cm⁻³, $\lambda(Cu K\alpha)=1.5418$ Å, $\mu(Cu K\alpha)=37.4$ cm⁻¹. A structural determination by X-ray diffraction resulted in a final residual $R=0.074$ for 1170 observed reflections. The hydrogen atoms were located from a difference map and were refined isotropically. The proflavine cations are stacked with considerable molecular overlap between nearest neighbor molecules. In the cation studied here, the protons are on the nitrogen atom of the central ring and on one amino group. The ring nitrogen atom is hydrogen bonded to a chloride ion.

Introduction

Detailed studies of the interaction of proflavine with DNA (Li & Crothers, 1969) have been made and it is believed that the interaction occurs *via* an intercalation mechanism. In order to obtain precise interatomic distances and angles and to determine the nature of the stacking of molecules in the crystalline state a crystal structure determination of the dihydrochloride dihydrate was done.

Crystals of proflavine dichloride dihydrate were prepared by dissolving commercial proflavine sulphate in water, neutralizing the solution with dilute aqueous ammonia, and extracting the free base into chloroform. Treatment of the dried extract with concentrated hydrochloric acid gave long needles of proflavine dihydrochloride dihydrate on standing. Unit-cell dimensions are listed in Table 1. Details of the data collection are given in Table 2.

Table 1. Unit-cell data for proflavine dichloride dihydrate

Formula	$C_{13}N_3H_{11} \cdot 2HCl \cdot 2H_2O$
Crystal system	Monoclinic
Space group	$P2_1/a$
Cell dimensions	$a=17.960$ (2) Å $b=7.156$ (1) $c=12.615$ (1) $\beta=109.47$ (1)° $V=1528.6$ (29) Å ³
Observed density	1.37 g cm ⁻³
Calculated density	1.38
Z	4
F.W.	318.21

Structure determination

The structure was solved by direct methods with the program *MULTAN* (Main, Woolfson & Germain,

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Table 2. Details of the data collection

Radiation used	Cu $K\alpha$ monochromatized
Diffraction used	Automated four circle
Systematic absences	$h0l$ when h odd, $0k0$ when k odd.
Scanning technique	$\theta-2\theta$ scan, variable rate
Range of $\sin \theta/\lambda$	0.05–0.61 Å ⁻¹
Number of reflections measured	2889
Number of theoretically accessible reflections	3502
Number of reflections below threshold value	1719
Criterion for threshold value	$I_{obs}=2.33 \sigma(I)$
Criterion for $\sigma(I)$	counting statistics
Criterion for $\sigma(F)$	$\sigma(F)=(F/2) \{[\sigma^2(I)/I^2] + \delta^2\}^{1/2}$; δ =measured instrumental uncertainty=0.0200
Intensity loss with time during data collection	22% over 166 h. This was corrected for.
Crystal dimensions	0.24 × 0.10 × 0.04 mm
Nature of absorption correction	Ellipsoid of revolution (Johnson, 1963)
Linear absorption coefficient	37.49 cm ⁻¹ (Cu $K\alpha$)
Other corrections	Lorentz, polarization factors, scale (from Wilson plot)

1971) and the hydrogen atoms were located from a difference map, by means of the X-RAY system of programs (Stewart, Kruger, Ammon, Dickinson & Hall, 1972). The parameters were refined by a full-matrix least-squares method (Gantzel, Sparks, Long & Trueblood, 1969). The non-hydrogen atoms were assigned anisotropic temperature factors and the hydrogen atoms were assigned isotropic temperature factors during the refinement. Weights of reflections were $1/[\sigma^2(F)]$ with zero weights for those reflections below the threshold value. The quantity minimized in the least-squares calculation was $\sum w\{|F_o| - |F_c|\}^2$. Scattering factors were those of Cromer & Mann (1968) for Cl, N, O, C and of Stewart, Davidson & Simpson (1965) for H, and anomalous dispersion corrections for Cl were those listed by Cromer & Liberman (1970). The

Table 3. *Final atomic parameters*

Positional parameters are given as fractions of cell edges $\times 10^4$ ($\times 10^3$ for hydrogen). Anisotropic temperature factors are expressed as $\exp[-\frac{1}{2}(h^2a^{*2}B_{11} + k^2b^{*2}B_{22} + l^2c^{*2}B_{33} + 2hka^*b^*B_{12} + 2hla^*c^*B_{13} + 2klb^*c^*B_{23})]$ and isotropic temperature factors as $\exp(-B \sin^2 \theta/\lambda^2)$ with B values given in \AA^2 . The standard deviations for each parameter, determined from the inverted full matrix, are given in parentheses and apply to the last specified digits. The numbering system of *Chemical Abstracts* is used with 4a, 8a, 9a and 10a replaced by 11–14 respectively.

	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
Cl(1)	1547 (1)	3590 (4)	0186 (2)	3.8 (1)	5.3 (1)	3.8 (1)	0.1 (2)	0.7 (2)	-0.4 (2)
Cl(2)	2536 (1)	-0146 (4)	6216 (2)	3.7 (1)	5.0 (1)	5.8 (1)	-0.5 (2)	2.1 (2)	-1.3 (3)
O(W1)	3302 (3)	2981 (9)	7966 (5)	6.3 (3)	6.2 (4)	4.8 (3)	-2.1 (7)	2.8 (6)	-1.1 (6)
O(W2)	2207 (4)	0575 (10)	3611 (6)	7.4 (4)	6.2 (5)	7.0 (4)	1.1 (7)	2.3 (8)	-0.7 (7)
N(10)	-0868 (3)	1695 (9)	3952 (5)	2.2 (2)	3.1 (4)	3.3 (3)	0.5 (5)	1.1 (5)	0.1 (6)
N(15)	-1805 (4)	0655 (10)	-0054 (5)	4.6 (3)	4.6 (5)	2.7 (3)	-0.3 (7)	1.2 (6)	0.6 (6)
N(16)	-0069 (4)	2783 (10)	7909 (5)	4.5 (3)	3.9 (4)	2.2 (3)	-1.1 (7)	0.4 (6)	-0.4 (6)
C(1)	0135 (4)	2419 (12)	1855 (8)	4.0 (4)	3.8 (5)	4.6 (4)	-0.4 (8)	2.7 (8)	-0.1 (8)
C(2)	-0472 (5)	1899 (13)	0887 (7)	4.5 (4)	3.4 (5)	4.1 (4)	0.7 (8)	1.5 (8)	0.6 (9)
C(3)	-1185 (5)	1298 (13)	0977 (7)	3.0 (4)	3.1 (5)	3.8 (4)	0.4 (8)	0.4 (7)	0.8 (8)
C(4)	-1343 (4)	1212 (13)	1955 (7)	3.6 (3)	4.2 (5)	3.0 (4)	0.3 (8)	1.9 (7)	0.6 (9)
C(5)	-0515 (4)	2189 (11)	5932 (6)	2.8 (3)	2.7 (4)	2.6 (4)	0.4 (7)	0.3 (6)	0.0 (7)
C(6)	0073 (4)	2739 (11)	6942 (7)	2.9 (3)	2.2 (4)	3.4 (4)	0.4 (7)	1.3 (7)	0.6 (7)
C(7)	0834 (5)	3341 (12)	6937 (7)	3.1 (4)	2.6 (5)	3.7 (4)	-0.1 (8)	0.5 (7)	0.0 (8)
C(8)	1014 (4)	3400 (13)	5999 (7)	1.7 (3)	3.2 (5)	4.5 (5)	-0.1 (8)	0.1 (7)	0.7 (9)
C(9)	0574 (5)	2935 (12)	3921 (7)	2.6 (3)	2.1 (4)	4.6 (5)	0.1 (7)	1.4 (8)	0.3 (8)
C(11)	-0734 (5)	1794 (12)	2947 (8)	3.0 (4)	1.9 (5)	3.4 (4)	-0.2 (8)	1.1 (8)	0.7 (8)
C(12)	0445 (5)	2854 (12)	4938 (8)	2.0 (3)	2.3 (4)	4.2 (5)	0.2 (7)	1.4 (8)	0.5 (8)
C(13)	0005 (5)	2367 (12)	2916 (8)	2.3 (3)	3.5 (5)	3.0 (5)	-0.2 (7)	0.8 (8)	0.7 (8)
C(14)	-0306 (4)	2252 (12)	4961 (7)	2.2 (3)	2.2 (4)	3.2 (4)	0.4 (7)	0.7 (7)	0.5 (7)

Table 3 (cont.)

	x	y	z	B
H(1)	68 (3)	294 (7)	182 (4)	12 (3)
H(2)	-33 (5)	155 (13)	59 (7)	3 (1)
H(4)	-189 (3)	78 (7)	201 (4)	4 (1)
H(5)	-130 (3)	157 (8)	588 (4)	1 (1)
H(7)	124 (3)	352 (9)	761 (5)	5 (2)
H(8)	156 (2)	375 (6)	598 (3)	7 (2)
H(9)	116 (3)	334 (8)	396 (4)	11 (3)
H(10)	-124 (5)	175 (13)	393 (7)	1 (1)
H(15)	-181 (5)	-79 (14)	-19 (7)	10 (2)
H(15')	-173 (3)	127 (9)	-56 (5)	3 (1)
H(15'')	-243 (4)	97 (11)	-13 (7)	10 (2)
H(16)	-59 (5)	237 (12)	788 (7)	4 (2)
H(16')	24 (4)	317 (10)	853 (5)	12 (3)
H(W1)	309 (3)	188 (9)	788 (5)	17 (4)
H(W1')	343 (5)	311 (13)	771 (7)	11 (3)
H(W2)	210 (5)	180 (16)	355 (8)	6 (2)
H(W2')	219 (5)	40 (15)	404 (8)	6 (3)

final residual was $R=0.074$ with a weighted R of 0.061. Final parameters are listed in Table 3.*

Discussion of structure

The bond lengths and interbond angles are shown in Fig. 1. The molecule is diionized, the two additional protons residing on the central nitrogen atom, N(10) and on one amino group, N(15). The other amino group N(16) only has two protons and the C–N distance, C(6)–N(16), is 1.33 \AA . This bond has consider-

able double-bond character [similar to the C–NH₂ bond in 9-aminoacridine (Talacki, Carrell & Glusker, 1974)] suggesting some positive charge on N(16) as well as on N(10) and N(15). The single-bond distance, C(3)–N(15) to the protonated amino group, is 1.48 \AA in length. The different states of protonation of N(15) and N(16) cause differences in bond lengths in the outer rings, as indicated by the lack of a vertical mirror plane in Fig. 1. The pK values for proflavine are 9.65 and 1.5 (Albert, 1966) representing ionization of the ring nitrogen atom and of one amino group respectively. The state of ionization is confirmed both by the hydrogen-atom positions (as located from the difference map) and by the hydrogen bonding (as shown in Table 4). As noted by the distances and angles in Table 4, the hydrogen bonding of H(W1) and H(W1') is not very good. The hydrogen atom, H(10), attached to N(10) in the central ring system is hydrogen bonded to a chloride ion, Cl(2¹). Two of the hydrogen atoms at N(15) are involved in hydrogen bonds to chloride ions and the third is involved in a hydrogen bond to a water molecule. The amino group, N(16), is hydrogen bonded to a water molecule through H(16) while the other hydrogen atom, H(16'), points towards a chloride ion although distances, as shown in Table 4, may be rather long for a hydrogen bond.

As shown in Figs. 2 and 3, the molecules stack above and below each other across the centers of symmetry along the b axis with interplanar spacings of 3.34 and 3.39 \AA . In solution it is believed that only dimerization occurs (Turner, Flynn, Lundberg, Faller & Sutin, 1972). Columns of proflavine molecules are held together in the crystal by the hydrogen-bond system.

As shown in Table 4 the three nitrogen atoms of proflavine lie in a straight line at 4.8 \AA apart. These

* A table of observed and calculated structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 30362 (23 pp., 1 microfiche). Copies may be obtained from the Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

hydrogen bond, as shown in Fig. 2, to the approximately linear system $\text{Cl}(1^{\text{II}}) \cdots \text{Cl}(2^{\text{I}}) \cdots \text{O}(\text{W}1^{\text{III}})$.

The vertical stacking of the proflavine ring systems results in the non-protonated amino group, N(16), lying over the outer ring system of another proflavine molecule. The protonated amino group, N(15), does not lie over a ring possibly because of the greater bulkiness of the non-planar $-\text{NH}_3^+$ group compared to that of the planar $=\text{NH}_2^+$ group.

The fused acridine ring system of the proflavine

molecule is nearly planar; the largest deviation from the least-squares plane is -0.032 \AA at N(10) (Table 5). Each of the three rings is planar within experimental error and the angle between the two outer rings is 1.3° . This may be compared with values of 1.2° for acridine (Phillips, 1956; Phillips, Ahmed & Barnes, 1960) and 1.0° for 9-aminoacridine (Talacki *et al.*, 1974). The nitrogen atom of the non-protonated amino group, N(16), lies 0.086 \AA out of the plane of the acridine moiety while that of the protonated amino group, N(15), lies 0.38 \AA from this plane.

Table 4. Hydrogen-bond system

Donor (D)	Acceptor (A)	$D \cdots A$	H-D	$H \cdots A$	$\angle A \cdots H-D$	$\angle A \cdots D-H$
N(10)—H(10) \cdots Cl(2 ^I)		3.135 (4) Å	0.66 (6) Å	2.55 (7) Å	149 (10)°	24 (8)°
N(15)—H(15) \cdots Cl(1 ^{II})		3.086 (7)	1.05 (10)	2.06 (7)	167 (6)	9 (4)
N(15)—H(15') \cdots O(W1 ^{III})		2.748 (8)	0.82 (6)	1.96 (6)	161 (6)	13 (4)
N(15)—H(15'') \cdots Cl(1 ^{II})		3.121 (5)	1.12 (5)	2.02 (5)	165 (7)	10 (4)
N(16)—H(16) \cdots O(W1 ^{III})		3.000 (6)	0.96 (6)	2.05 (6)	168 (7)	8 (5)
N(16)—H(16') \cdots Cl(1 ^I)		3.382 (6)	0.84 (6)	2.59 (6)	159 (5)	16 (4)
O(W1)—H(W1) \cdots Cl(2)		3.121 (6)	0.86 (6)	2.48 (6)	132 (5)	36 (4)
O(W1)—H(W1') \cdots O(W2 ^{II})		2.650 (9)	0.46 (7)	2.44 (9)	113 (11)	58 (10)
O(W2)—H(W2) \cdots Cl(2 ^{II})		3.094 (7)	0.90 (11)	2.27 (11)	152 (6)	20 (4)
O(W2)—H(W2') \cdots Cl(2)		3.182 (7)	0.56 (9)	2.64 (9)	165 (8)	13 (7)

A	B	C	$A \cdots B$	$B \cdots C$	$A \cdots C$	$A \cdots B \cdots C$
N(15)	N(10)	N(16)	4.822 (9) Å	4.775 (8) Å	9.594 (9) Å	177.2 (1)°
Cl(1 ^{II})	Cl(2 ^I)	O(W1 ^{III})	4.375 (3)	5.157 (6)	9.262 (6)	152.6 (1)

Superscript notation

None	x	y	z ;	iv	$-x$	$-y$	$-z$
i	$-x$	$-y$	$1-z$;	v	x	y	$1+z$
ii	$x-\frac{1}{2}$	$\frac{1}{2}-y$	z ;	vi	$\frac{1}{2}-x$	$\frac{1}{2}+y$	$1-z$
iii	$x-\frac{1}{2}$	$\frac{1}{2}-y$	$z-1$;	vii	$-x$	$1-y$	$-z$

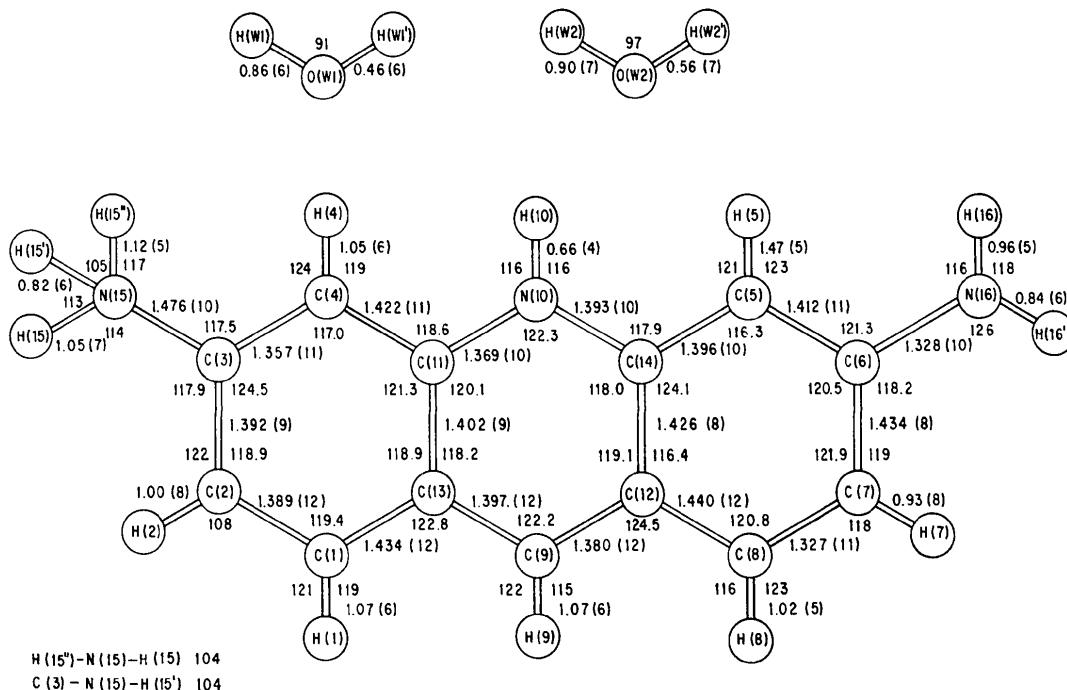


Fig. 1. Bond lengths and interbond angles in proflavine dichloride dihydrate. Estimated standard deviations for bond lengths are given in parentheses. Those for angles are 0.6° for C, N, O and $4-6^\circ$ if hydrogen atoms are involved in the angle.

We thank Dr D. E. Zacharias, Dr H. M. Berman and Dr N. Sutin for helpful discussions and Miss Roberta Talacki for technical assistance. This research was supported by grants CA-10925, CA-06927 and RR-05539

from the National Institutes of Health, U.S. Public Health Service, and by an appropriation from the Commonwealth of Pennsylvania.

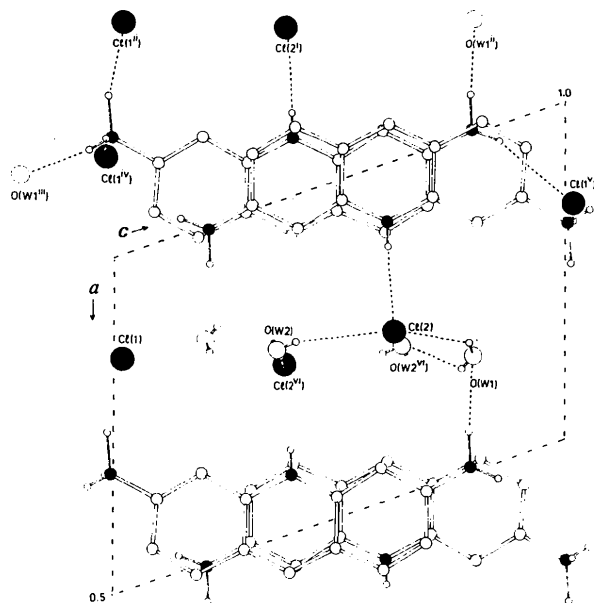


Fig. 2. Contents of the unit cell viewed down the *b* axis. Hydrogen bonds listed in Table 4 are shown.

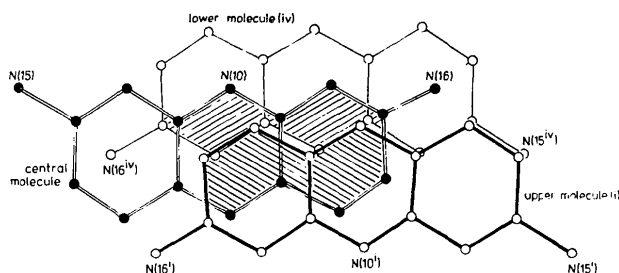


Fig. 3. Overlap of proflavine molecules. Three molecules in parallel planes approximately 3.35 Å apart are illustrated.

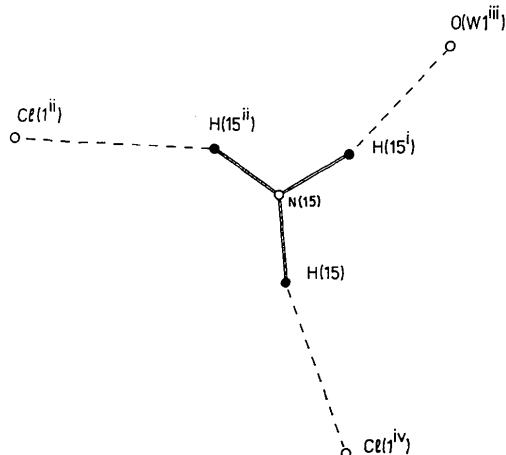


Fig. 4. Surroundings of N(15). This is a view directly down the C(3)-N(15) bond.

Table 5. Deviations of atoms from planes through parts of the molecule of proflavine dichloride dihydrate

	1	2	3	4
C(1)	-0.003*	-0.006*	-0.013	0.066
C(2)	0.024*	0.011*	0.014	0.099
C(3)	0.008*	-0.004*	0.008	0.069
C(4)	-0.006*	-0.008*	0.003	0.035
C(5)	0.018*	0.056	0.048	0.004*
C(6)	0.020*	0.068	0.050	-0.001*
C(7)	0.008*	0.055	0.028	-0.001*
C(8)	-0.011*	0.027	0.000	0.001*
C(9)	0.011*	0.028	0.011*	0.051
N(10)	-0.032*	-0.014	-0.013*	-0.018
C(11)	0.006*	0.014*	0.015*	0.040
C(12)	-0.018*	0.010	-0.008*	0.002*
C(13)	-0.014*	-0.006*	-0.014*	0.034
C(14)	-0.011*	0.017	0.009*	-0.004*
N(15)	-0.038	-0.060	-0.038	0.030
N(16)	0.086	0.143	0.125	0.044

* Included in the calculation of the plane e.s.d. (N)=0.010 Å, e.s.d. (C)=0.013 Å

Equations of planes

- (1) $-5.50480x + 6.73114y - 0.44375z = 1.47479$
- (2) $-5.50890x + 6.73947y - 0.34493z = 1.49797$
- (3) $-5.62321x + 6.72023y - 0.36551z = 1.49515$
- (4) $-5.33601x + 6.73943y - 0.62022z = 1.37757$

where *x*, *y* and *z* are fractional coordinates

Angle between planes 2 and 4 is 1.3°.

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