All the calculations were carried out on the FACOM 270-30 of this Institute and on the HITAC 8700/8800 at the Computer Centre of this University with a local version of the Universal Crystallographic Computation Program System (Crystallographic Society of Japan).

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The Crystal and Molecular Structure of Proflavine Hemisulphate Hydrate

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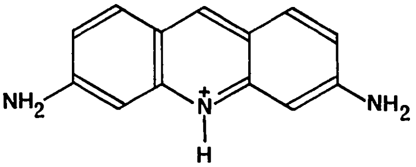
Proflavine hemisulphate (3,6-diaminoacridine hemisulphate hydrate), crystallizes in the monoclinic space group $P2_1/c$, with $a=12{\cdot}703$ (1), $b=19{\cdot}940$ (2), $c=21{\cdot}487$ (2) Å, $\beta=92{\cdot}24$ (2)°, and $Z=16$. The structure was solved by direct and search methods. The asymmetric unit contains four proflavine molecules, two sulphate ions and seven water molecules. The structure was refined to a final R of 0·074 for 4738 statistically significant reflexions, out of a total of 5601 measured on a diffractometer. The proflavine rings have slight deviations from equivalent geometry. They are not highly stacked. There are strong intermolecular interactions involving the positively-charged central nitrogens of the proflavine rings, and the sulphate anions, which tend to dominate the crystal structure. The complex hydrogen-bonding pattern in the structure involves all but three hydrogen-bond donors.

Introduction

The acridines derive much of their biological interest from the fact that they often show mutagenic and antitumour properties (Albert, 1966). Many of these compounds interact with nucleic acids; it is believed that these interactions are intimately related to the biological properties (Peacocke, 1973). The mode of binding to DNA has been well established for proflavine [(I), 3,6-diaminoacridine], which is perhaps the most extensively studied of all acridine–nucleic acid systems (Peacocke, 1973). Binding is of two types, a strong stacking interaction between planar aminoacridine rings and purine–pyrimidine base pairs (Lerman, 1961; Pritchard, Blake & Peacocke, 1966), and weaker electrostatic binding between aminoacridine cations and the charged phosphates on the exterior of the DNA double helix. One of the purposes of the present study was to obtain information relevant to the precise stereochemistry of these processes.



(I)

The structure of acridine itself has been established by Phillips (1956) and Phillips, Ahmed & Barnes (1960); several substituted acridines have also been studied (Talacki, Carrell & Glusker, 1974, and references therein; Courseille, Busetta & Hospital, 1973). Obendorf, Carrell & Glusker (1974) have recently reported the crystal structure of proflavine dihydrochloride in which the aminoacridine moiety is diprotonated. Proflavine is, however, normally studied and used medicinally as the hemisulphate, and it is this compound that is reported here. A preliminary account has been published (Neidle & Jones, 1975).

Experimental

Commercial proflavine hemisulphate (British Drug Houses Ltd) was crystallized from water in the dark. Large prismatic needles were obtained, which showed oblique extinction. Preliminary Weissenberg and precession photographs showed the crystals to be monoclinic. Systematic absences $h0l$: $l=2n+1$ and $0k0$: $k=2n+1$, showed the space group to be $P2_1/c$. Cell dimensions were obtained by least-squares refinement of 2θ values measured on a diffractometer.

Crystal data

$C_{13}H_{12}N_3^+0{\cdot}5(SO_4^{2-}).1{\cdot}75H_2O$, F.W.289·7. Monoclinic needles, elongated along **a**. $a=12{\cdot}703$ (1), $b=$

19·940 (2), $c=21\cdot487$ (2) Å, $\beta=92\cdot24$ (2)°, $V=5438\cdot45$ Å^3, $D_o=1\cdot43$ (2) g cm^{-3} (by flotation), $D_c=1\cdot415$ g cm^3 for $Z=16$. $F(000)=2440$, $\mu=18\cdot96$ cm^{-1} for Cu $K\alpha$ radiation ($\lambda_{K\bar{\alpha}}=1\cdot54178$ Å). Space group $P2_1/c$ (No. 14).

Intensities were collected on a Hilger and Watts automatic four-circle diffractometer, on-line to a PDP8 computer. The intensities of 5601 reflexions were measured with a variable ω–2θ scan and Ni-filtered Cu $K\alpha$ radiation to a limit of 120° in 2θ. Of these, 4738 were significantly above background [$I>2\cdot58\sigma(I)$].

Structure determination and refinement

The structure was solved with *MULTAN* (Germain, Main & Woolfson, 1971) and with various reciprocal-space search programs. The structure factors were placed on an absolute scale by a Wilson plot. Examination of the various parity groups showed no significant deviations of $\langle|E|^2\rangle$ from unity. An $I(\theta,\varphi)$ map (Tollin & Cochran, 1964) was calculated with 369 E values greater than 1·80 (Fig. 1). This map showed three peaks A,B,C at (44°, 78°), (60°, 147°) and (85°, 15°), with A twice the height of the others. On the assumption that A was due to two proflavine molecules, and B and C to one each, sum-rotation functions (Munns, Tollin & Jones, to be published) were calculated through these peaks with a model coordinate set calculated by Dr M. E. Davies. As expected, the sum function for A contained more peaks than for B and C. Finer scans were made around the three peaks arrowed in Fig. 2, and then Q functions (Tollin, 1966) were calculated. This meant evaluating $Q(X,Z)$ and $Q(Y)$ for the orientation defined by each of the peaks. For each of the orientations, at least one of the Q functions gave multiple peaks of equal height.

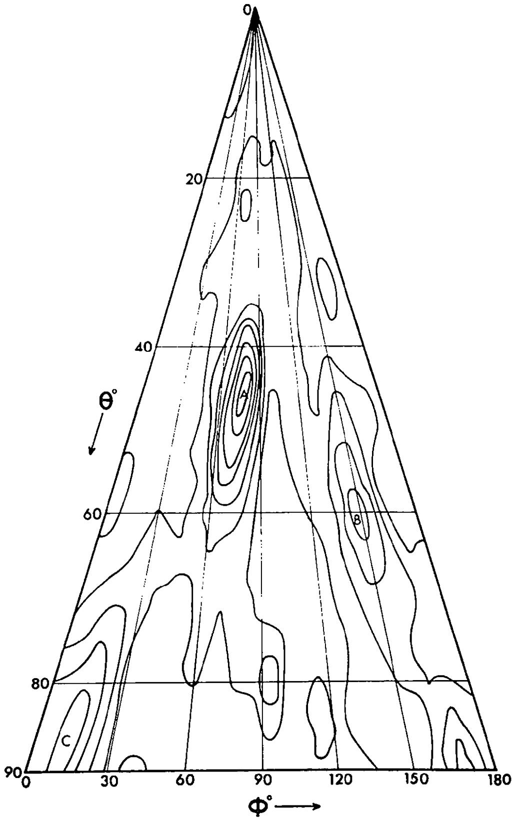


Fig. 1. $I(\theta,\varphi)$ plot.

Table 1. *Final positional parameters for the non-hydrogen atoms* (× 10^4), *as fractions of the unit-cell edges*

Standard deviations are in parentheses. The numbering scheme used for the carbon and nitrogen atoms is such that the first digit denotes the proflavine molecule number in the asymmetric unit.

	x	y	z
C(101)	12685 (5)	6102 (3)	1672 (3)
C(102)	12260 (6)	5707 (4)	2099 (4)
C(103)	11150 (6)	5749 (3)	2212 (3)
C(104)	10511 (5)	6181 (3)	1856 (3)
C(105)	10069 (5)	7884 (3)	293 (3)
C(106)	10479 (6)	8338 (4)	−115 (3)
C(107)	11610 (6)	8340 (4)	−193 (3)
C(108)	12242 (6)	7909 (4)	104 (3)
C(109)	12476 (5)	6992 (4)	866 (3)
N(110)	10337 (4)	7008 (3)	1054 (2)
C(111)	10945 (5)	6580 (3)	1408 (3)
C(112)	11831 (5)	7438 (3)	524 (3)
C(113)	12068 (5)	6567 (3)	1299 (3)
C(114)	10718 (5)	7438 (3)	617 (3)
N(115)	10758 (6)	5353 (4)	2661 (3)
N(116)	9892 (5)	8790 (3)	−442 (3)
C(201)	261 (6)	8218 (4)	2117 (3)
C(202)	105 (5)	8702 (4)	1700 (4)
C(203)	958 (5)	9012 (4)	1386 (3)
C(204)	1985 (5)	8769 (3)	1511 (3)
C(205)	4388 (6)	7288 (4)	2563 (3)
C(206)	4632 (7)	6781 (4)	2998 (3)
C(207)	3813 (7)	6506 (4)	3365 (4)
C(208)	2836 (8)	6745 (5)	3279 (3)
C(209)	1515 (6)	7484 (4)	2722 (3)
N(210)	3137 (4)	8002 (3)	2061 (2)
C(211)	2156 (5)	8263 (3)	1940 (3)
C(212)	2538 (6)	7250 (4)	2842 (3)
C(213)	1298 (6)	7974 (3)	2275 (3)
C(214)	3384 (6)	7508 (3)	2486 (3)
N(215)	777 (5)	9501 (3)	984 (3)
N(216)	5637 (6)	6554 (4)	3082 (3)
C(301)	8836 (6)	4234 (3)	329 (4)
C(302)	8752 (6)	4111 (4)	938 (4)
C(303)	8468 (6)	4620 (4)	1359 (3)
C(304)	8249 (6)	5263 (3)	1153 (3)
C(305)	8007 (5)	6868 (3)	−500 (3)
C(306)	8090 (5)	7042 (3)	−1130 (3)
C(307)	8368 (6)	6527 (4)	−1560 (3)
C(308)	8585 (6)	5899 (3)	−1388 (3)
C(309)	8732 (5)	5070 (3)	−542 (3)
N(310)	8151 (4)	6040 (2)	293 (2)
C(311)	8346 (5)	5406 (3)	513 (3)
C(312)	8516 (5)	5712 (3)	−752 (3)
C(313)	8640 (5)	4894 (3)	82 (3)
C(314)	8220 (5)	6212 (3)	−321 (3)
N(315)	8386 (6)	4484 (3)	1984 (3)
N(316)	7905 (6)	7673 (3)	−1328 (3)

Table 1 (*cont.*)

	x	y	z
C(401)	3955 (6)	6025 (4)	−327 (4)
C(402)	4270 (6)	6605 (5)	−553 (3)
C(403)	5048 (7)	7015 (4)	−232 (4)
C(404)	5482 (6)	6804 (4)	338 (3)
C(405)	5640 (6)	5241 (4)	2019 (3)
C(406)	5261 (6)	4673 (4)	2312 (4)
C(407)	4521 (6)	4233 (4)	1988 (4)
C(408)	4160 (6)	4379 (4)	1408 (4)
C(409)	4081 (5)	5177 (4)	525 (3)
N(410)	5535 (4)	5976 (3)	1150 (2)
C(411)	5137 (5)	6198 (4)	580 (3)
C(412)	4473 (5)	4978 (4)	1104 (3)
C(413)	4370 (5)	5775 (4)	259 (3)
C(414)	5231 (5)	5393 (4)	1429 (3)
N(415)	5386 (6)	7606 (4)	−476 (3)
N(416)	5605 (6)	4525 (4)	2903 (3)
W(1)	1389 (4)	1 (3)	9248 (2)
W(2)	3975 (6)	82 (4)	9070 (3)
W(3)	2539 (5)	608 (3)	8225 (3)
W(4)	1564 (5)	693 (3)	2209 (2)
W(5)	5063 (6)	1708 (3)	8659 (3)
W(6)	3077 (8)	1827 (4)	9502 (4)
W(7)	4236 (6)	6096 (4)	5216 (4)
S(1)	6898 (1)	5561 (1)	4329 (0)
O(1)	7003 (6)	5458 (4)	3657 (3)
O(2)	7303 (5)	4977 (3)	4653 (2)
O(3)	7556 (6)	6134 (4)	4449 (4)
O(4)	5877 (6)	5721 (4)	4491 (5)
S(2)	7636 (1)	7219 (0)	1811 (0)
O(5)	8161 (4)	6990 (2)	1246 (2)
O(6)	6644 (5)	6876 (4)	1847 (3)
O(7)	8303 (4)	7059 (3)	2360 (2)
O(8)	7495 (6)	7936 (3)	1787 (3)

Table 2. *Final anisotropic thermal parameters* ($\times 10^3$) *for the non-hydrogen atoms, with standard deviations in parentheses*

The thermal parameters are of the form: $\exp[-2\pi^2(h^2a^{*2}U_{11} + k^2b^{*2}U_{22} + l^2c^{*2}U_{33} + 2hka^*b^*U_{12} + 2hla^*c^*U_{13} + 2klb^*c^*U_{23})]$.

	U_{12}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C(101)	35 (5)	31 (5)	63 (6)	10 (4)	−2 (4)	−13 (4)
C(102)	47 (5)	45 (5)	60 (6)	11 (4)	−9 (4)	−7 (5)
C(103)	61 (6)	30 (5)	50 (5)	0 (4)	−3 (4)	−7 (4)
C(104)	36 (5)	36 (5)	48 (5)	2 (4)	1 (4)	0 (4)
C(105)	30 (4)	43 (5)	43 (5)	1 (4)	2 (4)	−6 (4)
C(106)	52 (5)	47 (5)	40 (5)	0 (4)	0 (4)	−7 (4)
C(107)	63 (6)	47 (5)	44 (5)	−5 (5)	19 (4)	1 (4)
C(108)	42 (5)	58 (6)	45 (5)	0 (4)	17 (4)	−3 (4)
C(109)	30 (4)	45 (5)	48 (5)	5 (4)	11 (4)	−17 (4)
N(110)	23 (3)	47 (4)	38 (4)	3 (3)	2 (3)	−9 (3)
C(111)	36 (5)	28 (4)	40 (5)	4 (4)	−3 (4)	−15 (4)
C(112)	35 (5)	41 (4)	41 (4)	4 (4)	9 (4)	−17 (4)
C(113)	30 (4)	38 (5)	44 (5)	5 (4)	1 (4)	−19 (4)
C(114)	37 (5)	31 (5)	34 (5)	0 (4)	5 (4)	−15 (4)
N(115)	70 (5)	66 (5)	71 (5)	1 (4)	0 (4)	18 (4)
N(116)	58 (5)	69 (5)	65 (5)	6 (4)	0 (4)	12 (4)
C(201)	37 (5)	42 (5)	60 (6)	−12 (4)	20 (4)	−21 (4)
C(202)	27 (4)	48 (5)	67 (6)	−1 (4)	3 (4)	−19 (5)
C(203)	36 (5)	45 (5)	44 (5)	−1 (4)	−3 (4)	−17 (4)
C(204)	31 (4)	39 (5)	37 (5)	−3 (4)	6 (4)	−3 (4)
C(205)	49 (5)	38 (5)	47 (5)	−1 (4)	−4 (4)	5 (4)
C(206)	79 (7)	51 (6)	44 (5)	−6 (5)	−18 (5)	−5 (5)
C(207)	84 (7)	60 (6)	40 (5)	−6 (5)	−8 (5)	4 (5)
C(208)	89 (7)	84 (7)	33 (5)	−34 (6)	13 (5)	1 (5)
C(209)	58 (5)	45 (5)	39 (5)	−16 (4)	18 (4)	−10 (4)
N(210)	35 (4)	35 (4)	45 (4)	−5 (3)	8 (3)	0 (3)
C(211)	29 (4)	46 (5)	35 (4)	−11 (4)	7 (3)	−16 (4)
C(212)	64 (5)	48 (5)	25 (5)	−14 (4)	4 (4)	0 (4)
C(213)	41 (5)	43 (5)	39 (5)	−8 (4)	13 (4)	−21 (4)
C(214)	48 (5)	37 (5)	33 (4)	−4 (4)	2 (4)	0 (4)
N(215)	38 (4)	61 (5)	73 (5)	2 (4)	−10 (4)	4 (4)
N(216)	37 (6)	82 (6)	67 (5)	12 (5)	−27 (4)	8 (5)
C(301)	44 (5)	28 (5)	65 (6)	0 (4)	−12 (4)	0 (4)
C(302)	50 (5)	31 (5)	67 (6)	0 (4)	−17 (5)	5 (4)
C(303)	47 (5)	53 (6)	40 (5)	−3 (4)	−13 (4)	16 (4)
C(304)	42 (5)	40 (5)	33 (5)	−4 (4)	−8 (4)	9 (4)
C(305)	44 (5)	34 (5)	25 (4)	−1 (4)	0 (4)	−2 (4)
C(306)	37 (5)	37 (5)	37 (5)	−6 (4)	1 (4)	2 (4)
C(307)	48 (5)	62 (6)	23 (4)	−13 (4)	7 (4)	−3 (4)
C(308)	47 (5)	36 (5)	34 (5)	−2 (4)	8 (4)	−4 (4)
C(309)	36 (5)	39 (5)	38 (5)	1 (4)	0 (4)	−12 (4)
N(310)	43 (4)	21 (3)	29 (3)	−1 (3)	−4 (3)	0 (3)
C(311)	28 (4)	31 (4)	39 (5)	−3 (4)	−8 (4)	−1 (4)
C(312)	32 (4)	51 (3)	33 (4)	−3 (4)	1 (3)	−8 (3)
C(313)	34 (4)	25 (4)	49 (5)	−3 (4)	−8 (4)	−6 (4)
C(314)	28 (4)	37 (4)	27 (4)	−6 (3)	0 (3)	−2 (4)
N(315)	106 (7)	42 (5)	60 (5)	2 (4)	−11 (5)	20 (4)
N(316)	86 (6)	40 (4)	35 (4)	2 (4)	5 (4)	11 (3)
C(401)	42 (5)	68 (6)	54 (6)	11 (5)	−8 (4)	−19 (5)
C(402)	54 (6)	81 (7)	42 (5)	26 (5)	−12 (4)	−9 (5)
C(403)	56 (6)	75 (7)	48 (6)	27 (5)	10 (5)	9 (5)
C(404)	42 (5)	67 (6)	45 (5)	10 (5)	2 (4)	9 (5)
C(405)	37 (5)	57 (6)	52 (5)	3 (4)	1 (4)	10 (5)
C(406)	43 (5)	62 (6)	59 (6)	13 (5)	14 (4)	7 (5)
C(407)	47 (5)	42 (5)	80 (7)	0 (4)	14 (5)	0 (5)
C(408)	37 (5)	51 (6)	73 (6)	1 (4)	6 (4)	−12 (5)
C(409)	31 (5)	45 (5)	56 (5)	5 (4)	−1 (4)	−21 (4)
N(410)	30 (4)	59 (5)	40 (4)	1 (3)	−1 (3)	2 (3)
C(411)	23 (4)	60 (6)	45 (5)	10 (4)	4 (4)	−4 (4)
C(412)	28 (4)	48 (6)	55 (5)	2 (4)	3 (4)	−15 (4)
C(413)	32 (4)	47 (5)	46 (5)	11 (4)	0 (4)	−15 (4)
C(414)	28 (4)	56 (5)	47 (5)	7 (4)	7 (4)	−3 (4)
N(415)	91 (7)	93 (7)	65 (6)	9 (5)	−3 (5)	28 (5)
N(416)	70 (6)	91 (7)	64 (5)	2 (5)	5 (4)	27 (5)
W(1)	49 (4)	108 (6)	60 (4)	−7 (4)	−10 (3)	14 (4)
W(2)	88 (6)	145 (8)	88 (6)	18 (5)	−1 (4)	−24 (5)
W(3)	66 (4)	73 (5)	105 (6)	−7 (4)	2 (4)	−9 (4)
W(4)	92 (5)	64 (4)	66 (4)	−13 (4)	24 (4)	−13 (4)
W(5)	99 (6)	72 (5)	112 (6)	−1 (4)	64 (5)	−3 (4)
W(6)	157 (9)	123 (8)	145 (8)	−39 (7)	−59 (7)	64 (7)
W(7)	105 (6)	90 (6)	141 (8)	8 (5)	47 (6)	−4 (5)
S(1)	29 (1)	47 (1)	26 (1)	1 (1)	0 (1)	1 (1)
O(1)	135 (7)	115 (7)	64 (5)	20 (6)	−3 (5)	3 (5)
O(2)	80 (4)	70 (4)	52 (4)	1 (4)	−8 (3)	25 (3)
O(3)	79 (6)	107 (6)	186 (9)	−42 (5)	−38 (6)	68 (7)
O(4)	99 (6)	94 (6)	222 (10)	28 (5)	108 (7)	28 (7)
S(2)	27 (1)	28 (1)	25 (1)	−3 (1)	2 (1)	−2 (1)
O(5)	42 (3)	60 (4)	33 (3)	3 (3)	4 (2)	−11 (3)
O(6)	56 (4)	152 (7)	74 (5)	−59 (5)	20 (4)	−34 (5)
O(7)	75 (4)	66 (4)	37 (3)	4 (3)	−4 (3)	−4 (3)
O(8)	155 (7)	53 (4)	67 (5)	30 (5)	6 (5)	−4 (4)

Attempts at a direct method solution were more successful, but our orientation information was of use as a check in the early stages. No attempt was made at directly incorporating orientation information into the direct method solution (Kroon & Krabbendam, 1970). With five symbolic phases chosen by inspection, several *E* maps were obtained which contained groups of peaks of approximately equal heights. In one of these maps three proflavine rings were identified, with θ, φ values of (45°, 76°), (45°, 77°) and (83·5°, 14°). The phase set for this map had the second highest absolute figure of merit, of 1·216 (Germain, Main & Woolfson, 1971), of all the phase sets examined. A structure-factor calculation based on these three rings with the largest 1400

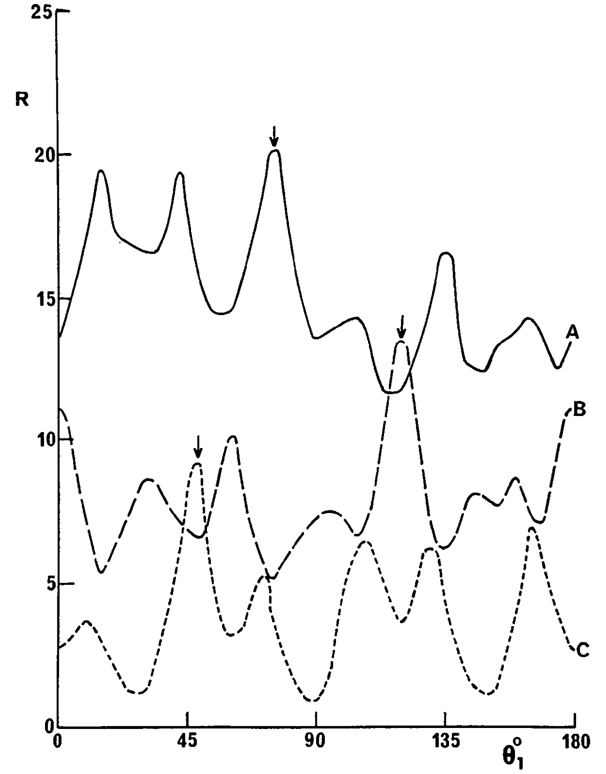


Fig. 2. Sum-function search plot. Searches *B* and *C* are shifted by −5 and −10 units respectively.

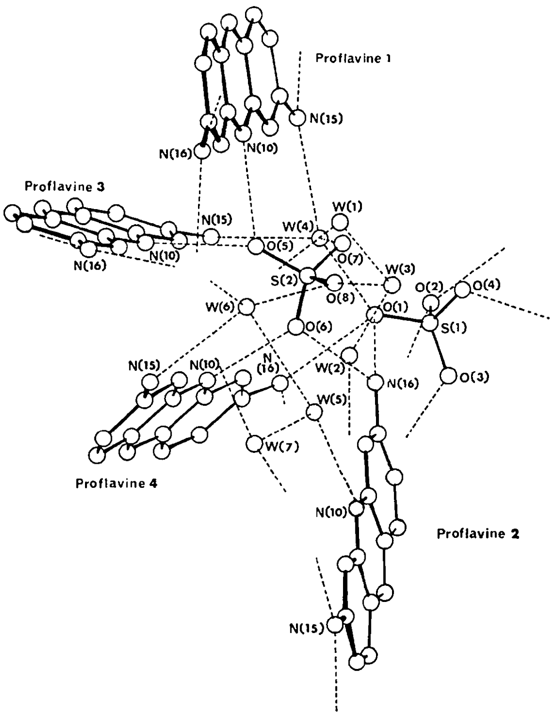


Fig. 3. The proflavine and sulphate ions, and water molecules in the asymmetric unit. Dashed lines represent hydrogen-bonded contacts.

Table 3. *Fractional coordinates* (× 10³) *of the hydrogen atoms in the asymmetric unit, together with their isotropic* (*U*) *thermal parameters* (× 10³ Å²), *and the length r* (*in* Å) *of the bonds to the non-hydrogen atoms*

Estimated standard deviations are in parentheses. The numbering scheme used is such that the first digit denotes the proflavine molecule number in the asymmetric unit, and the next one or two the number of the non-hydrogen atom to which each hydrogen is bonded.

	x	*y*	*z*	*U*	*r*
H(101)	1343 (5)	614 (3)	159 (3)	3 (18)	0·97 (7)
H(102)	1280 (8)	541 (5)	237 (4)	58 (32)	1·07 (9)
H(104)	984 (5)	625 (3)	197 (3)	2 (18)	0·90 (7)
H(105)	943 (6)	793 (3)	40 (3)	1 (17)	0·86 (6)
H(107)	1182 (5)	970 (3)	−43 (3)	4 (17)	0·91 (7)
H(108)	1303 (6)	793 (4)	5 (4)	34 (25)	1·01 (8)
H(109)	1333 (5)	702 (3)	80 (3)	2 (17)	1·10 (6)
H(110)	967 (6)	706 (4)	117 (3)	14 (21)	0·89 (7)
H(115)	1121 (9)	503 (6)	286 (5)	97 (44)	0·95 (12)
H(115′)	1020 (8)	538 (5)	278 (4)	58 (32)	0·77 (10)
H(116)	935 (5)	883 (3)	−41 (3)	0 (18)	0·69 (6)
H(116′)	1018 (9)	915 (6)	−64 (5)	87 (40)	0·91 (11)
H(201)	−28 (6)	792 (5)	231 (4)	34 (25)	1·01 (8)
H(202)	−49 (5)	880 (3)	159 (3)	7 (20)	0·80 (7)
H(209)	258 (5)	903 (3)	129 (3)	9 (20)	1·05 (7)
H(205)	491 (6)	743 (4)	236 (4)	30 (25)	0·85 (8)
H(207)	396 (8)	602 (5)	363 (5)	70 (35)	1·14 (10)
H(208)	230 (5)	656 (4)	347 (3)	12 (21)	0·89 (7)
H(204)	79 (7)	712 (5)	290 (4)	49 (29)	1·24 (9)
H(210)	362 (10)	801 (6)	176 (6)	92 (47)	0·91 (12)
H(215)	124 (6)	971 (6)	81 (3)	21 (23)	0·81 (8)
H(215′)	23 (5)	968 (4)	89 (3)	14 (21)	0·80 (7)
H(216)	621 (9)	671 (6)	288 (5)	90 (42)	0·92 (11)
H(216′)	591 (10)	634 (7)	342 (6)	120 (49)	0·91 (13)
H(301)	975 (5)	381 (5)	3 (4)	57 (32)	1·20 (10)
H(302)	899 (6)	364 (4)	108 (4)	30 (25)	1·03 (8)
H(304)	789 (6)	568 (4)	150 (3)	21 (22)	1·22 (8)
H(305)	779 (5)	726 (3)	−18 (3)	−3 (17)	1·08 (6)
H(307)	840 (6)	657 (4)	−186 (3)	11 (21)	0·64 (7)
H(308)	885 (6)	558 (4)	−171 (4)	31 (25)	1·01 (8)
H(309)	907 (6)	467 (4)	−86 (3)	16 (21)	1·14 (7)
H(310)	794 (7)	651 (5)	62 (4)	54 (31)	1·19 (9)
H(315)	805 (10)	484 (7)	232 (6)	129 (50)	1·10 (13)
H(315′)	856 (7)	406 (5)	212 (4)	45 (29)	0·92 (9)
H(316)	749 (7)	811 (5)	−102 (4)	49 (29)	1·24 (9)
H(316′)	797 (5)	778 (4)	−163 (3)	10 (21)	0·68 (7)
H(401)	347 (6)	576 (4)	−56 (3)	19 (22)	0·92 (7)
H(402)	390 (6)	673 (4)	−87 (4)	28 (24)	0·84 (8)
H(404)	626 (7)	704 (5)	51 (4)	52 (30)	1·14 (9)
H(405)	638 (6)	551 (4)	222 (4)	28 (24)	1·15 (8)
H(407)	428 (7)	373 (5)	215 (4)	50 (30)	1·10 (9)
H(408)	374 (4)	403 (3)	119 (3)	−1 (16)	0·99 (6)
H(409)	360 (6)	486 (4)	27 (3)	21 (23)	1·02 (7)
H(410)	625 (8)	634 (5)	146 (4)	63 (33)	1·31 (10)
H(415)	639 (11)	790 (7)	−59 (7)	170 (100)	1·43 (21)
H(415′)	567 (12)	795 (8)	−66 (7)	176 (104)	0·89 (21)
H(416)	615 (9)	485 (6)	316 (5)	97 (43)	1·08 (8)
H(416′)	537 (6)	431 (4)	308 (4)	22 (23)	0·64 (12)

structure factors, gave an *R* of 0·70. Because of the good agreement with the orientation search, we were confident that these rings had been located correctly. A series of difference syntheses was calculated in which the two sulphate ions were located, as well as the fourth proflavine molecule (with θ,φ values of 63° and 147°), and finally seven water molecules. At this stage, *R* was 0·17 over the whole data set. Six cycles of block-diagonal least-squares refinement with isotropic temperature factors for all atoms, and a 0·5 damping factor, were followed by six more cycles with anisotropic thermal parameters; R was reduced to 0·095. Reflexions were weighted by a factor of $1/[\sigma^2(F)]$, with those below the threshold given zero weight. The quantity minimized was $\sum w(|F_o|-|F_c|)^2$. The hydrogens attached to the proflavine rings were now located in a difference map; those attached to the water molecules could not be found. Four more cycles of refinement with anisotropic thermal parameters for the non-hydrogen atoms and variation of the hydrogen positional and isotropic

Table 4. *A comparison of the (averaged) bond lengths of the acridine rings found in proflavine hemisulphate, and those reported for some other acridine structures*

	Proflavine hemisulphate	Acridine II$_a$	Acridine III$_b$	2-[2-(6-Chloro-2-methoxy-9-acridinylamino)ethylamino]ethanol^c	ICR-170^d	2-[3-(6-Chloro-2-methoxy-9-acridinylamino)propylamino]ethanol^e	2-[3-(9-Acridinylamino)propylamino]ethanol^f	Quinacrine^g	Proflavine dichloride^h	9-Chloromethylacridine hydrochloride^i	9-Aminoacridine hydrochloride^j
C(1)—C(2)	1·332	1·357	1·370	1·367	1·363	1·370	1·362	1·359	1·389	1·351	1·362
C(1)—C(13)	1·436	1‘432	1·425	1·432	1·420	1·418	1·420	1·420	1·434	1·420	1·418
C(2)—C(3)	1·434	1·426	1·417	1·409	1·409	1·395	1·407	1·410	1·392	1·411	1·403
C(3)—C(4)	1·393	1·358	1·372	1·358	1·361	1·340	1·356	1·350	1·357	1·351	1·364
C(3)—N(15)	1·367*	–	–	–	–	–	–	–	1·476	–	–
C(4)—C(11)	1·392	1·427	1·431	1·423	1·433	1·422	1·422	1·414	1·422	1·418	1·410
C(5)—C(6)	1·395	1·358	1·372	1·369	1·360	1·345	1·351	1·364	1·412	1·357	1·357
C(5)—C(14)	1·378	1·427	1·431	1·434	1·428	1·425	1·429	1·413	1·396	1·408	1·412
C(6)—C(7)	1·443	1·426	1·417	1·386	1·406	1·396	1·404	1·402	1·431	1·421	1·408
C(6)—N(16)	1·353	–	–	–	–	–	–	–	1·328	–	–
C(7)—C(8)	1·333	1·357	1·370	1·365	1·357	1·359	1·360	1·357	1·327	1·350	1·363
C(8)—C(12)	1·422	1·432	1·425	1·424	1·437	1·418	1·428	1·430	1·440	1·423	1·416
C(9)—C(13)	1·386	1·395	1·389	1·425	1·425	1·441	1·438	1·431	1·397	1·409	1·434
C(9)—C(12)	1·389	1·395	1·389	1·435	1·419	1·421	1·430	1·449	1·380	1·403	1·439
N(10)–C(11)	1·371	1·345	1·344	1·358	1·352	1·356	1·362	1·362	1·369	1·354	1·364
N(10)–C(14)	1·371	1·345	1·344	1·349	1·341	1·350	1·352	1·356	1·393	1·342	1·357
C(11)–C(13)	1·446	1·432	1·432	1·422	1·421	1·414	1·414	1·408	1·402	1·431	1·410
C(12)–C(14)	1·432	1·432	1·432	1·426	1·437	1·428	1·426	1·425	1·426	1·433	1·409
⟨Standard deviation⟩											
	0·011	0·011	0·010	0·005	0·006	0·003	0·003	0·006	0·010	0·003	0·003

(*a*) Phillips (1956) (*b*) Phillips, Ahmed & Barnes (1960) (*c*) Glusker, Minkin & Orehowsky, (1972) (*d*) Berman & Glusker (1972) (*e*) Carrell (1972) (*f*) Glusker, Gallen & Carrell (1973) (*g*) Courseille, Busetta & Hospital (1973) (*h*) Obendorf, Carrell & Glusker (1974) (*i*) Zacharias & Glusker (1974) (*j*) Talacki, Carrell & Glusker (1974).

* The value for molecule 2 has been excluded from the averaging.

Table 5. *Deviations (in Å) of atoms from the least-squares planes for various parts of the proflavine molecules*

Plane	1	2	3	4	5	6	7	8
C(1)	−0·022	0·001	−0·010*	0·173*	−0·067	−0·007	−0·085*	−0·190*
C(2)	0·157	−0·014	0·018*	0·282*	−0·151	−0·011	−0·059*	−0·169*
C(3)	0·081	0·014	0·089*	0·381*	0·035	0·018	0·043*	−0·034*
C(4)	0·045	−0·002	0·067*	0·304*	0·047	−0·007	0·058*	0·020*
C(5)	−0·033	0·066*	0·037*	−0·002	−0·040	−0·131*	−0·046*	0·006
C(6)	0·037	0·189*	0·116*	−0·007	−0·046	−0·121*	−0·062*	−0·003
C(7)	0·088	0·279*	0·164*	0·010	0·001	−0·017*	−0·030*	−0·003
C(8)	0·036	0·211*	0·098*	−0·005	0·050	0·068*	0·016*	0·005
C(9)	−0·042	0·058*	−0·005	0·034*	0·026	0·067*	0·000	−0·060*
N(10)	−0·052	−0·025*	−0·007	0·091*	−0·004	−0·073*	−0·003	0·002*
C(11)	−0·018	−0·010	0·014	0·168*	0·022	−0·012	0·021	−0·011*
C(12)	−0·062	0·019*	−0·006	0·008	−0·008	−0·061*	−0·017	−0·004
C(13)	−0·035	0·011	−0·008	0·114*	−0·005	0·019	−0·020	−0·086*
C(14)	−0·039	0·079*	0·012	−0·005	0·042	0·047*	0·019	−0·002
N(15)	0·156*	0·036*	0·154*	0·528*	0·075*	0·041*	0·094*	0·011*
N(16)	0·080*	0·253*	0·173*	−0·005*	−0·077*	−0·189*	−0·090*	0·008*

Table 5 (*cont.*)

Plane	9	10	11	12	13	14	15	16
C(1)	−0·009	−0·005	−0·015*	−0·010*	0·033	−0·002	0·049*	0·312*
C(2)	−0·002	−0·001	−0·005*	−0·006*	0·068	0·006	0·071*	0·400*
C(3)	0·010	0·009	0·013*	0·003*	0·040	−0·002	0·044*	0·355*
C(4)	−0·010	−0·010	−0·005*	−0·018*	−0·012	−0·005	0·005*	0·228*
C(5)	0·000	0·012*	0·002*	−0·002	−0·003	0·160*	0·068*	−0·023
C(6)	0·009	0·025*	0·007*	0·009	0·102	0·295*	0·186*	0·023
C(7)	−0·014	0·004*	−0·022*	−0·010	0·061	0·236*	0·146*	−0·002
C(8)	0·000	0·016*	−0·010*	0·004	−0·028	0·100*	0·044*	−0·020
C(9)	0·187	0·029*	0·010	0·020*	−0·059	−0·013*	−0·015	0·084*
N(10)	0·002	0·008*	0·005	0·003*	−0·056	0·028*	−0·012	0·055*
C(11)	0·000	0·004	0·001	−0·005*	−0·026	0·009	0·004	0·159*
C(12)	−0·003	0·008*	−0·003	−0·004	−0·051	0·063*	0·006	0·001
C(13)	−0·004	0·003	−0·008	−0·005*	−0·021	−0·005	0·010	0·180*
C(14)	0·002	0·014*	−0·004	0·003	−0·050	0·045*	0·008	0·020
N(15)	0·018*	0·012*	0·024*	0·007*	0·045*	−0·026*	0·035*	0·414*
N(16)	0·028*	0·046*	0·028*	0·029*	0·218*	0·459*	0·315*	0·068*

Planes 1–4 refer to molecule 1, 5–8 to molecule 2, 9–12 to molecule 3, and 13–16 to molecule 4. Atoms marked (*) have been excluded from the calculation of the least-squares plane.

Table 5 (*cont.*)

Equations of the mean planes, in the form $px+qy+rz=s$

Plane	p	q	r	s
1	0·116	0·694	0·711	12·874
2	0·139	0·715	0·685	13·383
3	0·113	0·701	0·704	12·866
4	0·096	0·659	0·746	12·064
5	0·122	0·694	0·709	14·690
6	0·087	0·683	0·725	14·515
7	0·128	0·700	0·702	14·774
8	0·158	0·697	0·699	14·822
9	0·957	0·237	0·166	12·847
10	0·958	0·238	0·163	12·849
11	0·956	0·240	0·169	12·864
12	0·959	0·236	0·164	12·846
13	−0·757	0·487	0·436	1·629
14	−0·747	0·474	0·467	1·590
15	−0·757	0·480	0·444	1·582
16	−0·764	0·515	0·388	1·746

Angles (°) between the planes

Plane	Plane	Angle	Plane	Plane	Angle
2	3	2·0	14	15	1·5
2	4	5·3	14	16	5·2
3	4	3·5	15	16	3·8
6	7	2·9	1	5	0·5
6	8	4·4	1	9	66·1
7	8	1·7	1	13	57·8
10	11	0·4	5	9	66·4
10	12	0·1	5	13	57·4
11	12	0·4	9	13	123·6

thermal parameters, gave a final R of 0·074. These final parameters are listed in Tables 1–3.*

Unless otherwise stated, all calculations were performed with the X-RAY system of computer programs (Stewart, Kruger, Ammon, Dickinson & Hall, 1972), implemented on a CDC7600 computer. The scattering factors were taken from *International Tables for X-ray Crystallography* (1962).

Discussion

The molecular structure

Fig. 3. shows the arrangement of the molecules and ions in the asymmetric unit, and Figs. 4(*a*)–(*d*) and 5 detail the intramolecular bond lengths and angles, together with intermolecular hydrogen-bonding distances and angles involving the proflavines. The standard deviations quoted are probably underestimates because of the block-diagonal approximation employed in the refinement. The numbering scheme used for the proflavine rings and their substituents is as used by Obendorf *et al.* (1974) in their discussion of the structure of proflavine dichloride hemihydrate.

A comparison of the non-hydrogen bond lengths and angles shows that the four proflavine molecules in the asymmetric unit are equivalent, at least at the 2·5 σ significance level. (An apparent exception to this is C(3)–N(10) which is 6σ shorter in molecule 2 than in molecule 3.) Thus as expected (Albert, 1966), the molecules have m symmetry along C(9)–N(10) and are all protonated on the central ring nitrogens. This situation contrasts with the dicationic proflavine dichloride (Obendorf *et al.*, 1974), with the two protons located on the central ring nitrogen and a substituent amino nitrogen; this has the effect of making the molecule markedly dissymmetric (for example, C(3)–N(15) is of length 1·476 Å, and C(6)–N(16) is 1·328 Å).

The amino nitrogens in proflavine hemisulphate have induced changes in the ring geometry relative to compounds unsubstituted in these C(3), C(6) positions. In general, C(2)–C(3) and C(6)–C(7) have increased single-bond character compared with say, 9-chloromethylacridine hydrochloride (Zacharias & Glusker, 1974), with lengths of 1·411 and 1·421 Å for the two bonds in the latter. A similar situation of bond length-

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* A list of structure factors has been deposited with the British Library Lending Division as Supplementary Publication No. SUP 30848 (37 pp., 1 microfiche). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

ening for C(3)–C(4) and C(5)–C(6), and bond shortening (increased double-bond character) for C(4)–C(11) and C(5)–C(14) is evident. This pattern is repeated when proflavine hemisulphate is compared with other known acridine structures, as shown in Table 4. Although the differences in bond lengths are not large, they are thought to reflect real non-equivalence because of the relatively small spread of most of the values in the four molecules of the proflavine hemisulphate asymmetric unit.

Table 5 details the deviations of the various atoms from the least-squares planes calculated for the four proflavine molecules. It is apparent that these are not of equal planarity – molecules 1, 2 and 4 are the most

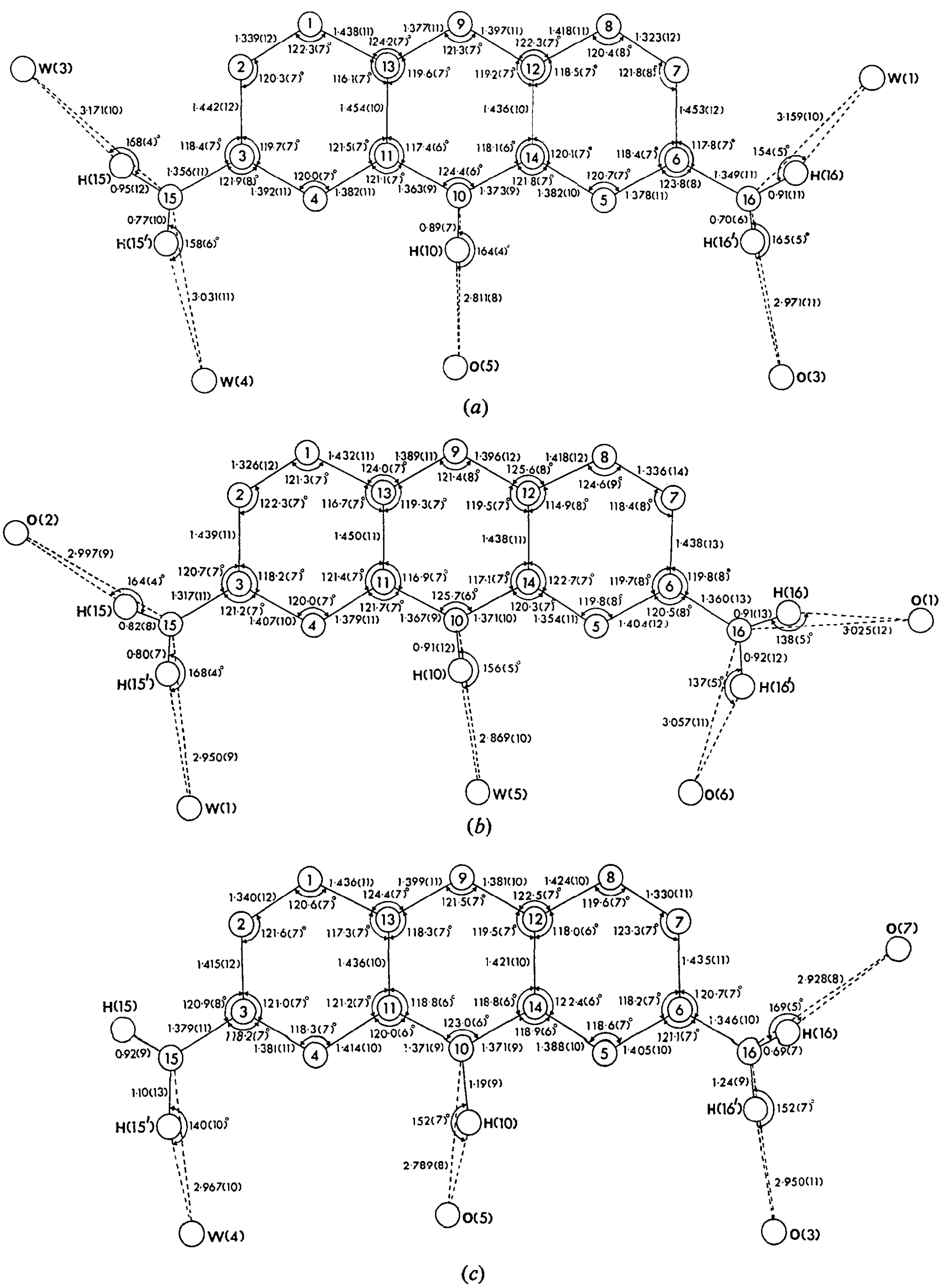
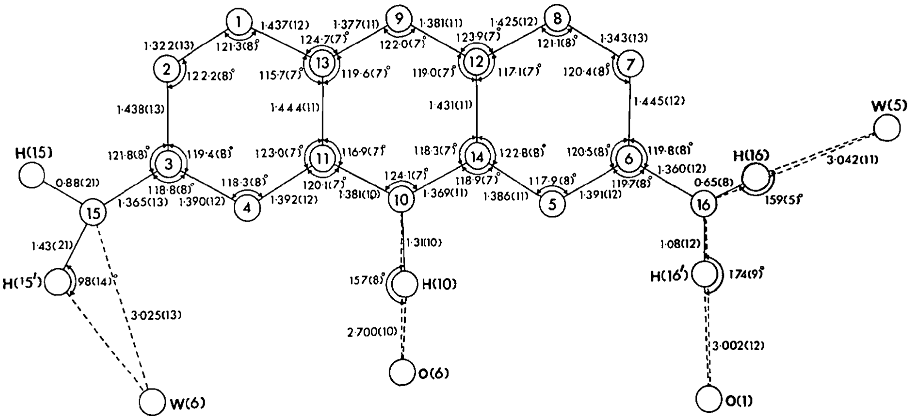


Fig. 4. (*a*)–(*d*) The intramolecular bond lengths and angles in the four proflavine molecules, with estimated standard deviations in parentheses. The geometry of the hydrogen bonds to the proflavines is also shown.



(*d*)

Fig. 4. (*cont.*)

ruffled, whereas 3 is very planar. For these first three, the deviations are considerably greater than in acridine itself (Phillips, 1956; Phillips *et al.*, 1960), and proflavine dihydrochloride (Obendorf *et al.*, 1974) [the maximum deviation from the least-squares plane of the ring is 0·032 Å for N(10)]. On the other hand, the more highly substituted acridines (referenced in Table 4), are invariably quite highly buckled; the compound ICR-170-OH has the best planes through its outer aromatic rings mutually inclined at 10·7°. The non-planarities observed in the present study are doubtless due to the influence of intermolecular interactions. However, we cannot correlate the bucklings with any specific interactions.

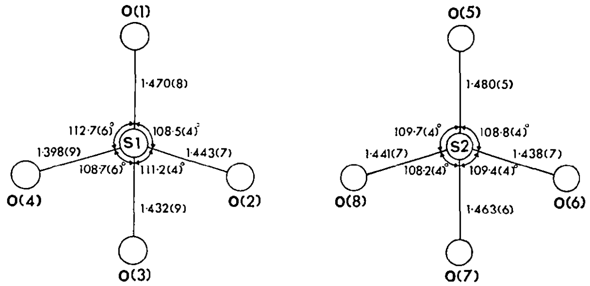
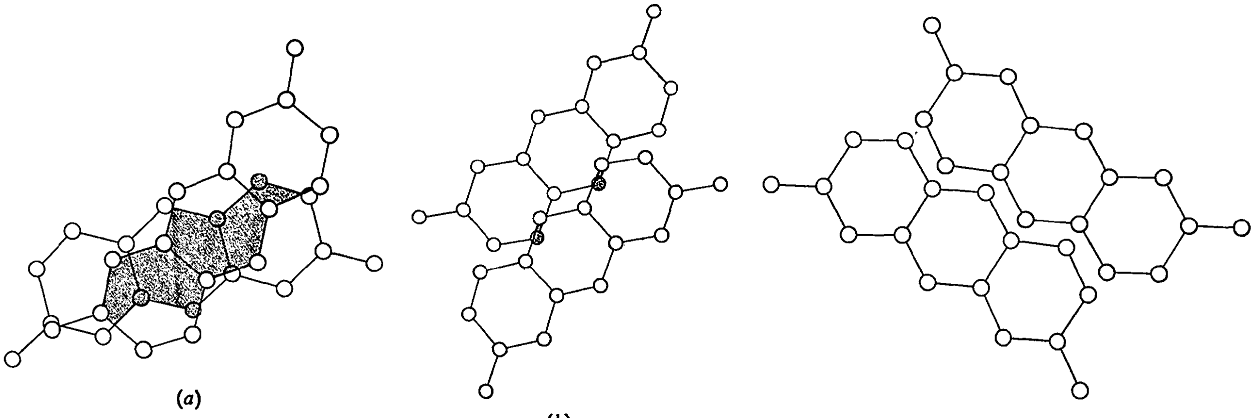


Fig. 5. The geometry of the sulphate ions. Angle O(1)–S(1)–O(3), 102·3(4)°; O(2)–S(1)–O(4), 112·5(4)°; O(5)–S(2)–O(7), 109·2(4)°; O(6)–S(2)–O(8), 111·5(4)°.

The C–H and N–H lengths and associated angles are mostly unexceptional (Table 3), and fall within the range of values commonly determined by X-ray analysis (Bonnett, Hursthouse & Neidle, 1972). The sulphate–oxygen bond lengths do show, however, some surprisingly large deviations from the 'standard' length of 1·47–1·48 Å for S–O bonds (Jönsson & Hamilton, 1970).

The crystal structure

Molecule 1 of the asymmetric unit forms a stacked pair with a molecule of type 2, which has been translated along **a**. A stacked pair [Fig. 6(*a*)] has the rings separated by 3·27 Å; one is rotated by 150° with respect to the other, so that the charged central N(10) nitrogens



(*a*)

(*b*)

(*c*)

Fig. 6. (*a*) The stacking of proflavine molecules 1 and 2. (*b*) Molecule 3 and its centrosymmetric mate. (*c*) Molecule 4 and its centrosymmetric mate.

are themselves not stacked on top of one another. The other proflavine molecules do not stack – Fig. 6(*b*) and (*c*) shows molecules 3 and 4 respectively, with their centrosymmetrically related mates. In neither case is there any real overlap, although the planes of the two rings are only 3·34 Å apart.

The four proflavine molecules within the asymmetric unit are clustered around a sulphate ion (Fig. 3), with the planes of the molecules mutually inclined at roughly 60° intervals [Table 5(*c*)]. The sulphate oxygens form strong hydrogen bonds with the N(10) nitrogens of molecules 1, 3 and 4, as does N(216). The four proflavines of the asymmetric unit all point in approximately the same direction, so that, viewed down **a**, the four asymmetric units in the cell have a cross-like appearance (Fig. 7). The sulphate ions and water molecules fit into the arms of the cross, forming a complex hydrogen-bonded network. There is a pronounced channel of water molecules and sulphate ions running through the middle of the cell, along **a**. All but three of the possible hydrogen-bond donors are involved in the network (Table 6). Fig. 8 shows this overall pattern in the unit cell. The shortest hydrogen-bonded distances are between the charged central nitrogen N(10) of proflavines, 1, 3 and 4, and the sulphate anion. However, as Table 6 shows, there are also several rather short contacts involving sulphate ions and water molecules.

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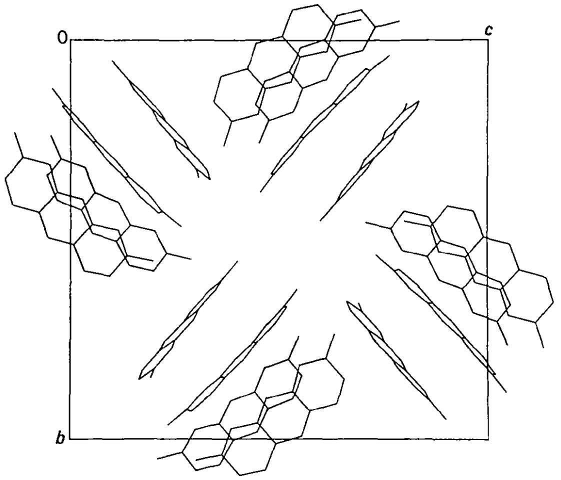

Fig. 7. The *a*-axis projection of a unit cell showing the 16 proflavine molecules in the cell.

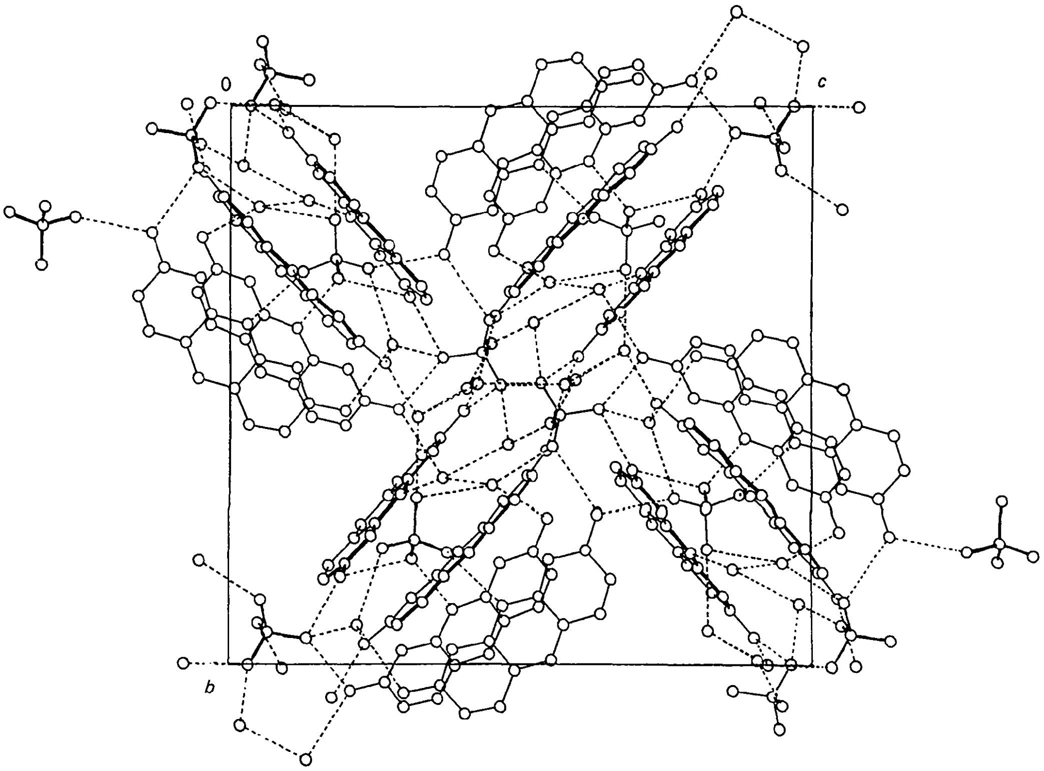

Fig. 8. The *a*-axis projection of the crystal structure: dashed lines represent hydrogen bonds. Only four asymmetric units are shown, for reasons of clarity.

Table 6. *Hydrogen-bonded contact distances in* Å

Estimated standard deviations are in parentheses.

The roman numerals refer to equivalent positions relative to the reference asymmetric unit at x,y,z.

I	$x,\ y,\ z$
II	$1+x,\ \frac{1}{2}-y,\ z-\frac{1}{2}$
III	$1-x,\ \frac{1}{2}+y,\ \frac{1}{2}-z$
IV	$x,\ 1\frac{1}{2}-y,\ z-\frac{1}{2}$
V	$1+x,\ 1+y,\ z-1$
VI	$1-x,\ 1-y,\ 1-z$
VII	$-x,\ 1-y,\ 1-z$
VIII	$x,\ \frac{1}{2}-y,\ z-\frac{1}{2}$
IX	$1-x,\ y-\frac{1}{2},\ 1\frac{1}{2}-z$
X	$x,\ \frac{1}{2}-y,\ \frac{1}{2}+z$
XI	$1-x,\ y-\frac{1}{2},\ \frac{1}{2}-z$

(*a*) Proflavine donors

Donor	Acceptor	Distance	Symmetry code
N(110)	O(5)	2·811 (8)	I
N(115)	*W*(3)	3·171 (10)	II
N(115)	*W*(4)	3·051 (11)	III
N(116)	O(3)	2·971 (11)	IV
N(116)	*W*(1)	3·159 (10)	V
N(210)	*W*(5)	2·869 (10)	VI
N(215)	*W*(1)	2·950 (9)	VII
N(215)	O(2)	2·997 (9)	III
N(216)	O(1)	3·025 (12)	I
N(216)	O(6)	3·057 (11)	I
N(310)	O(5)	2·789 (8)	I
N(315)	*W*(4)	2·967 (10)	III
N(316)	O(3)	2·950 (11)	VIII
N(316)	O(7)	2·928 (8)	VIII
N(410)	O(6)	2·700 (10)	I
N(415)	*W*(6)	3·025 (13)	VI
N(416)	O(1)	3·002 (12)	I
N(416)	*W*(5)	3·042 (11)	VIII

(*b*) Water donors

Donor	Acceptor	Distance	Symmetry code
W(1)	O(2)	2·831 (8)	IX
W(1)	*W*(3)	2·947 (10)	I
W(2)	O(4)	2·995 (12)	X
W(3)	*W*(2)	2·733 (11)	I
W(3)	O(8)	2·902 (10)	VI
W(4)	O(1)	2·694 (11)	XI
W(4)	O(7)	2·878 (9)	XI
W(5)	*W*(6)	3·171 (13)	I
W(5)	*W*(7)	2·818(12)	IX
W(6)	O(3)	2·790 (14)	IX
W(6)	O(8)	2·878 (12)	VI
W(7)	O(2)	2·922 (11)	VI
W(7)	O(4)	2·757 (13)	I

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