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The X-ray Crystal Structure of the Molecular Complex
Bis(lumiflavin–2,6-diamino-9-ethylpurine)–Ethanol–Water

BY F. E. SCARBROUGH,* HUEY-SHENG SHIEH† AND DONALD VOET‡

Department of Chemistry and the Laboratory for Research on the Structure of Matter, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

The deep-red complex (lumiflavin–2,6-diamino-9-ethylpurine)$_2$–ethanol–H$_2$O, C$_{42}$H$_{52}$N$_{20}$O$_6$, crystallizes in space group $P2_1/c$ with the unit-cell parameters $a = 16.479$ (7), $b = 13.613$ (5), $c = 22.214$ (8) Å, $\beta = 115.92$ (3)° and four complexes per unit cell. Intensity data were measured with an automated diffractometer and Cu $K\alpha$ radiation. The structure, which is largely composed of parallel planar hexagonal rings, was solved by direct methods only after all one- and two-dimensional reflections were deleted from the list of normalized structure factors, a Debye scattering correction had been applied to the E set in order to correct it for the non-random atomic distribution of the structure, and the parity groups of reflections were individually scaled to the condition $\langle E^2 \rangle = 1$. The structure was refined by full-matrix least-squares procedures to a final value of $R = 0.075$ based on the 3821 observed unique reflections with $|F_o| < 3.5$. The flavin and adenine derivatives form hydrogen-bonded base pairs. The molecules in the crystal also associate via extensively overlapped flavin/adenine and flavin/flavin interactions in which there are several closer than van der Waals contacts. This, together with the red color of the crystals, is indicative of a charge-transfer complex which may be of biological significance in the intramolecular interactions of the coenzyme FAD.

Introduction

Interactions between the flavin and adenine moieties of the oxidation–reduction coenzyme flavin adenine dinucleotide (FAD) have been studied by a variety of techniques. In the solid state, the X-ray structural studies of the crystalline complexes of riboflavin with 5′-bromo-5′-deoxyadenosine (Voet & Rich, 1971a,b) and adenine (Tomita, Fujii, Fujiki & Fujiwara, 1975) have provided direct observations of hydrogen bonding and stacking interactions that may serve as models for intramolecular flavin/adenine interactions in the oxidized form of FAD. However, considerably less is known about the complexing properties of reduced flavins. Nevertheless, it has been proposed that charge-transfer complexes of reduced flavocoenzymes might

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* Present address: Food and Drug Administration, Washington, DC 20204, USA.

† Present address: Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109, USA.

‡ Author to whom correspondence should be addressed.

possess the important function of stabilizing the planar conformation of the antiaromatic and normally bent 1,5-dihydroflavin nucleus (Massey & Ghisla, 1974; Hemmerich & Schuman-Jörns, 1973). Further proposals relate the reactivity of reduced flavins, especially oxygen reactivity, to the degree of planarity of these molecules (Tauscher, Ghisla & Hemmerich, 1973).

In an effort to observe the molecular associations of a reduced flavin with an adenine derivative we attempted to cocrystallize the relatively air-stable, reduced flavin 5-acetyl-1,5-dihydrolumiflavin (I) with 2,6-diamino-9-ethylpurine. Preliminary analyses, however, revealed that the oxidized flavin, lumiflavin (II), was the flavin species that actually crystallized. Yet the unexpected deep-red color of the crystals obtained (the flavin/adenine complexes that were previously crystallized were the yellow-brown color characteristic of oxidized neutral flavins; adenine derivatives are usually colorless), which indicated the probable existence of a charge-transfer complex, was of sufficient interest for the crystal-structure determination to be pursued.

(I)

(II)
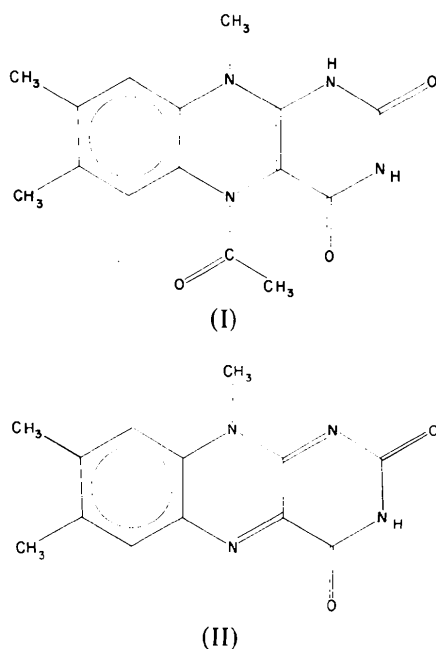
Slifkin (1971) ascribed the strong colors of the residues obtained upon evaporating aqueous solutions of purines and riboflavin to dryness to the presence of charge-transfer complexes. However, it appears that charge-transfer forces are of relatively minor importance in stabilizing flavin/adenine complexes (Slifkin, 1973). This conclusion is supported by the fact that the two previously mentioned crystalline flavin/adenine complexes show no indication of charge-transfer interactions. Yet even a minor influence could be enzymologically significant as it has been suggested that an intramolecular charge-transfer complex in FAD has a

catalytic role in certain flavoenzyme mediated reactions (Massey & Ghisla, 1974).

The crystal structure reported below represents the first direct structural evidence of flavin/adenine and flavin/flavin charge-transfer interactions. A discussion of the biological implications of the structure has appeared elsewhere (Scarbrough, Shieh & Voet, 1976). The present report is concerned with a discussion of the procedures used to solve the structure and a presentation of its molecular parameters.

Experimental

Lumiflavin, which was obtained following the procedure of Hemmerich, Fallab & Erlenmeyer (1956), was used in the synthesis of the relatively air-stable, reduced flavin derivative 5-acetyl-1,5-dihydrolumiflavin (Hemmerich & Erlenmeyer, 1957). The identities of the two synthesized flavin derivatives were confirmed by their IR spectra (Hemmerich, Prijs & Erlenmeyer, 1960).

Vapor diffusion of 50% ethanol or absolute ethanol into either dimethylformamide (DMF) or dimethyl sulfoxide (DMSO) solutions of 5-acetyl-1,5-dihydrolumiflavin in equimolar amounts with the adenine derivative 2,6-diamino-9-ethylpurine (Cyclo Chemical Co.), followed by the slow evaporation of the resulting mixture in air, reproducibly yielded deep-red tabular crystals. The UV spectrum of a DMSO solution of these crystals indicated that the crystals contained lumiflavin and 2,6-diamino-9-ethylpurine in an equimolar ratio. However, repeated attempts to cocrystallize lumiflavin with 2,6-diamino-9-ethylpurine under the crystallizing conditions described above failed, yielding instead yellow precipitates.

The crystal selected for data collection, which was grown from an absolute ethanol/DMSO solution, had approximate dimensions $0.3 \times 0.1 \times 0.1$ mm. Preliminary Weissenberg and precession photographs revealed that the crystal had monoclinic lattice symmetry. The observed systematic absences of $h0l$ reflections with l odd and of $0k0$ reflections with k odd are consistent with the space group $P2_1/c$. The diffraction pattern also exhibited a systematic weakness for $h0l$ reflections with h odd. This indicates the existence of a pseudo a glide plane. All subsequent X-ray measurements were made with a Picker FACS-I diffractometer, a pyrolytic graphite monochromator and Cu $K\alpha$ radiation ($\lambda = 1.5418$ Å). Unit-cell parameters (which are presented in Table 1) were determined by the least-squares analysis of the angular positions of 12 reflections. The density of the crystals (Table 1), as determined by flotation in a mixture of CCl_4 and n-hexane, is in excellent agreement with the calculated density for the crystal containing the complex (lumiflavin–2,6-diamino-9-ethylpurine)$_2$–

Table 1. *Crystal data for the complex* (*lumiflavin–2,3-diamino-9-ethylpurine*)$_2$–*ethanol*–H_2O

Molecular formula $C_{42}H_{52}N_{20}O_6$

$a = 16\cdot479\ (7)$ Å	FW $933\cdot0$
$b = 13\cdot613\ (5)$	$d_{obs} = 1\cdot382$ g cm^{-3}
$c = 22\cdot214\ (8)$	$d_{calc} = 1\cdot381$
$\beta = 115\cdot92\ (3)°$	$\mu(Mo\ K\alpha) = 8\cdot0$ cm^{-1}
Space group $P2_1/c$	$V = 4481\cdot9$ Å^3
$Z = 4$	$F(000) = 1968$

ethanol–H_2O in the asymmetric unit of its unit cell. The water molecule was apparently absorbed from the air by the hygroscopic solution from which the complex was crystallized.

The X-ray diffraction data were measured to the limit $2\theta = 125°$ with the $\theta/2\theta$ scan mode, a scan rate of $1°$ min^{-1}, a basic scan width of $1\cdot4°$ and a take-off angle of $2\cdot5°$. Stationary background counts of 20 s duration were taken at both limits of the scan. The three standard reflections that were scanned after every fiftieth measurement suffered no significant loss of intensity during data collection.

Structural determination and refinement

The measured intensities, I, were corrected for Lorentz and polarization effects. No absorption corrections were made owing to the small size of the crystal and to its low linear absorption coefficient for Cu $K\alpha$ radiation (Table 1). Standard deviations, $\sigma(I)$, were calculated (Stout & Jensen, 1968) for an instrumental instability factor of $0\cdot02$. Of the 7137 measured unique reflections, 4297 had $I > 2\cdot33\sigma(I)$ and were therefore considered to be observed.

The statistical distribution of the data revealed some aberrant features. Firstly, the Wilson plot was conspicuously non-linear. This suggested that the atoms in the crystal were arranged in an ordered manner. Secondly, the $N(Z)$ plot (Howells, Phillips & Rogers, 1950) indicated a hypercentric geometry. This observation is consistent with the presence of the pseudo a glide plane which, together with the crystallographic twofold screw axis, generates a pseudo center of symmetry. The hypercentric properties are also reflected in the distribution of the normalized structure factors, E (Karle & Hauptman, 1956). Thirdly, many of these E's have unusually large values (*e.g.* five have $|E| > 6$). These statistical features, which reflect the extended planarity of the complexes and the repetitive translations throughout the structure, were a forewarning of the difficulty to be encountered in the structure-determination procedure.

Initial attempts to determine the structure *via* direct methods utilized two different program systems, *REL* (Long, 1966) and *MULTAN* (Main, Woolfson & Germain, 1971), in combination with a wide variety of

modifications to the E set and to the starting phase set. Almost all resulting E maps contained large numbers of peaks that were covalent bond distances apart and which formed extensive planar hexagonal networks ('chicken wire'). These peaks either coincided with or fell midway between the $(40\bar{6})$ plane of the crystal, depending on the sign of $E(40\bar{6})$. However, packing considerations of the various reasonable models of the complex ruled out the possibility that the plane of the complex was coincident with the center of symmetry containing $(40\bar{6})$ plane. The hexagonal-net arrangements allowed many reasonable placements of the flavin molecules, but numerous models based on these possibilities failed to reveal the adenine molecules or to produce significantly reduced agreement indices.

Analysis of the Patterson map of the structure confirmed the planarity of the complex parallel to the $(40\bar{6})$ crystal plane, as well as indicating two probable orientations of the flavin ring system. Upon solution of the structure, the latter were found to correspond to the orientation of the two independent lumiflavin molecules. The Patterson map also revealed what later proved to be the correct y coordinate of the pseudo a glide plane. However, the translational positions of the flavin molecules could not be determined.

The structural evidence to this point suggested that a translational search with an R calculation as a test for correctness might be helpful in finding the position of the oriented flavin in the complex plane. However, the use of a centrosymmetric flavin fragment (and subsequently eight different models for hydrogen-bonded flavin/adenine complexes) as search fragments failed to produce a significantly low R value. This, however, is not surprising considering that the idealized model coordinates which were used differ somewhat from those that were eventually determined. Such a situation can lead to inordinately high R values (Milledge, 1962). Thus, for example, a trial structure of anthracene with errors in atomic positions ranging from $0\cdot10$ to $0\cdot55$ Å had $R = 0\cdot89$ (Sparks, 1961).

Karle's translation functions (Karle, 1972) were also applied in an effort to determine the structure. It was hoped by this method to correctly position an 18-atom flavin fragment. However, the many ambiguities that arose from the internal symmetry of the structure and from the numerous spurious peaks in the resulting maps could not be successfully resolved.

Vector-search methods (Nordman, 1966; Nordman & Schilling, 1970; Schilling, 1970), in which the flavin nucleus was used as a search group, were applied. The rotational search, as might be expected, was successful. In the subsequent translational search the highly regular structure, which is an almost endless hexagonal net, yielded many possible displacement vectors. However, none led to a successful structural determination. In retrospect, a judicious choice of a small subset of the interatomic vectors actually used might have been successful in revealing the structure. The

inclusion of a pair of nearly coincident vectors in the search essentially gives double weight to such a pair. This in turn limits the peak to noise discrimination of the technique (Nordman, 1972).

Preliminary versions of Thiessen & Busing's (1974) recently developed programs (ORTRAN and ORPHEX) to identify, and subsequently make use of, systematically aberrant phase relationships (see below) were also employed in this structure determination, again without success. Although the Fourier transform of a benzene ring or of a flavin fragment could be satisfactorily fitted to the $|E|$-weighted reciprocal lattice, phasing, starting from the best calculated orientations, failed to reveal the correct structure.

The crystal structure was eventually solved by direct methods, with the program MULTAN (Main, Woolfson & Germain, 1971), but only after the following three corrections were simultaneously applied to the calculation of the normalized structure factors: (1) all one- and two-dimensional reflections were removed from the data set (the **a** and **c** directions are uniquely determined in this structure by the pseudo a and c glide planes respectively), (2) Debye scattering corrections (Debye, 1915), in which the spherically averaged molecular transform was based on the supposition that the asymmetric unit contained two Watson–Crick paired lumiflavin–2,6-diamino-9-ethyl-purine complexes, were applied to the E's, and (3) scale factors were adjusted to impose the condition that $\langle E^2 \rangle = 1$ for each parity group.

Because of the indicated planar nature of the structure, considerable overlap of atoms in projection was highly probable. Under such conditions phase relationships involving the three principal zonal reflections are often unreliable (Delbaere & James, 1973; Karle, 1970) (probably for the same reason that three-dimensional reflections are unreliable in structures exhibiting translational regularities, but to a greater degree; see below) and thus correction 1 was applied. The Debye scattering correction employs a molecular scattering factor, i.e. a spherically averaged Fourier transform of the molecule (Zachariasen, 1945), in order to counteract the effect of the non-random distribution of atoms in the unit cell introduced by the repetitive interatomic translations found throughout the molecules in question. A rigorous theoretical justification is lacking for the separate scaling of parity groups in the calculation of E's. However, this technique, which seems intuitively reasonable, has been helpful in the elucidation of many crystal structures by direct methods.

The E map calculated with the phases, as determined by the MULTAN solution with the highest combined figure of merit, revealed the two flavin ring systems and parts of the purines. Alternate structure factor calculations followed by Fourier refinement gradually revealed 64 non-hydrogen atoms of the structure. The R value at this point was 0·37.

The structure was refined by full-matrix least-squares methods in which the quantity minimized was $\Sigma w(|F_o| - |F_c|)^2$. Here the summation is over the 4297 observed reflections and $w = I/\sigma^2(I) = 1/\sigma^2(F_o)$. The atomic scattering factors for non-hydrogen atoms were taken from Cromer & Waber (1965) and those for H from Stewart, Davidson & Simpson (1965).

The refinement of the overall scale factor and all atomic positional and isotropic thermal parameters converged at an R of 0·17. A difference Fourier map revealed five residual peaks which, because of their locations, were taken to represent solvent molecules. Of these, a lone peak represented the O atom of a water molecule while a cluster of four peaks were best described as the heavy atoms in a disordered ethanol molecule. Although methyl hydrogens were not located for this ethanol, an assignment of two half-weighted O positions was made, based on the relative orientation of the solvent molecule to the primary and secondary chelation sites of the lumiflavin molecules (Langhoff & Fritchie, 1970).

Further refinement, in which the anisotropic temperature parameters were varied, was performed in two sections because of limitations in computer memory storage. Two full cycles of such refinement reduced the R to 0·114. H atoms were located in a subsequent difference Fourier map. Two cycles of isotropic temperature refinement of the H atoms, followed by two additional cycles of anisotropic heavy-atom temperature refinement, yielded an R of 0·085. An examination of the distribution of R with respect to structure factor amplitude, $\sin \theta$ and parity of the reflections indicated a high value of R for those reflections with the lowest intensities. In view of the suspected unreliability of these weak reflections, the 476 with $|F_o| < 3·5$ were deleted from the subsequent least-squares refinement. A final cycle of anisotropic refinement yielded the agreement indices $R = 0·075$ and $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2]^{1/2} = 0·109$ for the remaining 3821 reflections.* The final parameter shifts were all less than the estimated standard deviations of their respective parameters. A final difference Fourier map had three peaks of approximately 0·4 e Å^{-3} associated with the disordered ethanol molecule. No other significant features were noted on this map.

Molecular structure

Fig. 1 shows the contents of the asymmetric unit of the unit cell together with the atomic-numbering scheme used in this report. Table 2 contains the final fractional
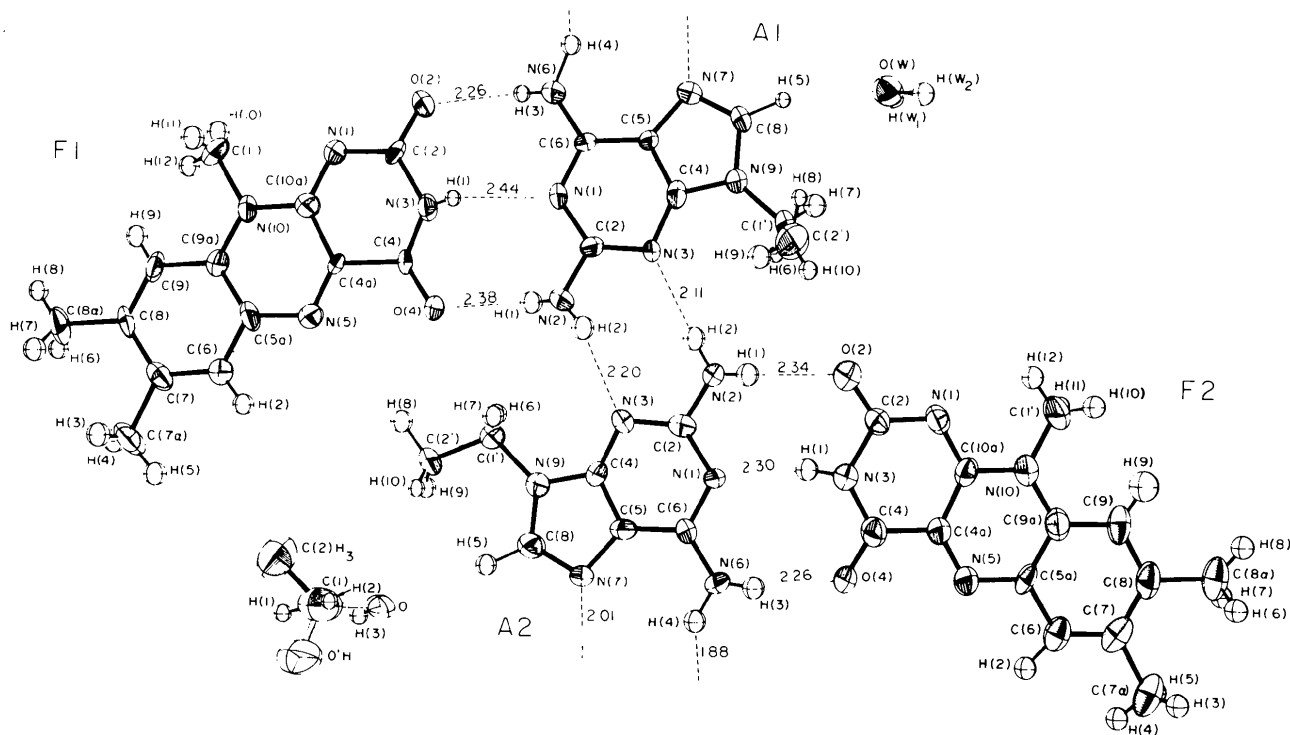
Fig. 1. A perspective drawing of the asymmetric unit of the crystalline complex (lumiflavin–2,6-diamino-9-ethylpurine)$_2$–ethanol–H$_2$O. The view is normal to the best least-squares plane of the asymmetric unit. Non-hydrogen atoms are represented by thermal ellipsoids at the 50% probability level. Non-solvent hydrogens are drawn as spheres at the 25% probability level, whereas solvent hydrogens have arbitrary radii. Hydrogen bonds are represented as dashed lines accompanied by the hydrogen···acceptor distances in Å. The atomic numbering scheme used in this report is shown. Standard nucleoside and coenzyme numbering systems have been followed where possible. The alternative positions of the disordered ethanol hydroxyl group are indicated by unfilled bonds.

coordinates for all atoms in the asymmetric unit and H thermal parameters together with the estimated standard deviations of these quantities. Fig. 2 presents the covalent bond distances and angles of the structure.

Lumiflavin

Wang & Fritchie (1973) have determined 'idealized' molecular dimensions for lumiflavin based on the weighted averages of three non-protonated structures. The bond distances in the present structure show an average deviation of 0·009 Å from the corresponding 'idealized' values. The average differences between the corresponding bond distances and angles of the two equivalent lumiflavin molecules in the asymmetric unit are 0·013 Å and 1·3° respectively, for bonds not involving H atoms. The average estimated standard deviations of these bond parameters are 0·008 Å and 0·5° respectively.

The lumiflavin molecules are approximately, but not strictly, planar;* the r.m.s. deviations of the non-hydrogen atoms of molecules F1 and F2 from their respective least-squares planes are 0·034 and 0·057 Å. The major distortion of lumiflavin F1 is a small twist

along its longitudinal molecular axis. Lumiflavin F2 exhibits a slight folding about its N(5)–N(10) axis. Both types of distortions have been observed in crystal structures of flavin derivatives (Kuo, Dunn & Fritchie, 1974; Wells, Trus, Johnston, Marsh & Fritchie, 1974, and references therein).

2,6-Diamino-9-ethylpurine

The covalent-bond parameters of the 2,6-diamino-9-ethylpurine molecules are in excellent agreement with the average values of the corresponding parameters in previously reported adenine-containing structures (Voet & Rich, 1970). The average differences between the corresponding bond distances and angles of the two chemically equivalent adenine derivatives in the asymmetric unit are 0·013 Å and 0·8° respectively. This is consistent with the finding that the average estimated standard deviations of these bond parameters are 0·007 Å and 0·5° respectively. The 2,6-diamino-9-ethylpurine molecules are very nearly planar;* the r.m.s. deviations of the non-hydrogen atoms of molecules A1 and A2 from their respective least-squares planes are 0·014 and 0·015 Å.

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* See deposition footnote.

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* See deposition footnote.

Table 2. *Positional parameters* ($\times 10^4$, *for* H *and ethanol atoms* $\times 10^3$) *and* H *thermal parameters for the complex*
(lumiflavin–2,6-diamino-9-ethylpurine)$_2$*–ethanol–*H_2O

The positional parameters are expressed in fractions of the unit-cell edges. Isotropic temperature factors have the functional form T $\exp(-B \sin^2\theta/\lambda^2)$. Standard deviations, as determined from the variance–covariance matrices of the final cycles of the least squares refinement, are given in parentheses and refer to the least significant digits of their corresponding parameters. The prefixes $F1$, $F2$, $A1$, $A2$, W and E refer to lumiflavin molecule numbers 1 and 2, adenine derivative numbers 1 and 2, the water molecule and the ethanol molecule respectively (refer to Fig. 1).

	x	y	z		x	y	z
F1N(1)	3353 (3)	6424 (3)	4702 (2)	A2C(8)	6117 (4)	66 (4)	6386 (3)
F1C(2)	4161 (3)	6450 (4)	5246 (3)	A2N(9)	6168 (3)	1056 (3)	6459 (2)
F1O(2)	4533 (3)	7230 (3)	5492 (2)	A2C(1')	5566 (4)	1834 (4)	6029 (3)
F1N(3)	4605 (3)	5560 (4)	5535 (2)	A2C(2')	4720 (5)	1416 (5)	5461 (4)
F1C(4)	4276 (3)	4655 (4)	5361 (3)	WO	10855 (5)	7528 (6)	9626 (5)
F1O(4)	4665 (2)	3916 (3)	5648 (2)	EC(2)H₃	240 (1)	−9 (1)	454 (9)
F1C(4a)	3360 (3)	4624 (4)	4763 (2)	EC(1)	293 (1)	−83 (2)	498 (1)
F1N(5)	2998 (3)	3767 (3)	4561 (2)	EO	385 (1)	·90 (1)	532 (1)
F1C(5a)	2177 (4)	3757 (5)	4016 (3)	EO'H	259 (1)	−163 (2)	493 (2)
F1C(6)	1777 (4)	2851 (5)	3775 (3)				
F1C(7)	967 (4)	2766 (5)	3208 (3)		x	y	z	B (Å²)

	x	y	z		x	y	z	B (Å²)
F1C(7α)	549 (5)	1764 (6)	2973 (4)	F1H(1)	492 (4)	569 (4)	574 (3)	1 (1)
F1C(8)	530 (3)	3651 (5)	2873 (3)	F1H(2)	207 (4)	236 (5)	399 (3)	3 (1)
F1C(8α)	−361 (4)	3584 (6)	2251 (3)	F1H(3)	3 (6)	178 (7)	309 (4)	8 (2)
F1C(9)	930 (3)	4547 (5)	3108 (3)	F1H(4)	49 (5)	168 (5)	253 (4)	3 (2)
F1C(9a)	1760 (4)	4612 (5)	3672 (3)	F1H(5)	88 (5)	124 (5)	320 (4)	6 (2)
F1N(10)	2173 (3)	5508 (3)	3918 (2)	F1H(6)	−25 (4)	322 (5)	190 (3)	4 (1)
F1C(1')	1744 (5)	6427 (5)	3595 (4)	F1H(7)	−73 (7)	326 (8)	234 (5)	12 (3)
F1C(10a)	2985 (4)	5572 (4)	4468 (3)	F1H(8)	−52 (4)	427 (5)	197 (3)	5 (1)
A1N(1)	6381 (3)	5760 (3)	6708 (2)	F1H(9)	66 (4)	502 (5)	283 (3)	2 (1)
A1C(2)	6808 (3)	4942 (4)	7053 (3)	F1H(10)	206 (6)	689 (7)	357 (5)	9 (2)
A1N(2)	6383 (3)	4073 (4)	6803 (3)	F1H(11)	131 (5)	652 (6)	369 (4)	7 (2)
A1N(3)	7574 (3)	4882 (3)	7617 (2)	F1H(12)	162 (4)	637 (5)	310 (3)	4 (1)
A1C(4)	7902 (3)	5783 (4)	7831 (3)	A1H(1)	619 (7)	409 (8)	645 (5)	10 (3)
A1C(5)	7576 (3)	6672 (4)	7531 (3)	A1H(2)	666 (5)	359 (5)	700 (3)	4 (1)
A1C(6)	6755 (3)	6639 (3)	6938 (3)	A1H(3)	597 (4)	742 (4)	627 (3)	1 (1)
A1N(6)	6351 (3)	7448 (3)	6612 (3)	A1H(4)	654 (4)	813 (5)	678 (3)	1 (1)
A1N(7)	8126 (3)	7443 (3)	7895 (2)	A1H(5)	932 (3)	735 (4)	878 (2)	2 (1)
A1C(8)	8775 (3)	7008 (4)	8406 (3)	A1H(6)	983 (5)	561 (5)	913 (3)	3 (2)
A1N(9)	8699 (3)	6010 (3)	8386 (2)	A1H(7)	935 (3)	482 (4)	867 (3)	1 (1)
A1C(1')	9276 (4)	5304 (5)	8894 (3)	A1H(8)	878 (3)	558 (4)	959 (3)	3 (1)
A1C(2')	8870 (6)	4989 (6)	9330 (4)	A1H(9)	828 (4)	467 (5)	915 (3)	6 (1)
F2N(1)	11431 (3)	2201 (4)	10204 (2)	A1H(10)	899 (4)	454 (5)	957 (3)	5 (1)
F2C(2)	10599 (4)	2164 (4)	9672 (3)	F2H(1)	988 (6)	138 (7)	903 (4)	10 (2)
F2O(2)	10170 (3)	2907 (3)	9417 (2)	F2H(2)	1287 (5)	−187 (5)	1098 (4)	5 (2)
F2N(3)	10198 (3)	1250 (4)	9389 (3)	F2H(3)	1458 (6)	−226 (7)	1255 (5)	8 (2)
F2C(4)	10594 (4)	369 (5)	9618 (3)	F2H(4)	1409 (7)	−272 (7)	1173 (5)	9 (3)
F2O(4)	10229 (3)	−406 (3)	9357 (2)	F2H(5)	1492 (5)	−229 (6)	1205 (4)	6 (2)
F2C(4a)	11486 (4)	398 (4)	10199 (3)	F2H(6)	1512 (6)	−68 (7)	1310 (4)	5 (2)
F2N(5)	11888 (3)	−427 (4)	10436 (3)	F2H(7)	1570 (5)	−72 (5)	1270 (4)	5 (1)
F2C(5a)	12715 (3)	−386 (5)	10986 (3)	F2H(8)	1546 (7)	23 (8)	1292 (5)	15 (3)
F2C(6)	13154 (4)	−1290 (5)	11260 (3)	F2H(9)	1416 (5)	111 (6)	1204 (4)	7 (2)
F2C(7)	13980 (5)	−1291 (6)	11812 (4)	F2H(10)	1322 (5)	241 (6)	1169 (4)	6 (2)
F2C(7α)	14416 (5)	−2283 (7)	12095 (4)	F2H(11)	1354 (4)	241 (5)	1112 (3)	4 (1)
F2C(8)	14373 (4)	−393 (6)	12103 (3)	F2H(12)	1271 (5)	295 (5)	1100 (3)	7 (2)
F2C(8α)	15269 (4)	−366 (7)	12723 (3)	A2H(1)	868 (5)	290 (5)	859 (4)	4 (2)
F2C(9)	13971 (4)	491 (6)	11834 (3)	A2H(2)	810 (6)	350 (7)	805 (5)	7 (3)
F2C(9a)	13133 (4)	508 (5)	11270 (3)	A2H(3)	887 (5)	−59 (6)	847 (4)	3 (2)
F2N(10)	12691 (3)	1377 (4)	10975 (2)	A2H(4)	828 (4)	−119 (5)	790 (3)	4 (1)
F2C(1')	13138 (4)	2325 (6)	11224 (4)	A2H(5)	562 (4)	−27 (4)	600 (3)	5 (1)
F2C(10a)	11856 (4)	1358 (4)	10451 (3)	A2H(6)	585 (4)	217 (5)	581 (3)	4 (1)
A2N(1)	8454 (3)	1209 (3)	8181 (2)	A2H(7)	533 (4)	224 (5)	629 (3)	2 (1)
A2C(2)	8022 (4)	2039 (4)	7884 (3)	A2H(8)	437 (4)	203 (5)	518 (3)	6 (2)
A2N(2)	8379 (4)	2884 (4)	8209 (3)	A2H(9)	486 (4)	99 (5)	516 (3)	2 (1)
A2N(3)	7248 (3)	2126 (3)	7314 (2)	A2H(10)	438 (3)	98 (4)	564 (3)	4 (1)
A2C(4)	6939 (3)	1247 (4)	7039 (2)	WH(1)	1122 (7)	750 (9)	948 (5)	21 (4)
A2C(5)	7319 (4)	348 (4)	7277 (3)	WH(2)	1098 (7)	740 (8)	1017 (5)	13 (4)
A2C(6)	8124 (4)	342 (4)	7883 (3)	EH(1)	255 (4)	−102 (5)	446 (3)	5 (1)
A2N(6)	8544 (4)	−492 (4)	8165 (3)	EH(2)	289 (7)	−69 (7)	535 (4)	4 (2)
A2N(7)	6779 (3)	−419 (3)	6861 (2)	EH(3)	369 (7)	−109 (7)	494 (5)	6 (2)

(a)

(b)
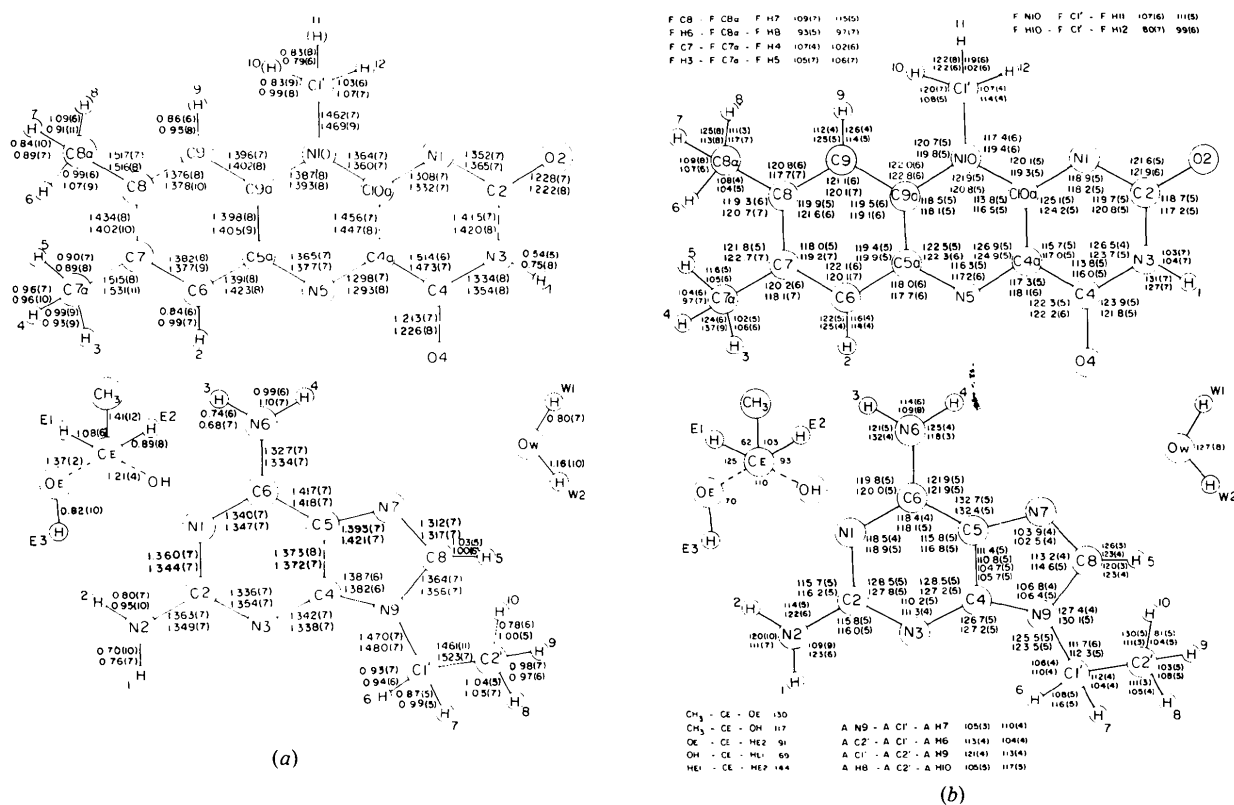
Fig. 2. Schematic drawings indicating (a) the covalent-bond distances (Å), and (b) the covalent-bond angles (°) for the complex (lumiflavin–2,6-diamino-9-ethylpurine)₂–ethanol–H₂O. The parameters of molecules F1 and A1 are placed above the corresponding values of molecules F2 and A2. Standard deviations, as estimated from the variance–covariance matrices of the final cycles of least-squares refinement average 0·008 Å and 0·5° respectively, for the distances and angles involving non-solvent, non-hydrogen atoms. The corresponding quantities involving H atoms are 0·07 Å and 5° respectively.

The intermolecular interactions

The structure is composed of centrosymmetrically related layers of base-paired molecules. These layers are essentially parallel to, and fall half-way between, the (406) crystallographic planes. The molecules composing the asymmetric unit of the unit cell are virtually coplanar. The dihedral angle between the A1 least-squares plane and that of F1 is 3·5°, that between A2 and F2 is 6·6°, and that between the members of the A1–A2 base pair is 7·6°. The 62 non-hydrogen atoms comprising the four heterocyclic molecules shown in Fig. 1, less adenine atoms C(2'), have a r.m.s. deviation of 0·143 Å from their collective least-squares plane. Although the degree of coplanarity of the individual bases is typical for such molecules (Voet & Rich, 1970), the coplanarity of the entire asymmetric unit is remarkable considering its large lateral extent.

The component molecules of the structure participate in an extensive array of hydrogen bonds, the parameters of which are given in Table 3. Flavin F2 participates in a triply hydrogen-bonded Watson–Crick-type base-pairing interaction with adenine derivative A2. The amine substituent to atom C(2) of

Table 3. Hydrogen-bond parameters

Roman numerals accompanying atom numbers refer to atoms related to those in Table 2 by the following symmetry operations: (i) x, y + 1, z; (ii) x, y − 1, z.

D–H···A	D···A (Å)	H···A (Å)	D–H···A (°)
A1N(6)–A1H(3)···F1O(2)	2·957 (6)	2·26 (6)	157 (6)
A1N(2)–A1H(1)···F1O(4)	2·880 (6)	2·38 (11)	131 (11)
A2N(6)–A2H(3)···F2O(4)	2·883 (6)	2·26 (5)	155 (9)
A2N(2)–A2H(1)···F2O(2)	2·998 (6)	2·34 (7)	145 (7)
F1N(3)–F1H(1)···A1N(1)	2·960 (6)	2·43 (5)	164 (8)
F2N(3)–F2H(1)···A2N(1)	2·955 (6)	2·30 (8)	147 (9)
A1N(2) A1H(2)···A2N(3)	2·988 (7)	2·20 (7)	167 (6)
A2N(2)–A2H(2)···A1N(3)	3·060 (7)	2·11 (10)	173 (6)
A1N(6)–A1H(4)···A2N(7) (i)	2·982 (6)	2·01 (7)	165 (5)
A2N(6)–A2H(4)···A1N(7) (ii)	2·894 (7)	1·88 (8)	151 (6)

the adenine derivatives permits the formation of a third hydrogen bond that is not possible in the normally doubly hydrogen-bonded adenine–uracil base pairs. Flavin F1 and adenine A1 form a similar triply hydrogen-bonded base pair, but one in which the orientation of A1 with respect to F1 is reversed relative to the A2–F2 interaction. The geometry of these base
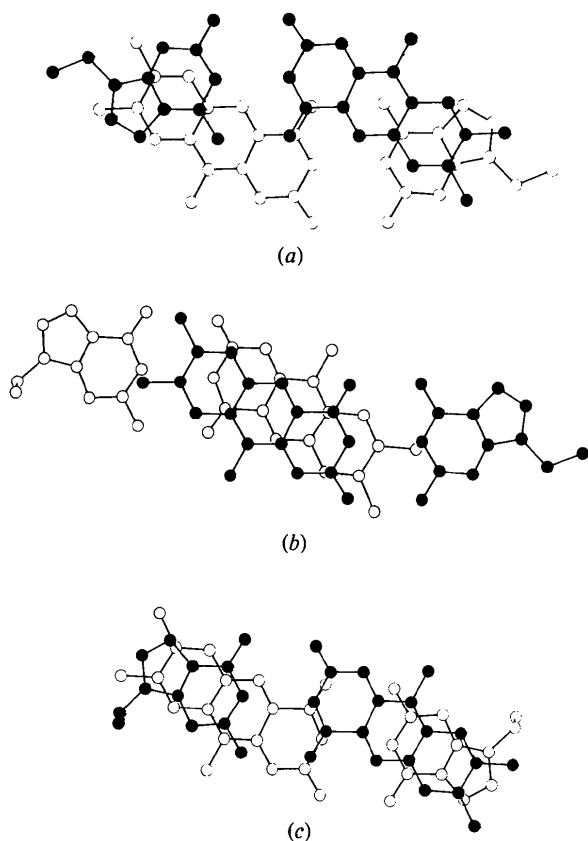
(a)

(b)

(c)

Fig. 3. Projections onto the least-squares planes of the aromatic rings of two consecutive molecular layers. Illustrated are the stacking relationships between (a) two centrosymmetrically related $F2$–$A2$ complexes; (b) the pseudo centrosymmetrically related $F2$–$A2$ and $F1$–$A1$ complexes; and (c) two centrosymmetrically related $F1$–$A1$ complexes. Atoms are represented as spheres of arbitrary radii. H atoms have been omitted for the sake of clarity.

Table 4. *Intermolecular contacts less than* $3 \cdot 4$ Å

Target-atom-transformation indicators refer to atoms related to those in Table 2 by the following symmetry operations (1) $2 - x$, $-y$, $2 - z$; (2) $1 - x$, $1 - y$, $1 - z$; (3) $x - 1$, $\frac{1}{2} - y$, $z - \frac{1}{2}$.

Origin atom	Target atom	Target-atom transformation	Distance
$F2C(4)$	$F2O(4)$	1	$3 \cdot 118$ Å
$F2C(4)$	$F2C(4)$	1	$3 \cdot 260^*$
$F1N(3)$	$F1C(4)$	2	$3 \cdot 264$
$F1N(5)$	$F2C(10a)$	3	$3 \cdot 275$
$F1C(4)$	$F2C(9a)$	3	$3 \cdot 317^*$
$F1N(10)$	$A1C(2)$	2	$3 \cdot 319$
$F1C(6)$	$F2C(2)$	3	$3 \cdot 335^*$
$F1C(2)$	$F2C(6)$	3	$3 \cdot 338^*$
$F1C(5a)$	$A1C(6)$	2	$3 \cdot 338^*$
$F2C(5a)$	$A2C(6)$	1	$3 \cdot 357^*$
$F2N(3)$	$F2O(4)$	1	$3 \cdot 360$
$F1C(2)$	$F1O(4)$	2	$3 \cdot 361$
$F1C(10a)$	$F2N(5)$	3	$3 \cdot 366$
$F2O(4)$	$F2C(4a)$	1	$3 \cdot 376$
$F2C(6)$	$A2N(1)$	1	$3 \cdot 384$
$F1C(7)$	$A1N(7)$	2	$3 \cdot 394$
$F2C(5a)$	$A2N(1)$	1	$3 \cdot 394$
$F1O(4)$	$F2C(9)$	3	$3 \cdot 396$
$F1N(5)$	$F2N(10)$	3	$3 \cdot 399$

* Less than accepted minimal van der Waals interatomic contacts ($3 \cdot 4$ Å for C—C, $3 \cdot 2$ Å for C—N and $3 \cdot 1$ Å for C—O).

pairs was expected in light of the chemical similarity between uracil and the pyrimidinoid ring of flavins together with the fact that in the solid state 2,6-diaminopurine derivatives have invariably been observed to associate with adenine derivatives by one of the foregoing types of base-pairing modes (Voet & Rich, 1970; Sobell, 1972). It should be noted that the alternation of the orientations of the adenine derivatives together with the placement of the water and ethanol molecules constitute the greatest local deviations of the structure from a-glide symmetry.

There are also base-pairing interactions between the adenine derivatives, resulting in endless parallel $\cdots$—$A1$–$A2$–$A1$–$A2$–$\cdots$ ribbons running through each structural layer. The cyclic dimer involving the N(6)—H(4)$\cdots$N(7) hydrogen bonds is a characteristic mode of adenine self-association (Voet & Rich, 1970). That involving the N(2)—H(2)$\cdots$N(3) hydrogen bonds has often been observed in structures of 2,6-diaminopurine and 2-aminopurine derivatives (Voet & Rich, 1970;

Sobell, 1972). As can be seen in Fig. 1, both types of self-pairing interactions lie across pseudo centers of symmetry that are generated by the pseudo a-glide plane in combination with the crystallographic twofold screw axis.

All the hydrogen bonds that the flavin and adenine molecules are capable of forming have been formed. They all fall within the normally observed ranges for such interactions with respect to length and linearity (Voet & Rich, 1970; Hsu & Craven, 1974).

The structure exhibits stacking interactions which are among the most extensive that have been observed in structures containing nucleic acid base-type molecules (Bugg, Thomas, Rao & Sundaralingam, 1971). These are illustrated in Fig. 3. Each lumiflavin molecule is bounded on one side by a symmetry-non-equivalent lumiflavin molecule and on the other side by a purine ring. In this way the order of stacking of the molecules is $A2/F2[-x,-y,-z]/F1[-x, -\frac{1}{2} + y, \frac{1}{2} - z]/A1[x, \frac{1}{2} - y, -\frac{1}{2} + z]$. Table 4, which lists the interatomic contacts less than $3 \cdot 4$ Å, indicates that there are numerous closer than van der Waals interactions within these stacks.

The geometry of the foregoing stacks has been discussed in detail elsewhere (Scarbrough, Shieh & Voet, 1976). The extent and the regular geometry of the stacks together with their numerous close interatomic contacts as well as the red coloration of the crystals lead to the conclusion that the crystalline complex exhibits flavin/flavin as well as flavin/adenine charge-transfer interactions. The possibility that the red coloration of the crystals is due to the presence of
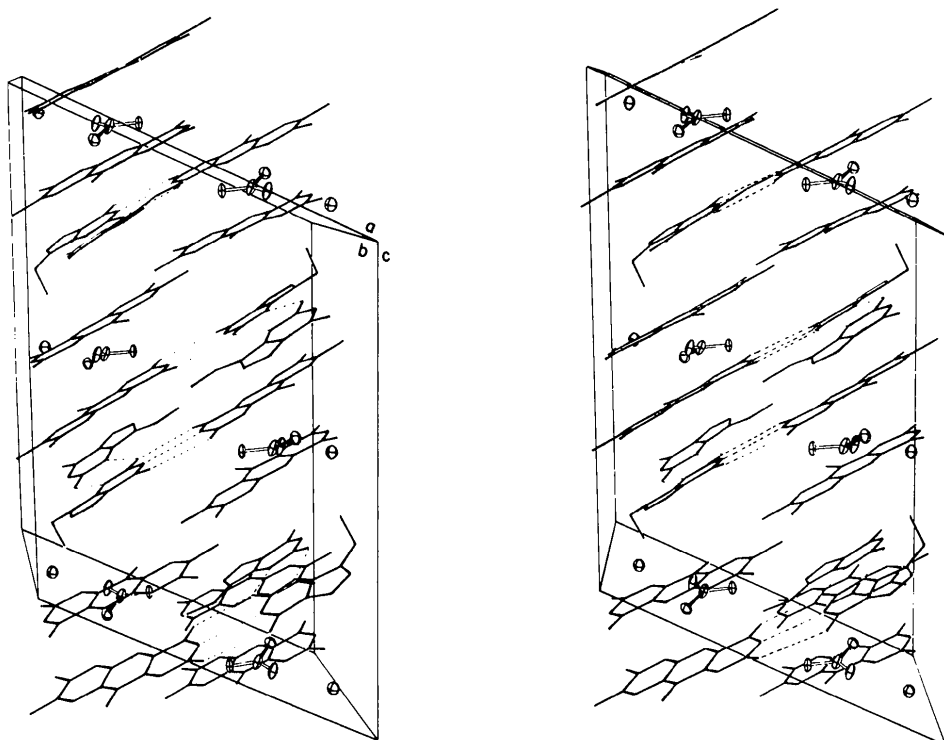
Fig. 4. A stereographic view of the crystalline complex (lumiflavin–2,6-diamino-9-ethylpurine)$_2$–ethanol–H$_2$O illustrating the packing of the molecules in the unit cell. The view is roughly along **b** with **a** and **c** extending horizontally and vertically respectively. Hydrogen bonds are represented as dashed lines. H atoms have been omitted for the sake of clarity. The cocrystallized solvent molecules are drawn as thermal ellipsoids at the 25% probability level. The alternate positions of the disordered ethanol hydroxyl groups are indicated by unfilled bonds.

diradicals, a hypothesis that is suggested by the failure of the red crystalline complex to form in solutions containing lumiflavin, must be rejected on the grounds that EPR measurements, both at liquid-helium and at room temperatures, proved the crystals to be diamagnetic (Scarbrough, Shieh & Voet, 1976).

Fig. 4 shows the contents of the unit cell. This provides an alternate view of the hydrogen bonding and stacking patterns in the crystal structure of the complex. Fig. 4 also shows the location of the cocrystallized solvent molecules within the unit cell. Each of the two half-weighted, disordered oxygen positions of the ethanol molecule exhibits a potential hydrogen bond. The EO atom is 2·738 Å away from the carbonyl oxygen $F10(2)$, whereas the distance from $EO'H$ to the solvent water oxygen WO is 2·904 Å. These solvent molecules apparently participate in no other hydrogen-bonding contacts [although the water O atom is 2·965 Å distant from the potential hydrogen-bond acceptor atom $F2O(4)$]. Therefore, it seems that the solvent molecules are merely filling the cavities that occur between the stacked flavin and adenine layers. The lack of forces holding the solvent molecules in place is probably the cause of the disorder observed in the ethanol molecule and accounts for the difficulty in locating its H atoms.

Discussion

The chemical and biological implications of this structural study, the first to present information pertaining to the nature of flavin–flavin and flavin–adenine charge-transfer interactions, have been discussed elsewhere (Scarbrough, Shieh & Voet, 1976). In light of the increasing use of direct methods, and particularly of the program $MULTAN$ {more than 80% of the structures presented at the 1976 Summer Meeting of the American Crystallographic Association were solved with this program [Abstracts, American Crystallographic Association Summer Meeting, 1976]} it is of interest to include in this report a summary of the direct-method trials used in the solution of the structure.

Extended chains or networks of peaks are frequently observed in the E maps of crystal structures exhibiting non-crystallographic translational regularities (e.g. Lai & Marsh, 1974). Bürgi & Dunitz (1971) have demonstrated that 'amplitude termination effects' in such E maps are responsible for the spurious peaks that often cause the difficulties in sorting out the correct atomic positions of the molecules comprising such crystal structures. This comes about because the largest E's in a regular structure arise from reflections whose inter-

planar spacings coincide, both in magnitude and direction, to the vectors generating the non-crystallographic translationally repeating pattern. The irregularities in the repeating pattern, which bestow the identity of the structure, tend to manifest themselves in the reflections with lower E values. Hence, an E map of a regular structure that is based only upon those reflections with the largest E values (as is almost always the case), even if it is properly phased, will tend to reveal only the regularities of the structure but not its identity.

In addition, it has been shown (Hauptman & Karle, 1955, 1959; Cochran, 1958) that the simpler phase relationships do not rigorously hold for structures containing several coincident or nearly coincident interatomic vectors in the asymmetric unit. Indeed, for the Fourier transform of a benzene ring or several fused benzene rings some of the strongest triple products yield phase indications that are uniquely wrong (Thiessen & Busing, 1974). Thus the fact that the structure, including the hydrogen-bonded regions, is composed almost exclusively of translationally repeated hexagonal rings (with the most notable exception being the reasonably similar pentagonal imidazole rings of the purines); the coplanarity of the entire asymmetric unit; and the stacking regularities that cause neighboring layers of hexagonal units to project onto one another in interlocking patterns; all combined to create considerable difficulty in the eventual solution of this structure by direct methods.

As was stated earlier, after three corrections were made to the calculation of the normalized structure factors (the Debye scattering correction, separate scaling of parity groups and the removal of one- and two-dimensional reflections) the structure was solved with the program $MULTAN$. The 500 reflections with the highest E were used to generate 2000 Σ_2 relationships. The program selected the origin and three starting-set reflections, thus creating eight phase sets. There was good discrimination among the various sets with the combined figure of merit as the criterion; the set with the highest combined value (second-highest absolute figure of merit) produced an E map in which most of both flavins and fragments of the purines could be recognized.

As a test, the E values were recalculated for all data (*i.e.* with one- and two-dimensional reflections included) but again applying Debye scattering corrections and parity-group scaling. $MULTAN$ was run as before only this time the program was constrained to select the same origin and starting-set reflections used in the successful phasing procedure. The eight phase sets showed almost no discrimination in their combined figure of merit values. The set corresponding to the successful starting-set phases of before now had the third-highest combined figure of merit (fifth-highest absolute figure of merit) but produced an E map in which the same molecular fragments as before were

discernable. Although there were additional spurious peaks in the map, the structure would probably have been solved by a diligent investigator using this set of $MULTAN$ outputs.

Three additional tests were made with all data for the E calculation. In the first, Debye scattering corrections were made but no parity-group scaling was done, in the second, no Debye scattering corrections were employed while the parity groups were scaled separately, and in the third, neither Debye scattering nor parity-group scaling corrections were made. With each of the three sets $MULTAN$ was again constrained to select the same origin and starting-set reflections that had been successful, and with each case eight possible phase sets were produced. In the first test none of the eight sets produced an E map from which the correct structure could have been recognized. However, in the second case (*i.e.* no Debye scattering correction, parity groups scaled separately) the set with the third-highest combined figure of merit gave an E map that, although crowded with spurious peaks, revealed enough of the correctly positioned molecular fragments to allow eventual solution of the structure. Test three failed. It gave no discrimination and produced no successful E maps.

For structures containing molecules composed of fused six-membered rings, Laing (1976) has recently proposed a simple approach to direct methods involving a judicious choice of origin and starting-set reflections. According to Laing, the plane of one origin-determining reflection should lie in the approximate plane of the molecule and have an interplanar spacing of 3·5 Å. The planes of the other two origin-determining reflections should be perpendicular to the molecular plane, should parallel two different sides of the fused hexagons, and should have interplanar spacings of about 1·2 Å. It is of interest to compare the origin-determining reflections selected by $MULTAN$ for the eventually successful phase set against these criteria. The $(8,1,\bar{1}\,\bar{1})$ plane is in the approximate plane of the molecule $(4,0,\bar{6})$ but its interplanar spacing is only 1·7 Å. Planes $(11,6,1)$ and $(14,6,\bar{3})$ are approximately perpendicular to the molecular plane and have interplanar spacings of 1·1 and 1·0 Å respectively. The $(11,6,1)$ plane parallels one side of the hexagonal ring system. However, the $(14,6,\bar{3})$ plane falls relatively close to the same plane. This is a situation that Laing cautions should be avoided.

The origin-determining reflections selected from the convergence mapping routine of $ORPHEX$ (Thiessen & Busing, 1974) with, for example, the Fourier transform of a centrosymmetric flavin fragment were 1,16,19, 15,5,$\bar{4}$ and 669. None of these three reflections fall in the molecular plane, and their respective d spacings of 0·93, 0·99 and 1·18 Å fail to satisfy Laing's criteria. The resulting E map contains the familiar 'chicken-wire' arrangement of peaks. A similar E map was obtained when, with the same flavin

Fourier transform, the convergence mapping routine was constrained to select the successful *MULTAN* origin set. Tracing the phasing procedure back through the convergence map reveals that 41 reflections were correctly phased before $1,11,\bar{1}$ (a reflection involved in several uniquely wrong triple-product relationships) is incorrectly phased. Subsequently, 39 reflections among the remaining 250 E values were assigned wrong phases. It therefore appears that too few phase relationships were used to permit *ORPHEX* to generate the statistical information necessary to correctly identify all triple product rule violations (W. E. Thiessen, personal communication).

Lessinger (1976) has made a comprehensive study of various *MULTAN* failures from which he has been able to suggest several tactics to be used with difficult cases. Most prominent among those proposed are using the known information about the molecular structure in the calculation of E values (Debye scattering corrections), increasing the number of starting-set reflections, and using as many Σ_2 relationships as possible with as few E values as necessary. It is interesting to note that a *MULTAN* attempt utilizing Debye-corrected E values, expanded to use 4000 Σ_2 relationships (rather than the usual 2000), and employing five starting-set reflections produced 32 phase sets that all yielded E maps containing extended sheets of interlocking hexagons. The program-selected origin-determining reflections were 767, 425 and 415 which clearly violate Laing's criteria for interplanar spacings and mutual perpendicularity. A similar *MULTAN* attempt in which no Debye scattering correction was applied to the E calculation also failed to produce a solution among 32 phase sets. In this case the origin-determining reflections were 10,6,3, 758 and 767. The latter two reflections again fall on essentially the same plane and thereby fail to 'fix' the molecules within the crystal lattice.

From the foregoing discussion it appears that a judicious choice of origin combined with the counter-action of the abnormal intensity distribution by individual parity class scaling is sufficient to allow direct methods to correctly phase the present structure. However, it should be noted that the order of the reflections in the convergence map, the criterion used by *MULTAN* to choose the starting set of reflections, was dependent on both the use of Debye scattering correction and on the deletion of one- and two-dimensional reflections. Thus a perhaps fortunate combination of tactics was needed to allow the phasing to start and to continue on a proper track. Therefore, the moral of this story is that even if sound strategies are followed in phasing by direct methods, many variations in tactics might be required before the crystal structure of a highly symmetric molecule will reveal itself.

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Structure Cristalline du Dioxodichlorobis(hexaméthylphosphoramide)molybdène(VI)

PAR B. VIOSSAT

Laboratoire de Chimie III, Université Pierre et Marie Curie, 4 place Jussieu, 75230 Paris Cédex 05, France

ET P. KHODADAD ET N. RODIER

Laboratoire de Chimie Minérale, Faculté de Pharmacie, rue J. B. Clément, 92290 Chatenay-Malabry, France

$MoO_2Cl_2\{OP[N(CH_3)_2]_3\}_2$ crystallizes in the tetragonal system, space group $I4_1cd$, with $a = b = 14\cdot466$ (7), $c = 24\cdot31$ (1) Å. The structure has been refined by the least-squares method from single-crystal diffractometer data with 630 independent structure factors. $R = 0\cdot044$. The two hexamethylphosphoramide ligands occupy *cis* positions in the octahedral Mo coordination. The P atom is in an almost regular tetrahedral environment.

Introduction

En milieu très acide (HCl, 8M) le molybdate de sodium Na_2MoO_4 réagit avec l'hexaméthylphosphoramide (HMPA) et donne le composé de formule MoO_2Cl_2.2HMPA. La méthode de préparation a déjà été décrite ainsi que la symétrie du réseau et les paramètres cristallins (Khodadad & Viossat, 1976). Il est dit, dans ce mémoire, que le complexe étudié cristallise dans le système orthorhombique, groupe spatial *Fdd*2, et que la maille, de dimensions $a_1 = b_1 = 20,46$, $c_1 = 24,34$ Å, contient 16 unités formulaires.

Au cours de la présente étude, nous avons observé les deux faits suivants qui n'avaient pas été notés précédemment: (1) les réflexions *hkl* n'existent que pour $l = 2n$, (2) aux erreurs d'expérience près, les intensités des réflexions *hkl* et *khl* sont égales.

Ceci indique que la symétrie du réseau n'est pas celle indiquée déjà. Nous avons été conduits à décrire le composé dans une maille quadratique de dimensions: $a = b = 14,466$ (7), $c = 24,31$ (1) Å, reliée à la précédente par les relations: $\mathbf{a} = (\mathbf{a}_1 + \mathbf{b}_1)/2$, $\mathbf{b} = (-\mathbf{a}_1 + \mathbf{b}_1)/2$ et $\mathbf{c} = \mathbf{c}_1$.

Les conditions qui limitent l'existence des réflexions s'écrivent alors: *hkl*: $h + k + l = 2n$; *0kl*: $l(k) = 2n$; et *hhl*: $2h + l = 4n$. Elles correspondent à un seul groupe spatial, le groupe $I4_1cd$.

Mesure de l'intensité des réflexions

Le monocristal utilisé a été prélevé dans le produit obtenu après recristallisation du produit brut dans de l'acétone anhydre. Sa forme est approximativement celle d'un cube dont l'arête mesure environ 130 μm. Il faut, pour éviter son altération, le conserver à l'abri de la lumière et de l'humidité.